EPA United States Environmental Protection Agency

Capabilities and Evaluation of the US EPA's HTTK (High Throughput Toxicokinetics) R package

Webinar Presentation to European Chemical Agency

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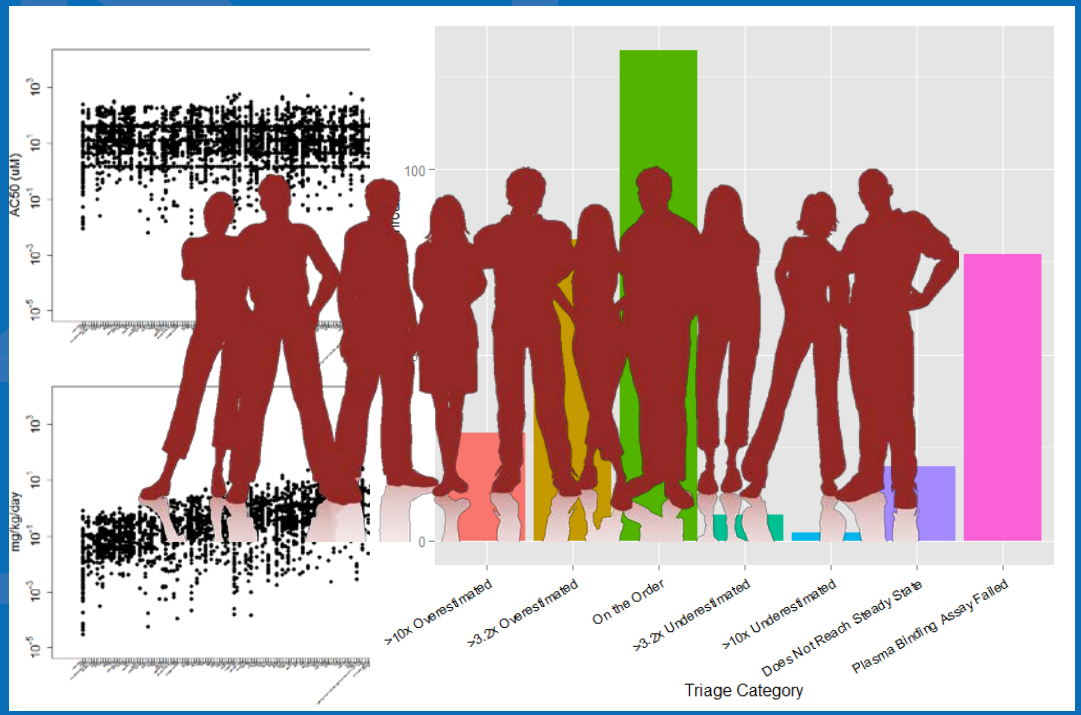

Figure includes image from Thinkstock

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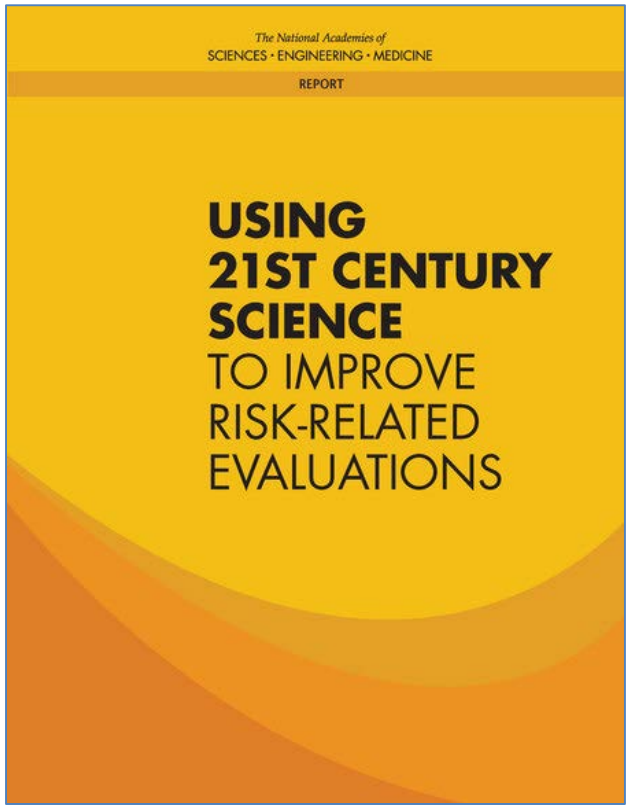
Introduction

Park *et al*. (2012): At least 3221 chemicals in pooled human blood samples, many appear to be exogenous

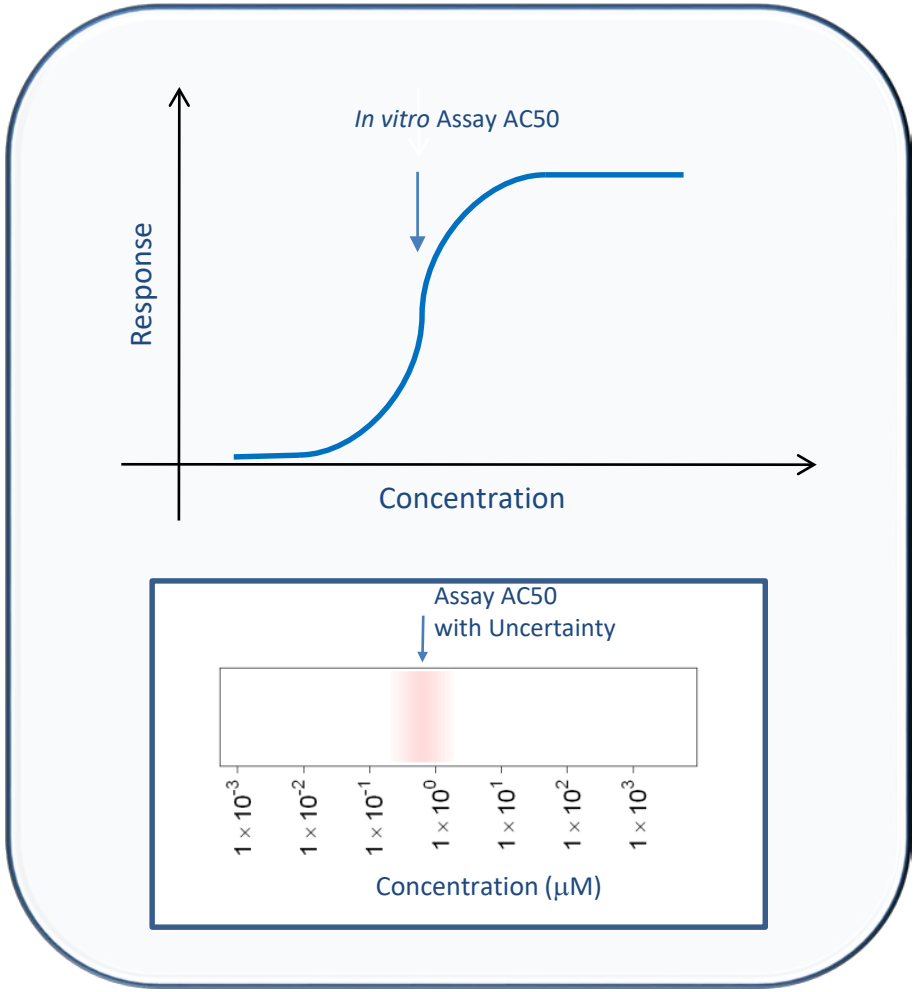


National Academy of Sciences, January, 2017: "Translation of high-throughput data into risk-based rankings is an important application of exposure data for chemical priority-setting. Recent advances in high-throughput toxicity assessment, notably the ToxCast and Tox21 programs… and in high-throughput computational exposure assessment… have enabled first-tier risk-based rankings of chemicals on the basis of margins of exposure…"

November 29, 2014

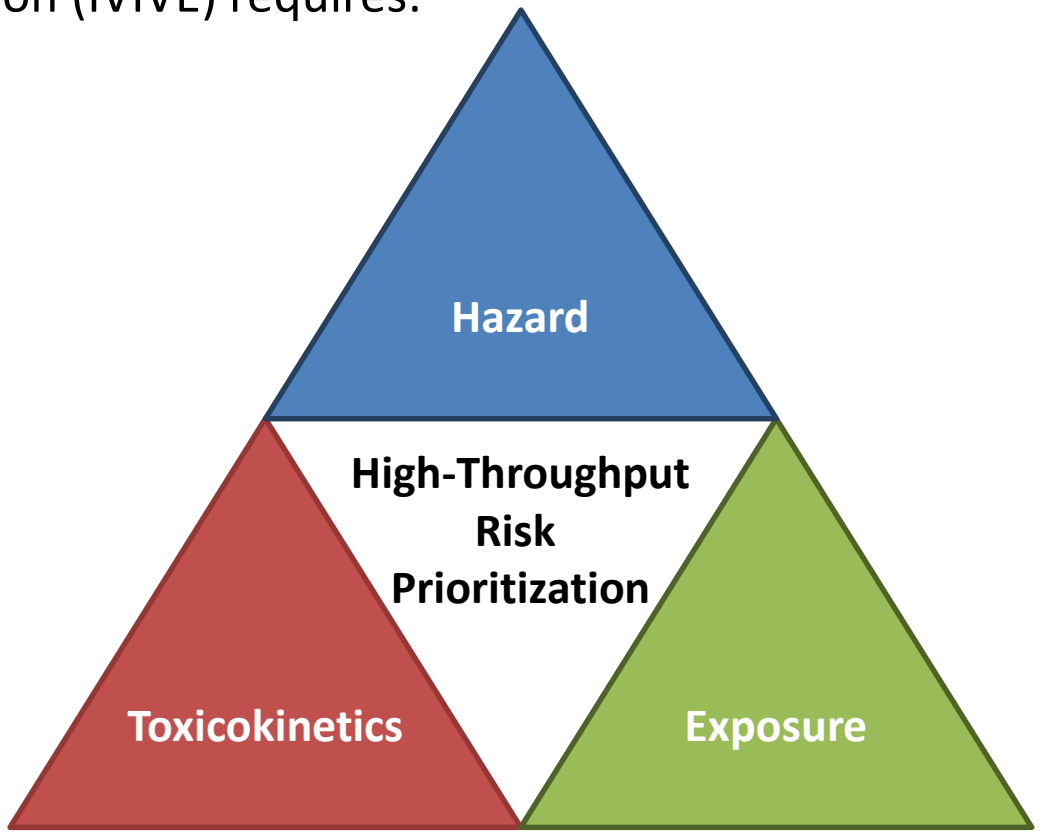
High-Throughput Screening

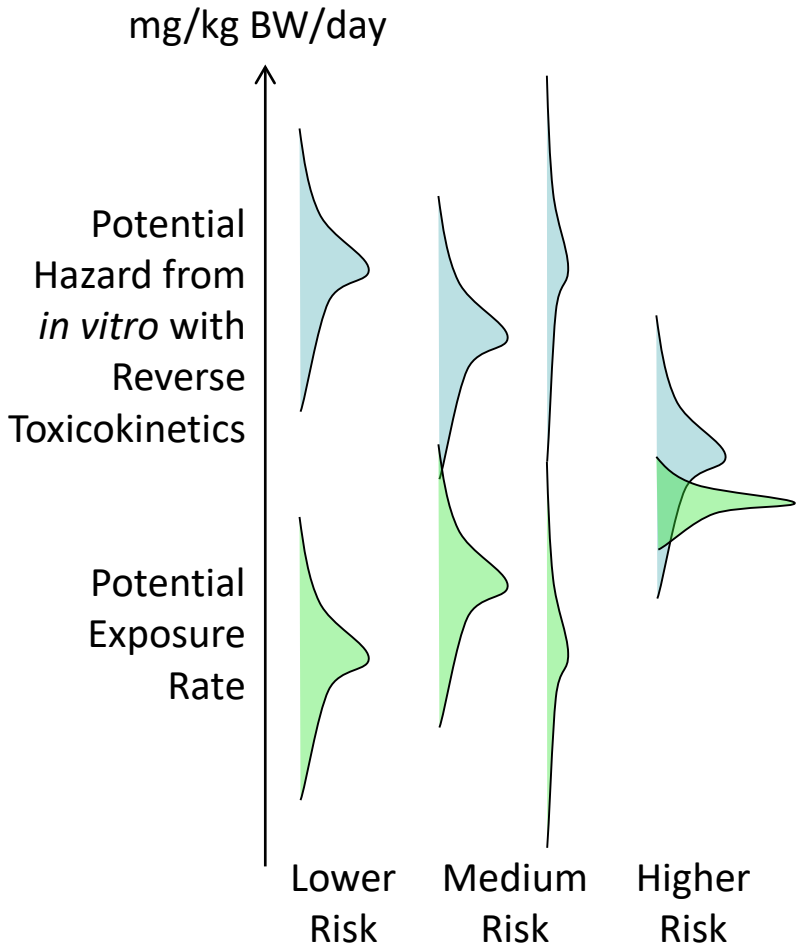
- **Tox21**: Examining >8,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)
- **ToxCast**: For a subset (>2000) of Tox21 chemicals ran >1100 additional assays (Judson *et al.*, 2010)
- Most assays conducted in dose-response format (identify 50% activity concentration – AC50 – and efficacy if data described by a Hill function, Filer *et al.*, 2016)
- How do we relate *in vitro* concentration to *in vivo* doses? *In vitro-in vivo* extrapolation (IVIVE)



High-Throughput Risk Prioritization

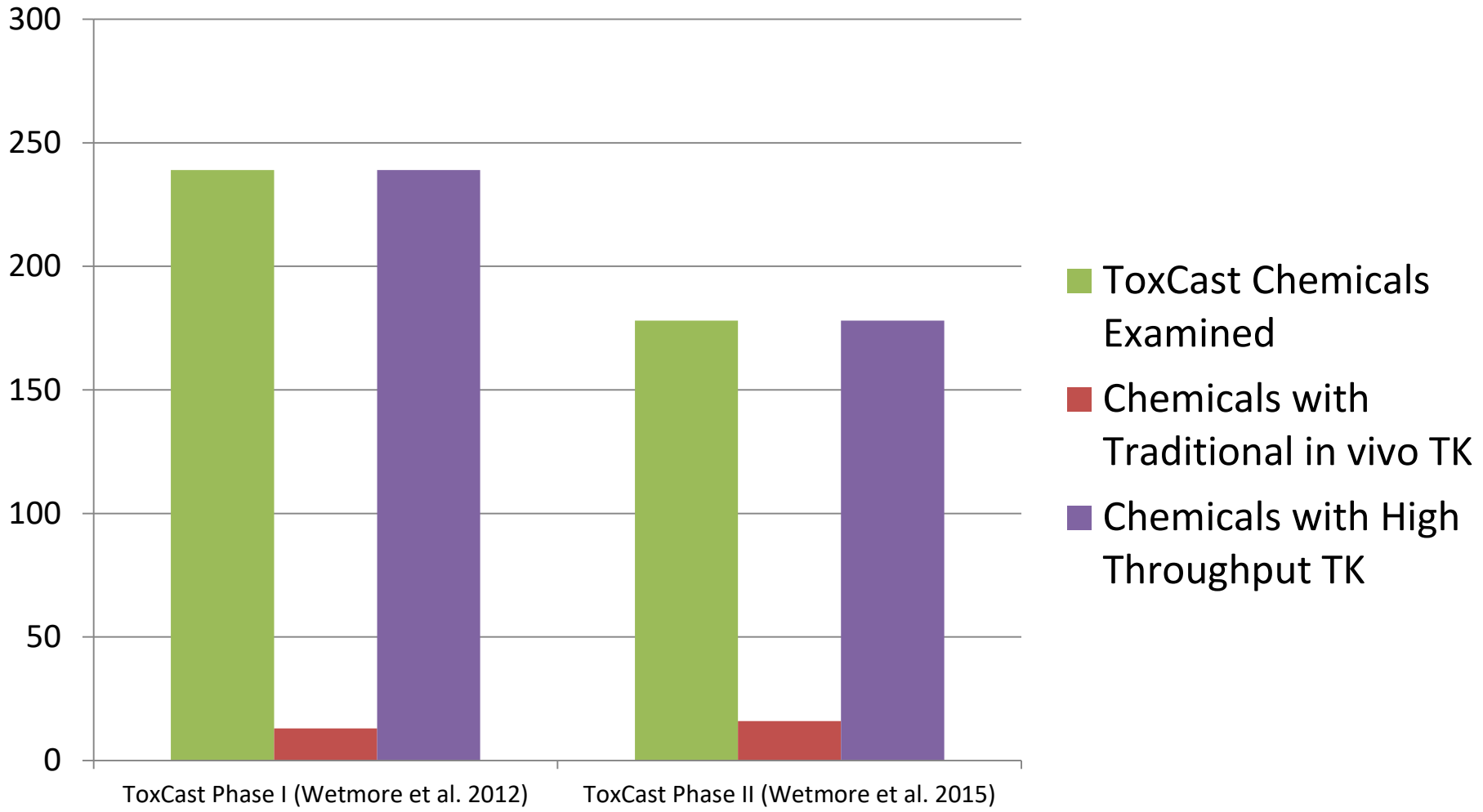
- High throughput risk prioritization based upon *in vitro-in vivo* extrapolation (IVIVE) requires:





Most chemicals do not have TK data – Wetmore et al. (2012…) use *in vitro* methods adapted from pharma to fill gaps

The Need for *In Vitro* Toxicokinetics



- Studies like Wetmore et al. (2012, 2015), address the need for TK data using *in vitro* methods

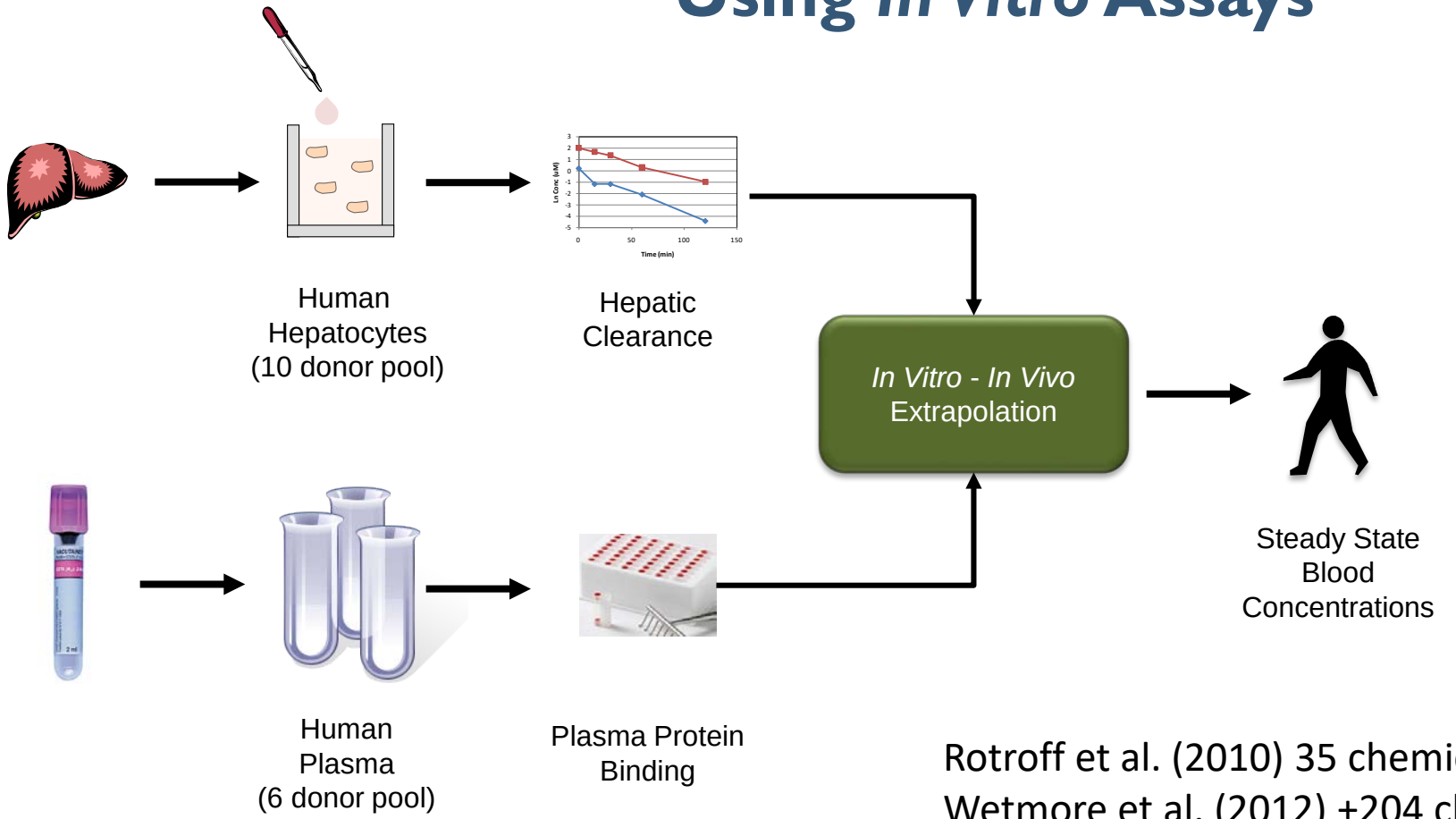
High Throughput Toxicokinetics (HTTK)

- Toxicokinetics (TK) provides a bridge between toxicity and exposure assessment by predicting tissue concentrations due to exposure
  - However 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 (starting off with Rotroff, *et al*., 2010)

- A new EPA open source R package (“httk”) is freely available on CRAN allows RTK and other statistical analyses of 553 chemicals (more coming)

Characterizing Human *In Vivo* Toxicokinetics Using *In Vitro* Assays



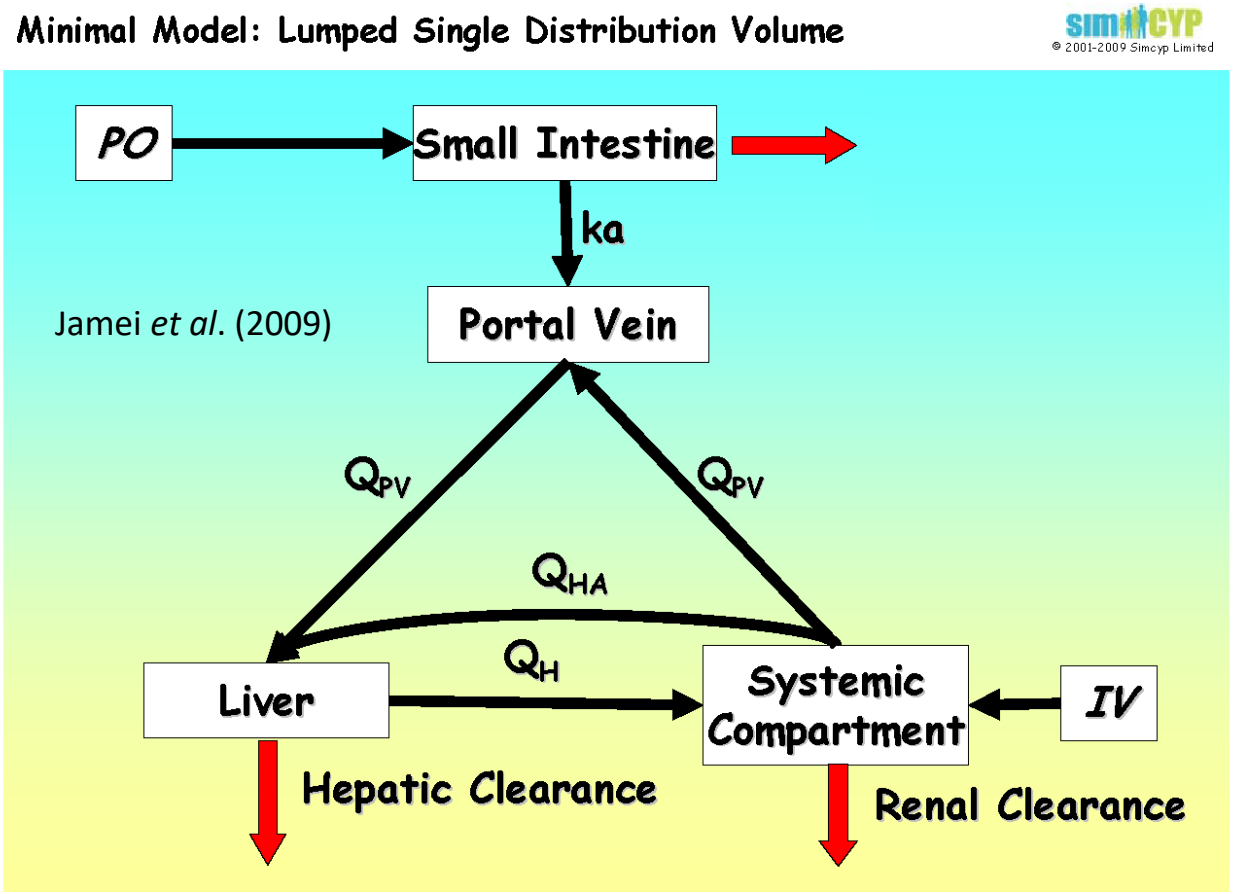
Rotroff et al. (2010) 35 chemicals
Wetmore et al. (2012) +204 chemicals
Wetmore et al. (2015) +163 chemicals

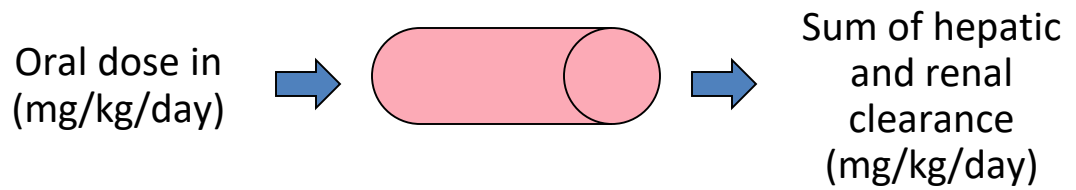
Slide from Barbara Wetmore

A Basic Model for 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

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



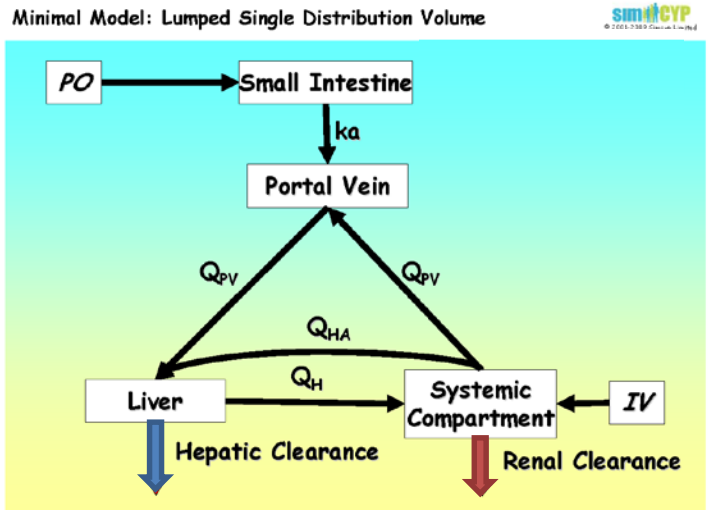


Variability in the Basic Steady-State TK Model

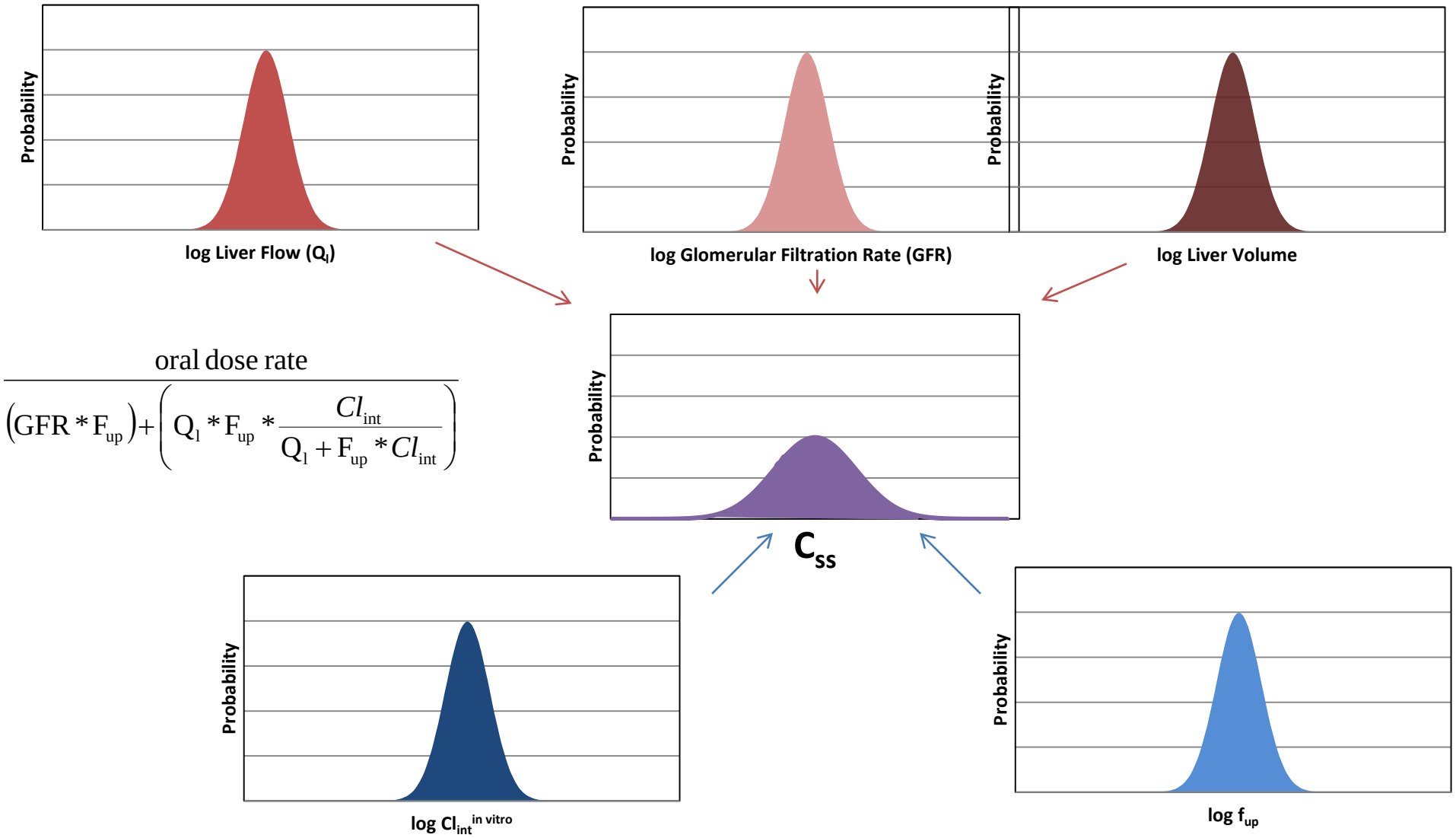
- *In vitro* clearance (µL/min/10^6 hepatocytes) is scaled to a whole organ clearance using the density of hepatocytes per gram of liver and the volume of the liver (which varies between individuals)
- Glomerular filtration rate (GFR) and blood flow to the liver (Q_l) both vary from individual to individual

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

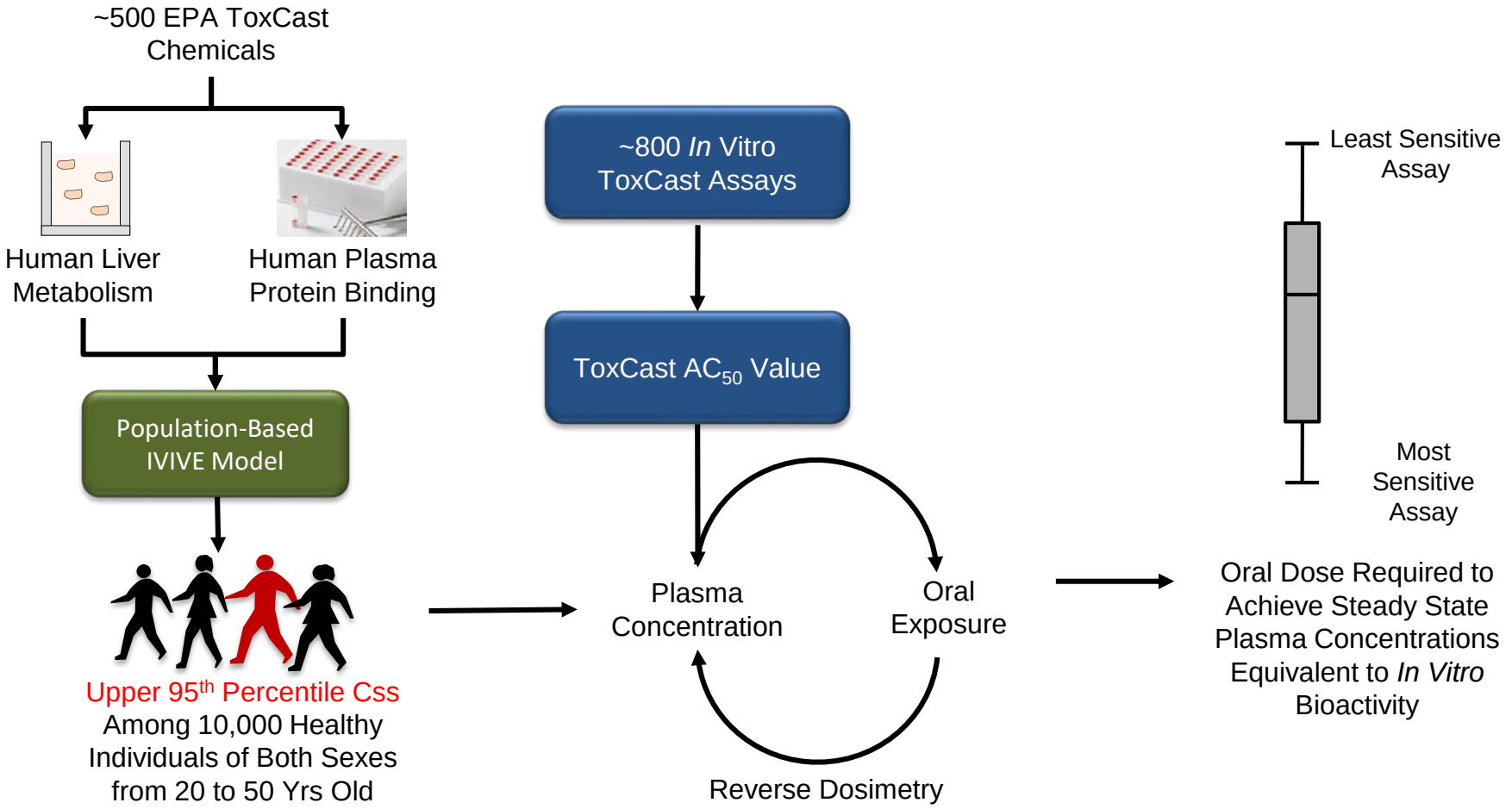
Jamei *et al*. (2009)



Monte Carlo (MC) Approach to Variability



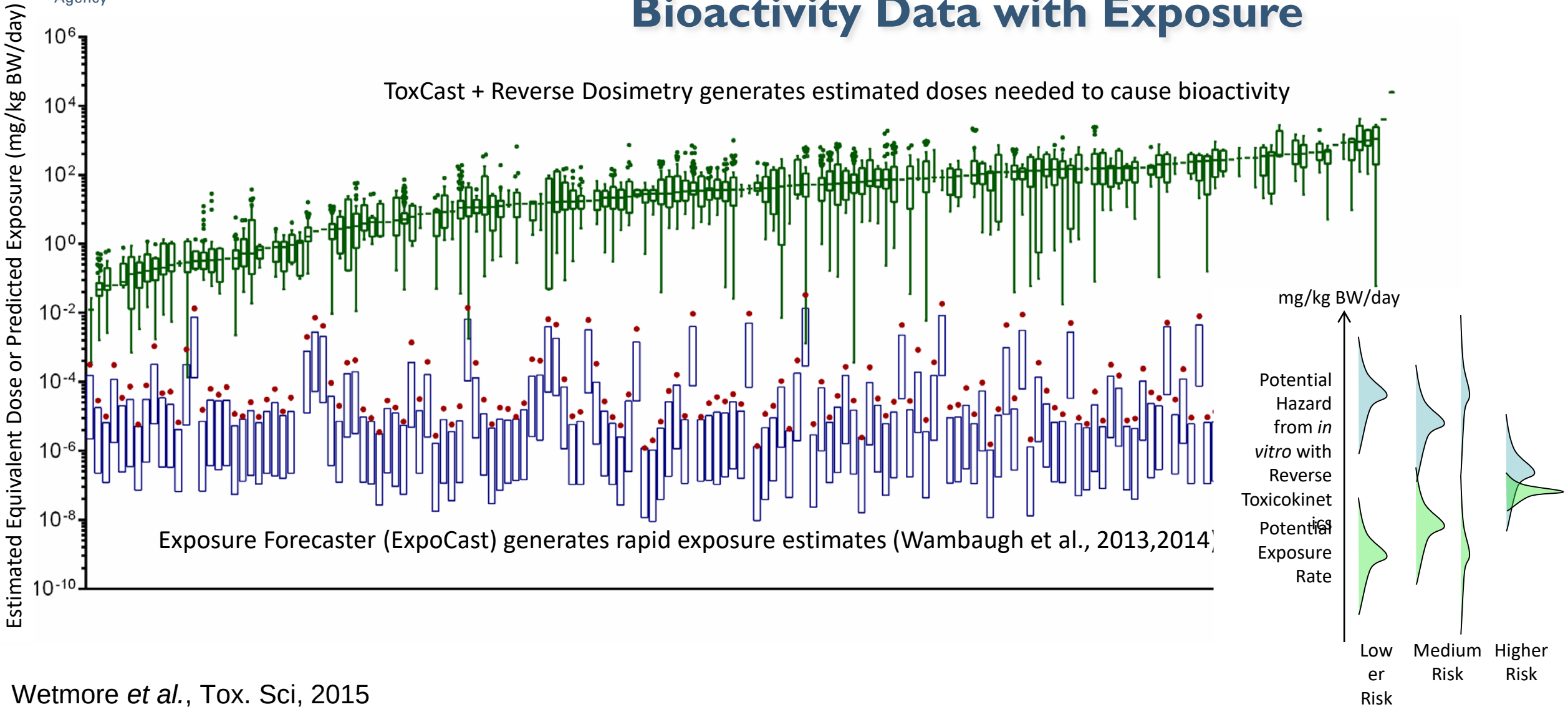
Integrating Human Dosimetry and Exposure with ToxCast *In Vitro* Assays



Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012
Wetmore et al., *Tox Sci,* 2015

Slide from Barbara Wetmore

Incorporating Dosimetry-Adjusted ToxCast Bioactivity Data with Exposure



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

Goals for HTTK

- In order to address greater numbers of chemicals we collect *in vitro*, high throughput toxicokinetic (HTTK) data (Wang, 2010, Rotroff et al., 2010, Wetmore et al., 2012, 2015)
- The **primary goal** of HTTK is to provide a human dose context for bioactive *in vitro* concentrations from HTS (*i.e., in vitro-in vivo* extrapolation, or **IVIVE**)
  - This allows direct comparisons potentially bioactive doses with exposure (Rotroff et al., 2010, Wetmore et al., 2012, 2014, 1015)
- **Secondary goal** is to provide **open source data and models** for evaluation and use by the broader scientific community (Pearce et al, 2017a)
  - This includes population variability Monte Carlo based upon NHANES physiology (Ring et al., 2017)
- An free R statistical package ("httk") allows us to evaluate *in vitro* predictions two ways (Wambaugh et al., 2015):
- We have expanded the tools offered in the "httk" package beyond the "reverse dosimetry" application to parameterize simple PBPK models (Ring et al., 2017, Pearce et al., 2017b)

High Throughput Toxicokinetics (HTTK) for Statistical Analysis

Download R:

https://www.r-project.org/

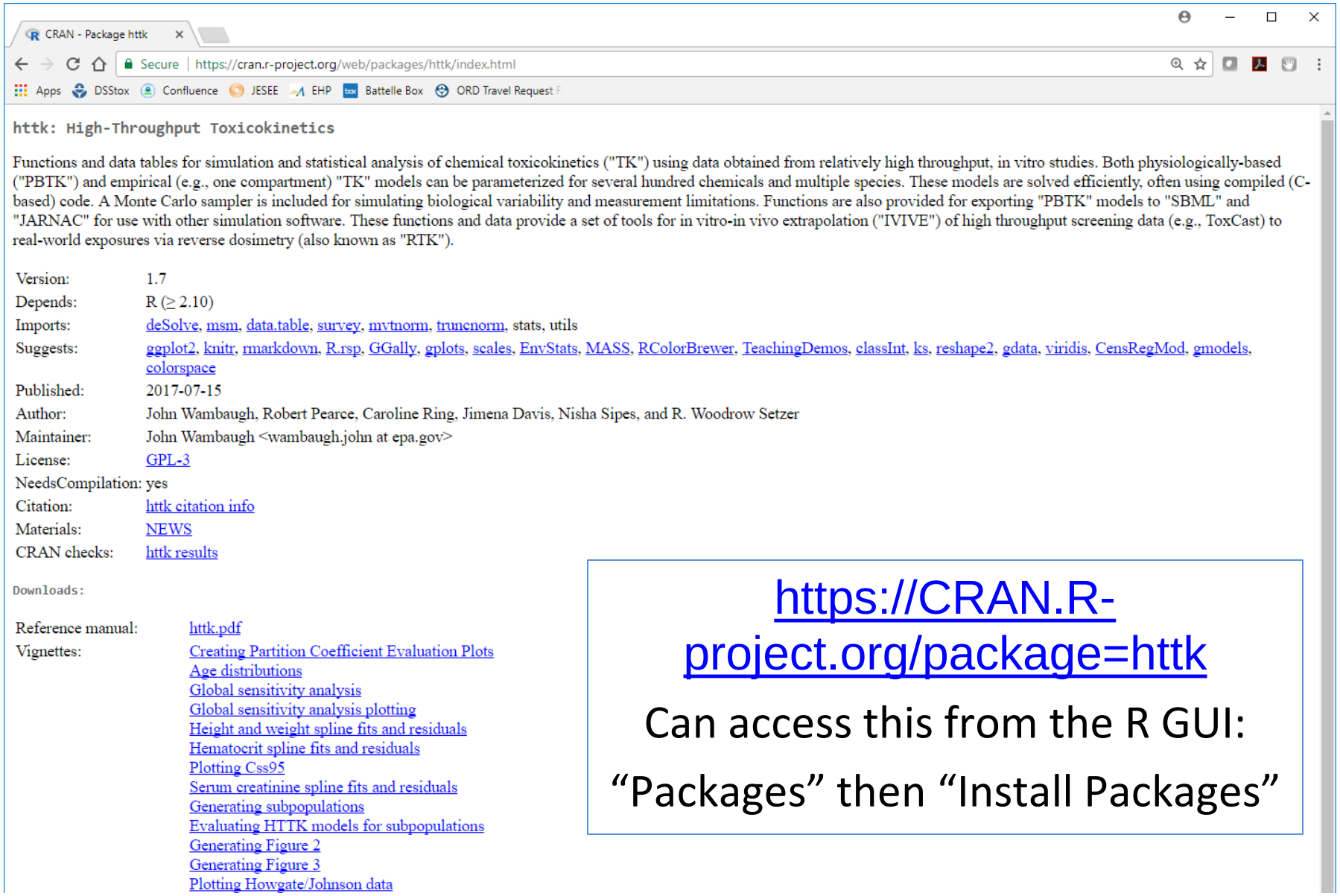
within R, type:

```
install.packages("httk")
```

Then

```
library("httk")
```

- "httk" R Package for IVIVE and **PBTK**
- 553 chemicals to date
- 100's of additional chemicals being studied
- Pearce *et al.* (2017a) provides documentation and examples
- Built-in vignettes provide further examples of how to use many functions



https://CRAN.R-project.org/package=httk

Can access this from the R GUI:

"Packages" then "Install Packages"

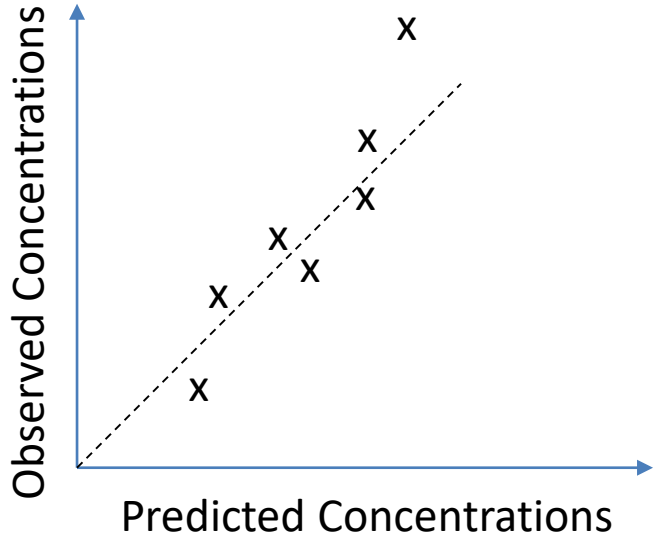
Why Do Statistical Analysis of HTTK?

- If we are to use HTTK, we need confidence in predictive ability
- 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 most compounds in the environment there will be no clinical trials
- Uncertainty must be well characterized
  - We compare to *in vivo* data to get **empirical estimates of HTTK uncertainty**
  - Any approximations, omissions, or mistakes should work to increase the estimated uncertainty when evaluated systematically across chemicals

Model Evaluation

- In order to evaluating a **chemical-specific TK model** for “chemical x” you can compare the predictions to *in vivo* measured data
  - Can estimate bias
  - Can estimate uncertainty
  - Can consider using model to extrapolate to other situations (dose, route, physiology) where you don’t have data

- However, we do not typically have TK data



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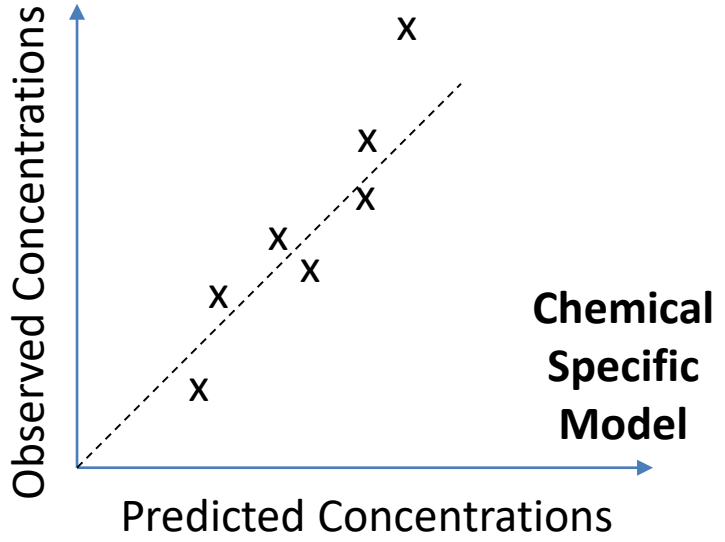
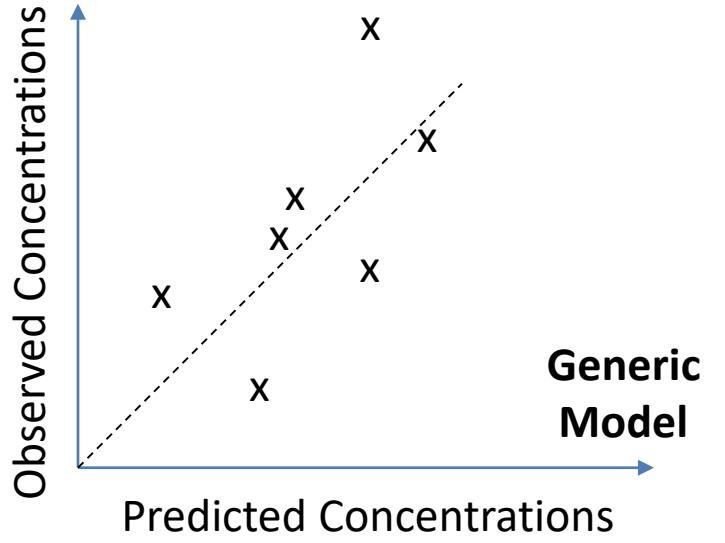
- We can parameterize a **generic TK model**, and evaluate that model for as many chemicals as we do have data
  - We do expect larger uncertainty, but also greater confidence in model implementation
  - Estimate bias and uncertainty, and try to correlate with chemical-specific properties
  - Can again consider using model to extrapolate to other situations (chemicals without *in vivo* data)





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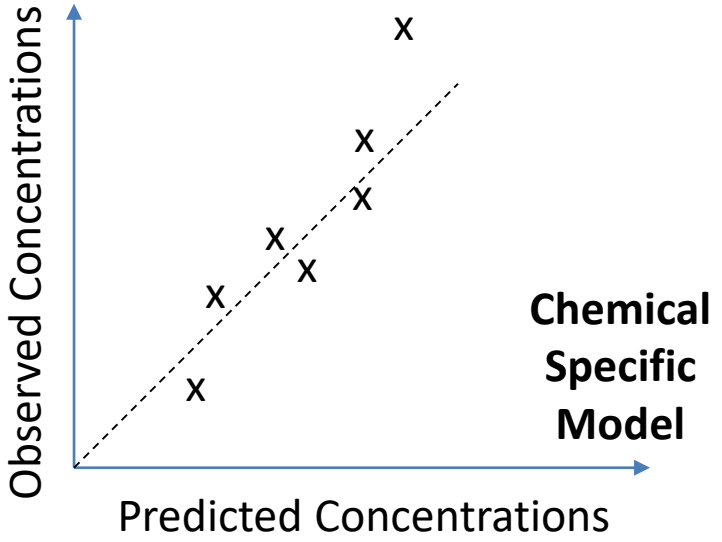

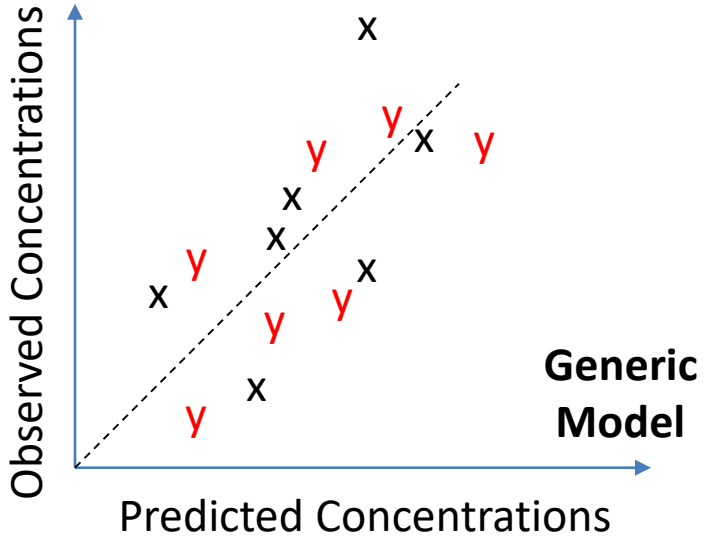

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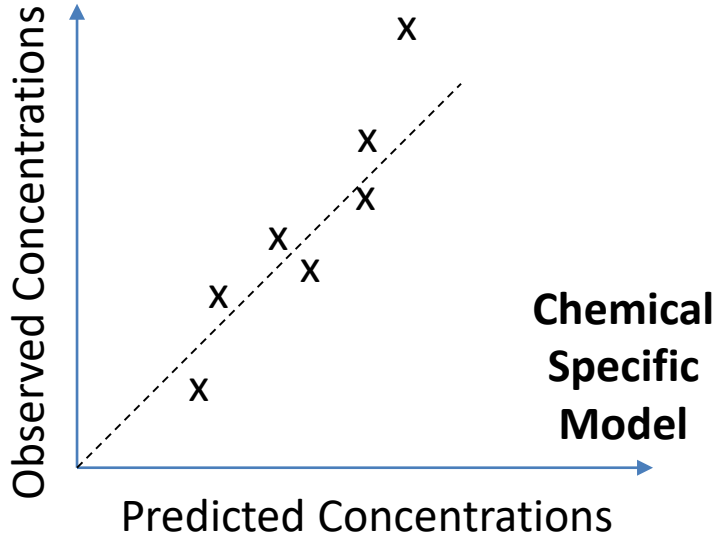
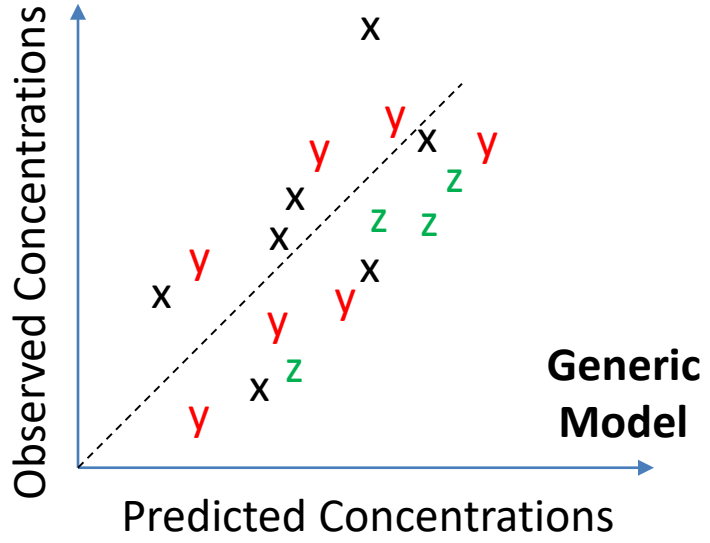




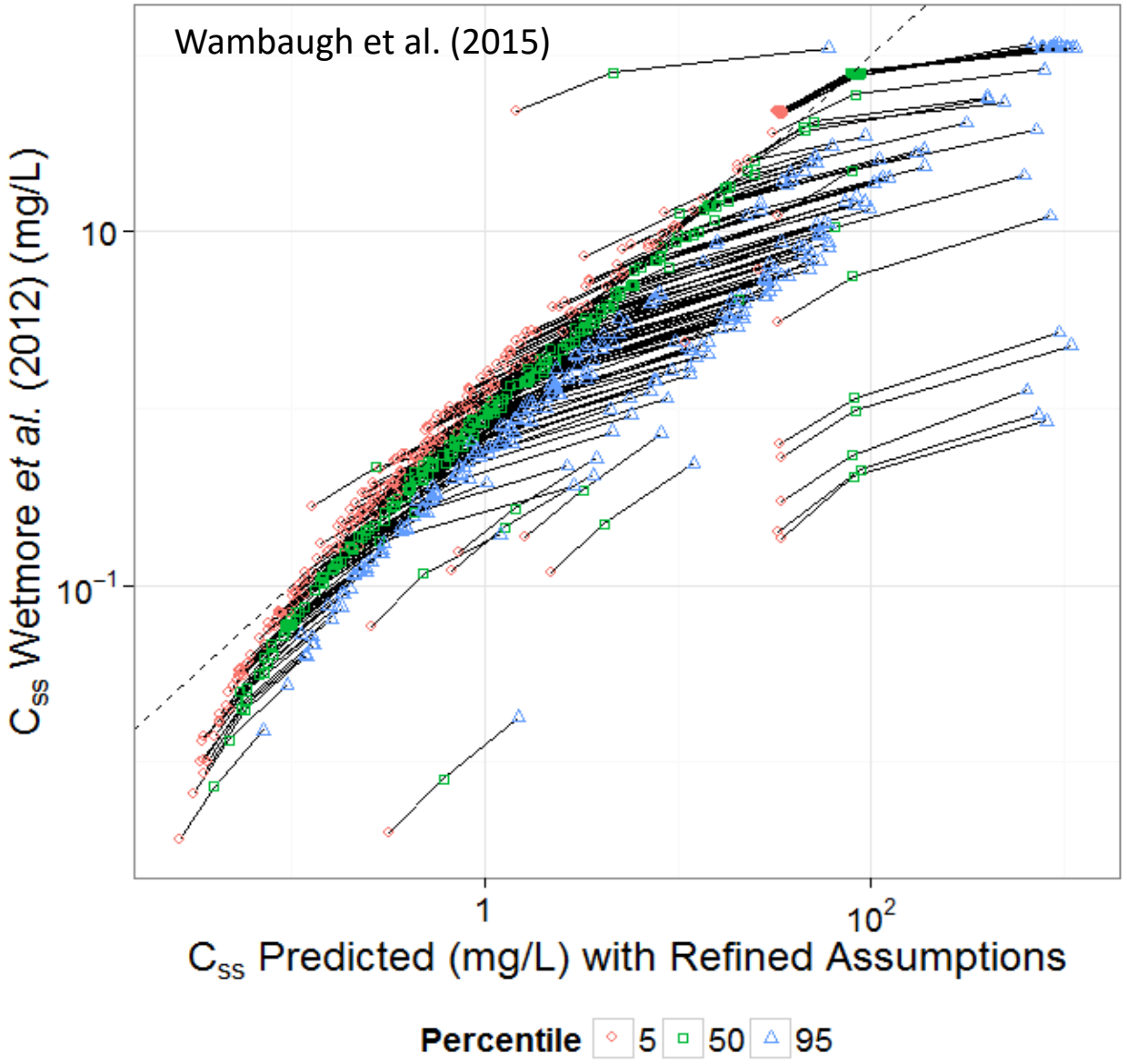
Why Build Another Generic PBTK Tool?

	SimCYP	ADMET Predictor / GastroPlus	MEGen	IndusChemFate	httk
Maker	SimCYP Consortium / Certara	Simulations Plus	UK Health and Safety Laboratory	Cefic LRI	US EPA
Availability	License, but inexpensive for research	License, but inexpensive for research	Free: http://xnet.hsl.gov.uk/megen	Free: http://cefic-lri.org/lri_toolbox/induschemfate/	Free: https://CRAN.R-project.org/package=httk
Open Source	No	No	**Yes**	No	**Yes**
Default PBPK Structure	**Yes**	**Yes**	No	**Yes**	**Yes**
Expandable PBPK Structure	No	No	**Yes**	No	No
Population Variability	**Yes**	No	No	No	**Yes**
Batch Mode	**Yes**	**Yes**	No	No	**Yes**
Graphical User Interface	**Yes**	**Yes**	**Yes**	Excel	No
Physiological Data	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Chemical-Specific Data Library	Many Clinical Drugs	No	No	15 Environmental Compounds	543 Pharmaceutical and ToxCast Compounds
Ionizable Compounds	**Yes**	**Yes**	Potentially	No	**Yes**
Export Function	No	No	Matlab and AcslX	No	SBML and Jarnac
R Integration	No	No	No	No	**Yes**
Easy Reverse Dosimetry	**Yes**	**Yes**	No	No	**Yes**
Future Proof XML	No	No	**Yes**	No	No

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

Comparison Between httk and SimCYP

- In the Rotroff *et al*. (2010) and Wetmore et al. (2012,2013,2014,2015) papers SimCYP was used to predict distributions of C_{ss} from *in vitro* data
    - We show that "httk" 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.
- The RED assay for measuring protein binding fails in some cases because the amount of free chemical is below the limit of detection
    - A default value of 0.5% free was used
    - Now we use random draws from a uniform distribution from 0 to 1%.



Installing "httk"

```
install.packages("httk")

library(httk)

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for human for Acetochlor (published value):
calc_mc_css(chem.cas="34256-82-1")

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.95 quantile, for Acetochlor (calculated value):
calc_mc_oral_equiv(0.1,chem.cas="34256-82-1")

# Should produce error:
calc_mc_css(chem.name="34256-82-1")

#Capitalization shouldn't matter:
calc_mc_css(chem.name="acetochlor")
calc_mc_css(chem.name="Acetochlor")

# What's going on?
help(calc_mc_css)
```

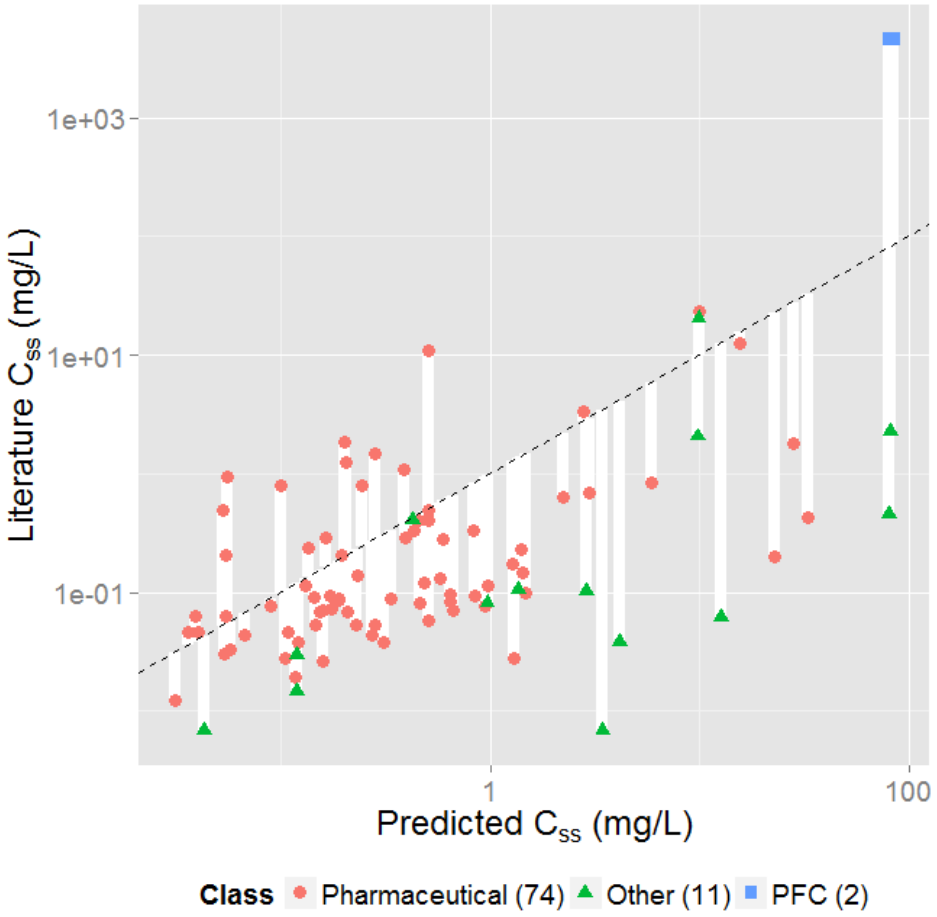
Help Files

Every function has a help file

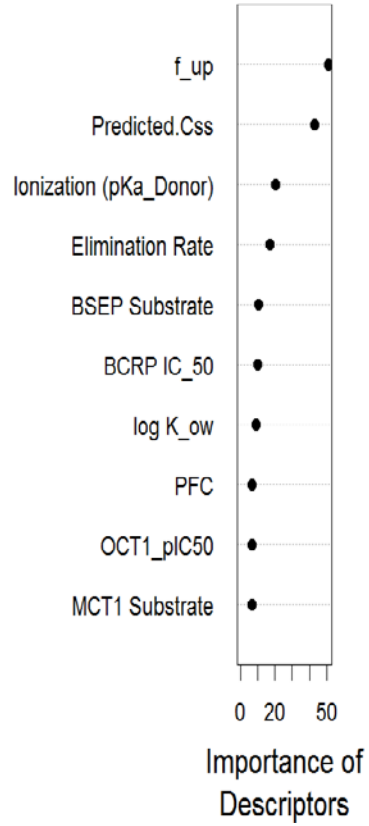
```
help(add_chemtable)

Add a table of chemical information for use in making httk predictions.

Description
This function adds chemical-specific information to the table
chem.physical_and_invitro.data. This table is queried by the model parameterization
functions when attempting to parameterize a model, so adding sufficient data to this table
allows additional chemicals to be modeled.
Usage
add_chemtable(new.table, data.list, current.table=NULL, reference=NULL,species=NULL,
overwrite=F)
Arguments

  new.table          Object of class data.frame containing one row per chemical, with each chemical minimally by
                     described by a CAS number.

  data.list          This list identifies which properties are to be read from the table. Each item in the list should
                     point to a column in the table new.table. Valid names in the list are: 'Compound', 'CAS',
                     'DSSTox.GSID' 'SMILES.desalt', 'Reference', 'Species', 'MW', 'logP', 'pKa_Donor', 'pKa_Accept',
                     'logMA', 'Clint', 'Clint.pValue', 'Funbound.plasma', 'Fgutabs', 'Rblood2plasma'. Note that
                     Rblood2plasma (Ratio blood to plasma) is currently not used.
```


Pearce et al. (2017a)

Using *in vivo* Data to Evaluate RTK



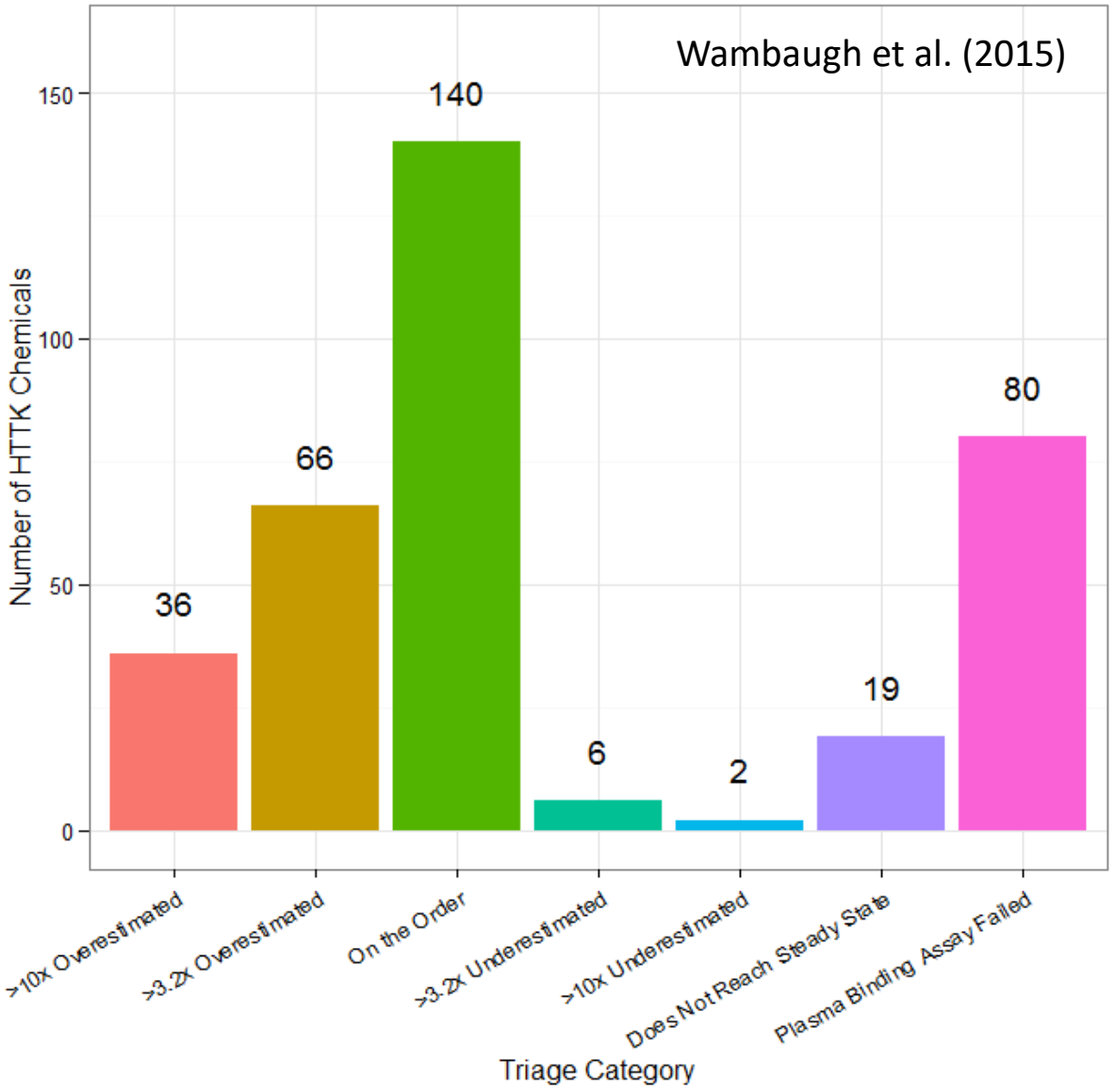
- When we compare the C_{ss} predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate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



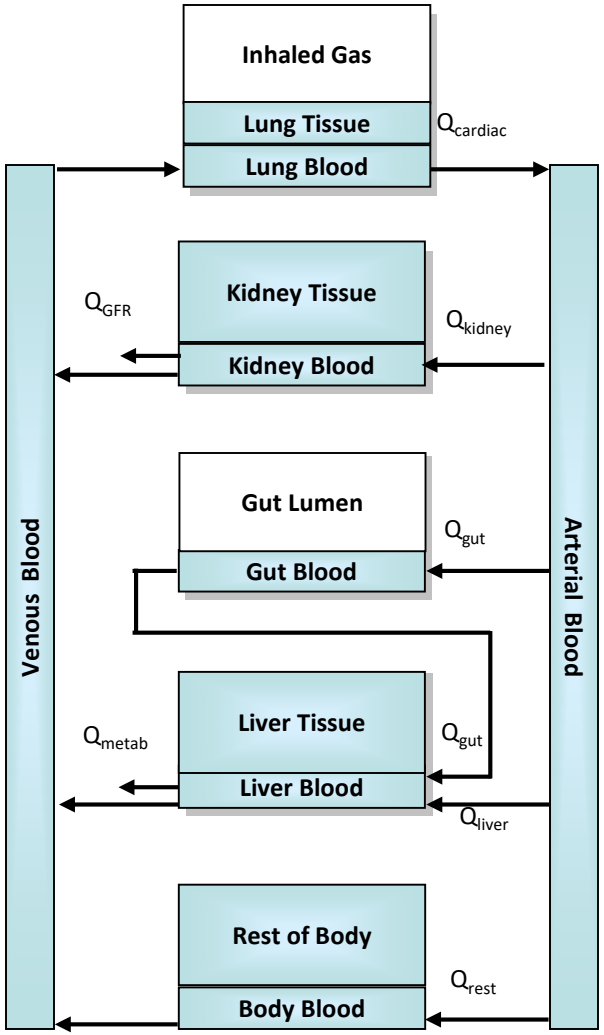
Wambaugh et al. (2015)

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



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).

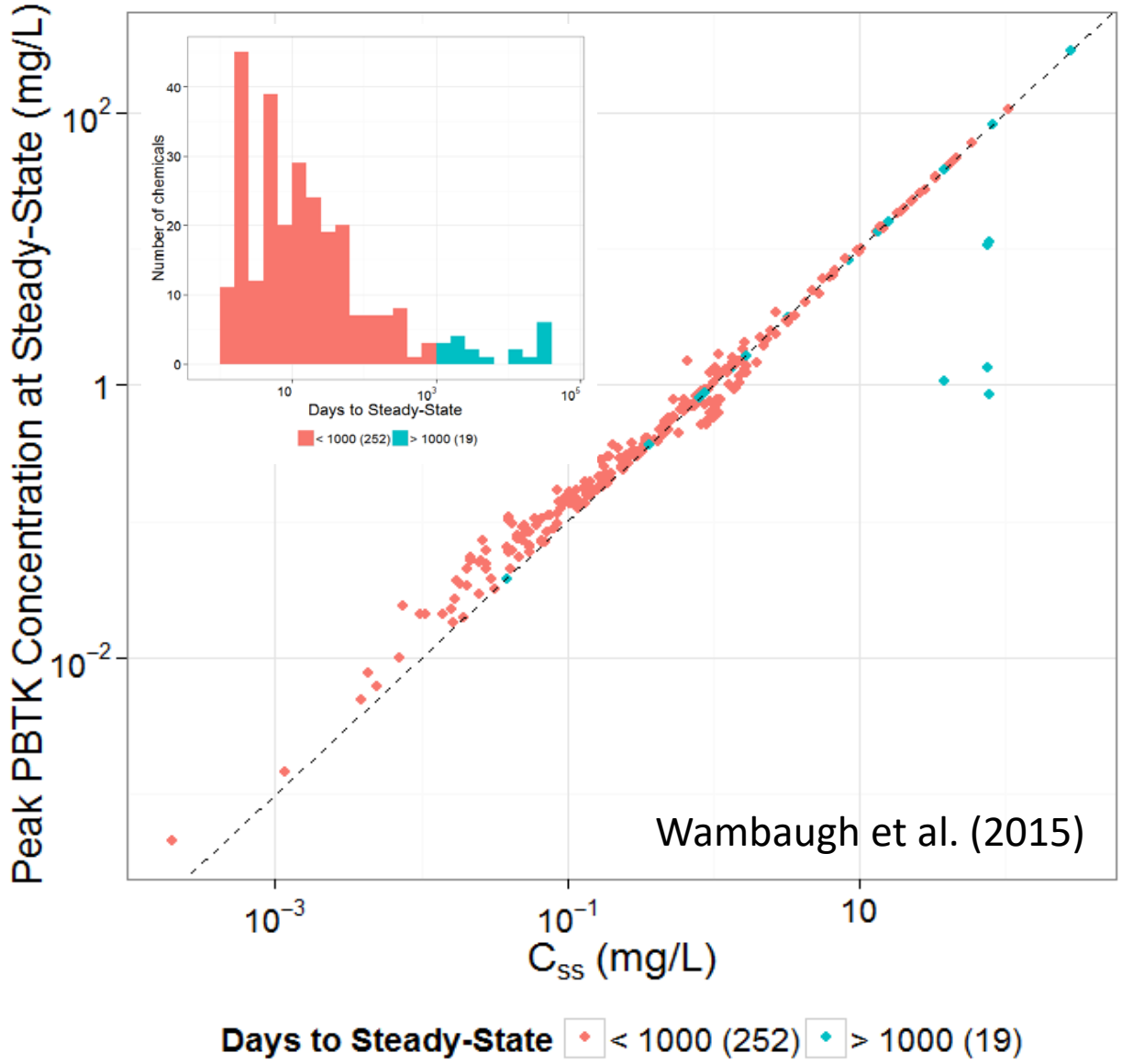
Basic PK Statistics Examples

```
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)
    Human plasma concentrations returned in uM units.
    AUC is area under plasma concentration curve in uM * days units with Rblood2plasma =
    0.79 .
    $AUC
    [1] 44.82138
    $peak
    [1] 23.16455
    $mean
    [1] 1.600764
# 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",default.to.human=T)
```

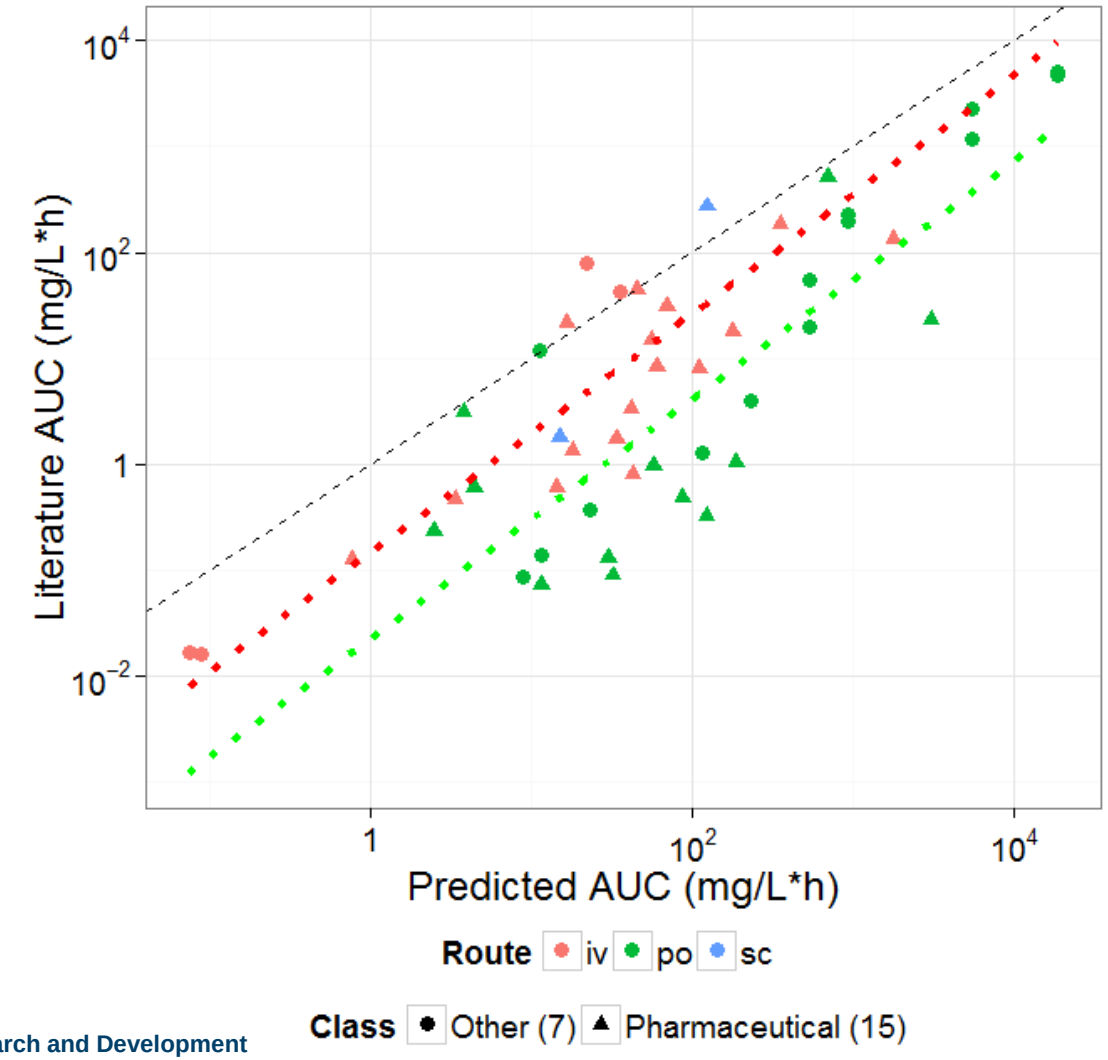
Peak Concentration vs. C_{ss}

- Peak serum concentrations from the HT-PBTK 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}



Evaluating *In Vitro* 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)

Using the PBPK Solver Directly

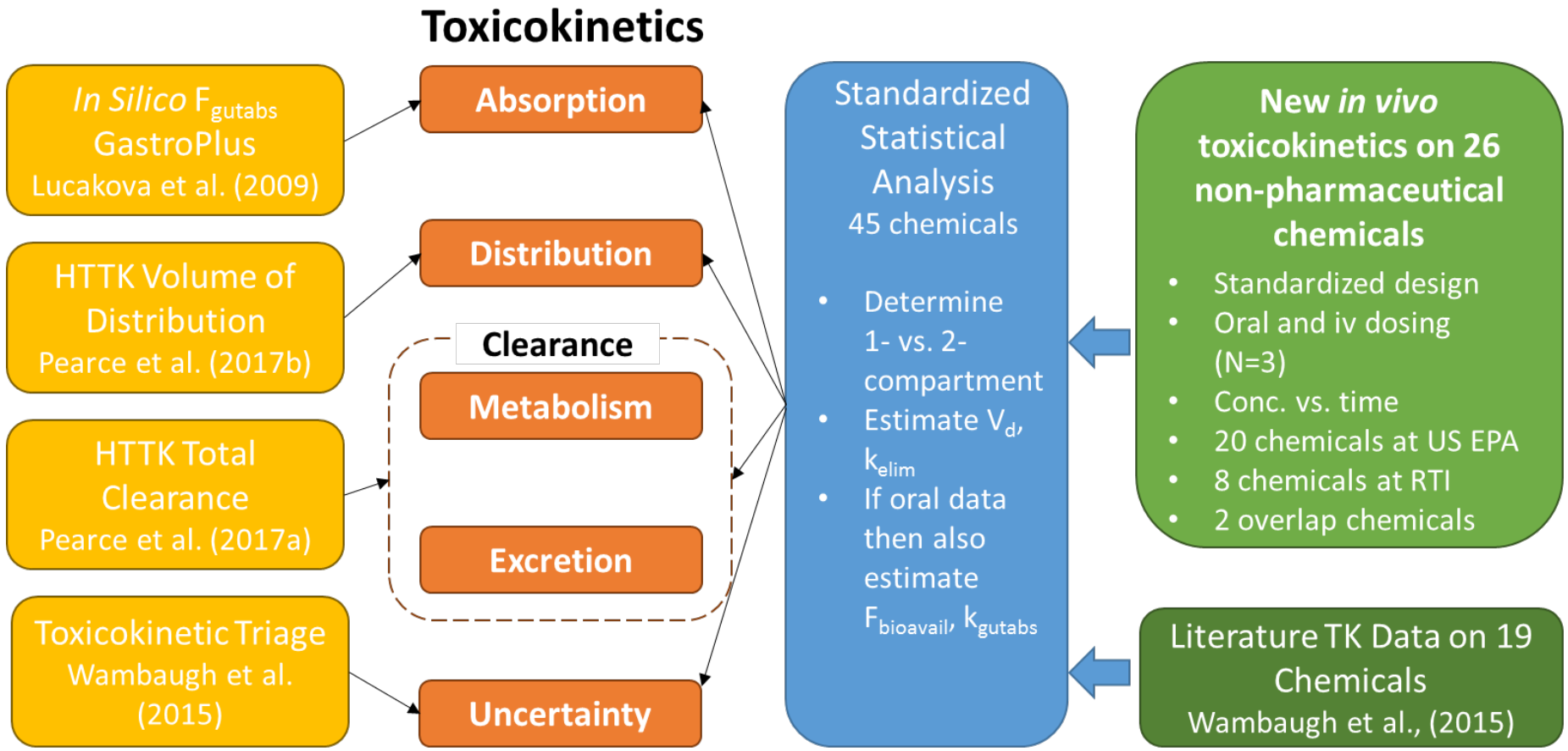
```
library(httk)
solve_pbtk(chem.name="bisphenol a")
Human values returned in uM units.
AUC is area under plasma concentration curve in uM * days units with Rblood2plasma = 13.829 .
```

	time	Agutlumen	Cart	Cven	Clung	Cgut	Cliver	Ckidney	Crest	Ametabolized	Atubules	Cplasma	AUC
[1,]	0.00000000	3.066275e+02	0.0000000	0.0000000	0.0000000	0.0000000	0.000000	0.000000	0.0000000	0.0000000	0.000000000	0.00000000	0.0000000000
[2,]	0.01041667	2.388017e+02	0.5991529	0.6287457	1.3199744	21.5143390	16.400297	3.233837	0.1914032	0.6152291	0.001766711	0.04546572	0.0002027523
[3,]	0.02083333	1.859790e+02	1.0004073	1.0083651	2.1406984	21.2910531	23.929492	5.969930	0.8381364	2.3122408	0.009141183	0.07291668	0.0008494912
[4,]	0.03125000	1.448406e+02	1.0588194	1.0574935	2.2507541	18.6383943	23.805194	6.461686	1.6078696	4.2587907	0.018747032	0.07646924	0.0016399193
[5,]	0.04166667	1.128020e+02	0.9900774	0.9858431	2.1000346	15.6437008	21.093573	6.086786	2.3205218	6.0701074	0.028321968	0.07128808	0.0024132951
[6,]	0.05208333	8.785027e+01	0.8881710	0.8835210	1.8825725	12.9223287	17.876882	5.473438	2.9227548	7.6352470	0.037100155	0.06388898	0.0031178197
[7,]	0.06250000	6.841785e+01	0.7883695	0.7841762	1.6709261	10.6387106	14.905516	4.859989	3.4111465	8.9492093	0.044931086	0.05670518	0.0037452376
[8,]	0.07291667	5.328387e+01	0.7019889	0.6984803	1.4881848	8.7907797	12.394544	4.324754	3.7991362	10.0424589	0.051886143	0.05050836	0.0043026722
[9,]	0.08333333	4.149753e+01	0.6310281	0.6281916	1.3382326	7.3221169	10.355693	3.883444	4.1039821	10.9532118	0.058100464	0.04542565	0.0048013867
[10,]	0.09375000	3.231830e+01	0.5741708	0.5719161	1.2181499	6.1656716	8.732407	3.529201	4.3419895	11.7173422	0.063712849	0.04135627	0.0052525642
[11,]	0.10416667	2.516952e+01	0.5291804	0.5274035	1.1231570	5.2594857	7.452953	3.248636	4.5270631	12.3653625	0.068845520	0.03813749	0.0056659289
[12,]	0.11458333	1.960204e+01	0.4938045	0.4924101	1.0484744	4.5511975	6.449790	3.027926	4.6705414	12.9221223	0.073599630	0.03560705	0.0060494826
[13,]	0.12500000	1.526609e+01	0.4660733	0.4649812	0.9899344	3.9982940	5.665391	2.854874	4.7814699	13.4074338	0.078056481	0.03362362	0.0064096387
[14,]	0.13541667	1.188924e+01	0.4443620	0.4435072	0.9441034	3.5669375	5.052878	2.719379	4.8669831	13.8369184	0.082280440	0.03207080	0.0067514674
[15,]	0.14583333	9.259350e+00	0.4273671	0.4266978	0.9082280	3.2304670	4.574870	2.613319	4.9326758	14.2228237	0.086322084	0.03085528	0.0070789492
[16,]	0.15625000	7.211189e+00	0.4140571	0.4135327	0.8801305	2.9679880	4.201883	2.530261	4.9829214	14.5747234	0.090221004	0.02990328	0.0073951988
[17,]	0.16666667	5.616079e+00	0.4036218	0.4032104	0.8581008	2.7631742	3.910801	2.465151	5.0211325	14.9000872	0.094008099	0.02915686	0.0077026468
[18,]	0.17708333	4.373808e+00	0.3954277	0.3951043	0.8408012	2.6032874	3.683555	2.414033	5.0499698	15.2047384	0.097707470	0.02857070	0.0080031886
[19,]	0.18750000	3.406325e+00	0.3889798	0.3887250	0.8271873	2.4783968	3.506044	2.373818	5.0715067	15.4932160	0.101337911	0.02810940	0.0082983022
[20,]	0.19791667	2.652848e+00	0.3838923	0.3836909	0.8164447	2.3807648	3.367276	2.342097	5.0873584	15.7690549	0.104914056	0.02774538	0.0085891383
[21,]	0.20833333	2.066041e+00	0.3798646	0.3797048	0.8079387	2.3043633	3.258686	2.316992	5.0987829	16.0350089	0.108447302	0.02745713	0.0088765933
[22,]	0.21875000	1.609034e+00	0.3766622	0.3765349	0.8011748	2.2444970	3.173600	2.297042	5.1067604	16.2932235	0.111946540	0.02722791	0.0091613663
[23,]	0.22916667	1.253117e+00	0.3741028	0.3740007	0.7957679	2.1975087	3.106820	2.281105	5.1120540	16.5453689	0.115418684	0.02704466	0.0094440009

Evaluating HTTK Predictions

We collected new *in vivo* data for 26 chemicals more commonly associated with non-therapeutic and/or unintentional exposure

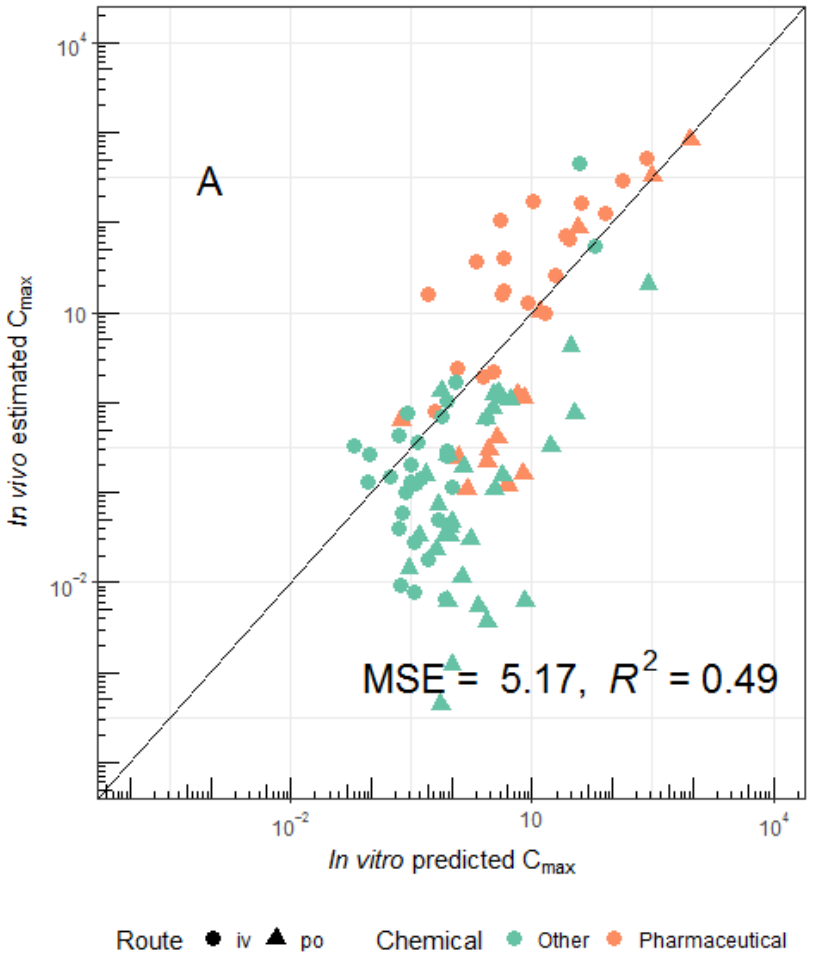
Minimal design – six animals per study (3 dosed per oral / 3 iv)



In Vivo Work led by Mike Hughes (EPA/NHEERL) and Tim Fennell (RTI)

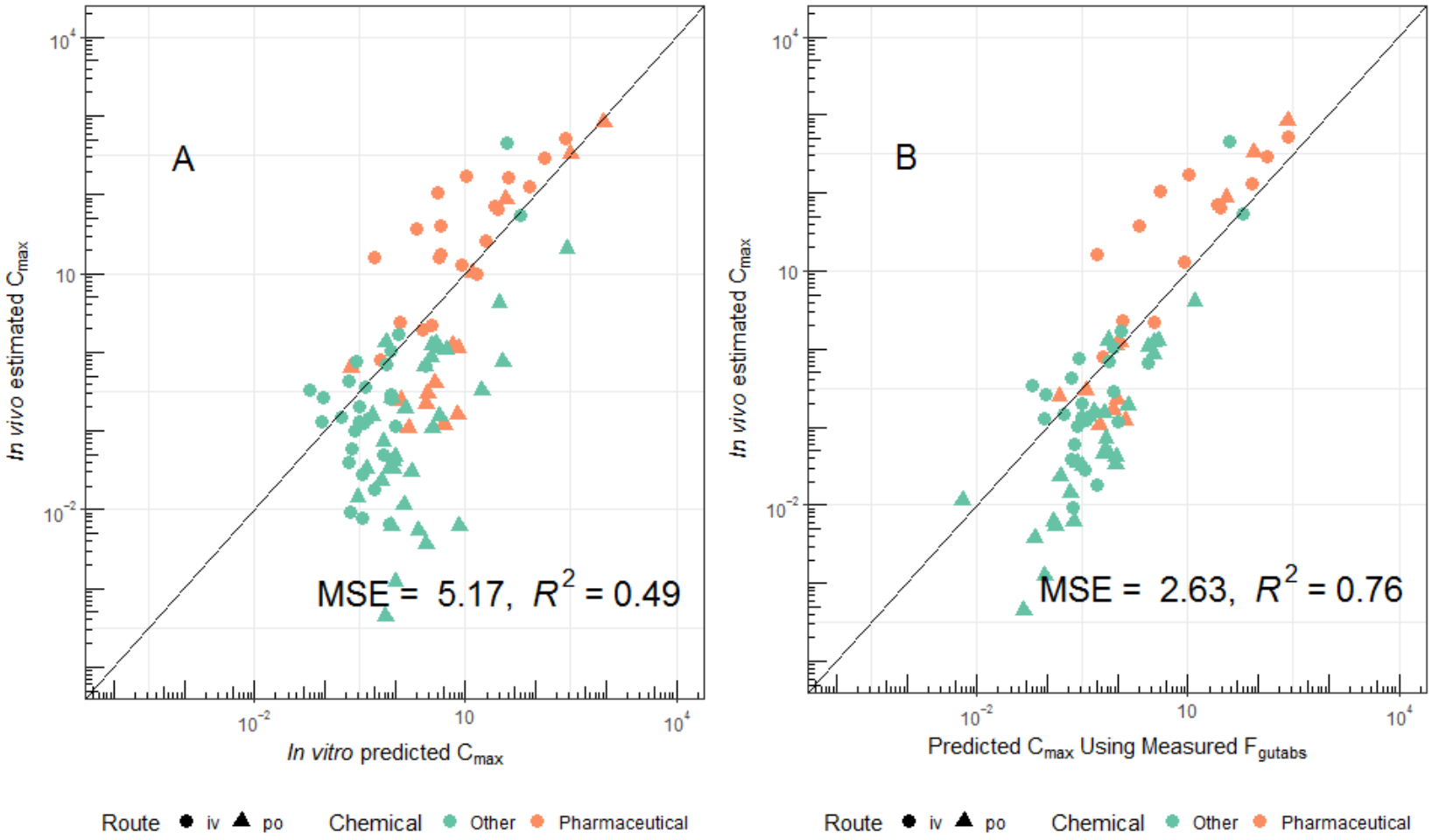
Evaluating *In Vitro* PBTK Predictions with *In Vivo* Data

- PBTK predictions can be made for maximum plasma concentration (C_{max}) and 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

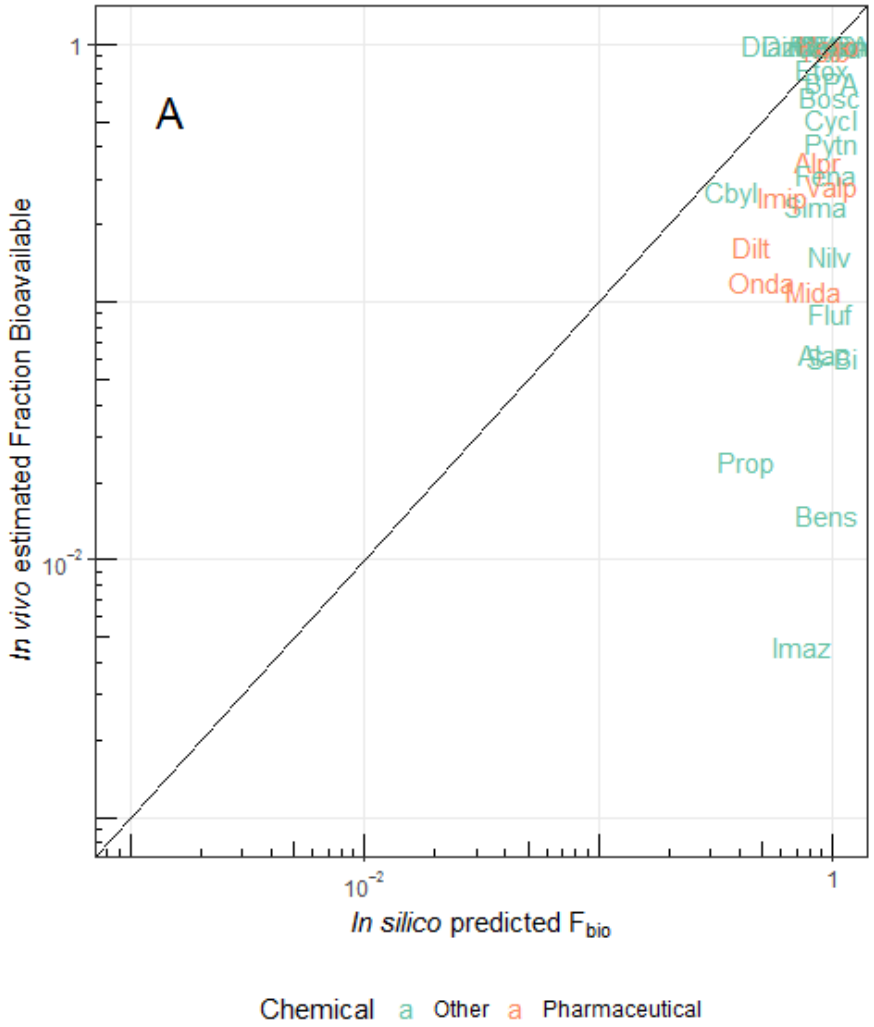


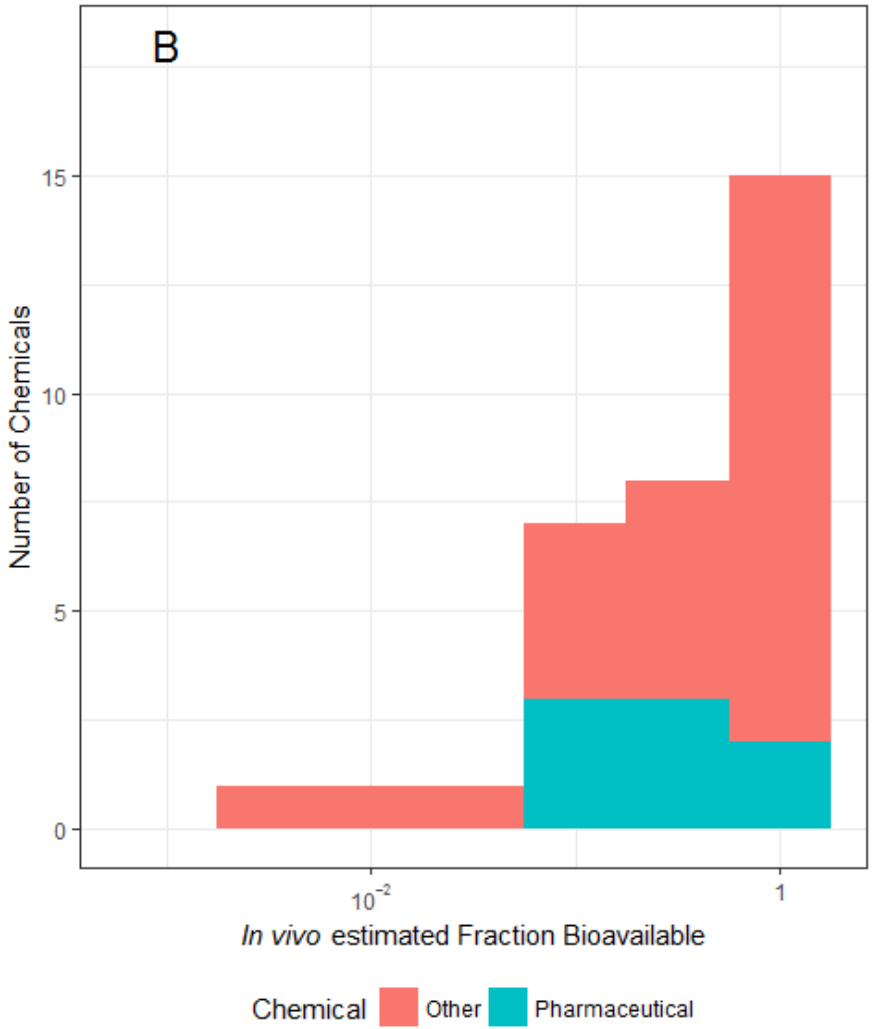
Evaluating *In Vitro* PBTK Predictions with *In Vivo* Data

- PBTK predictions can be made for maximum plasma concentration (C_{max}) and 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
- Inclusion of oral bioavailability data improves predictions ("httk" assumes default of 100%)



Evaluating *In Silico* Oral Bioavailability Predictions with *In Vivo* Data





- *In silico* methods developed for pharmaceuticals do not seem to do a good job of predicting oral bioavailability for environmental chemicals
- Predictions were made without the benefit of in vitro assays that can inform absorption (i.e., CACO-2 membrane permeability)
- CACO-2 permeability is now being measured for HTTK chemicals (Derek Angus, Cyprotex)

Bioavailability predictions from GastroPlus (Nisha Sipes)

Population simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters

- Body weight
- Tissue masses
- Tissue blood flows
- GFR (kidney)
- Hepatocellularity

Source of data: CDC NHANES

Large, ongoing CDC survey of US population: demographic, body measures, medical exam, biomonitoring (health and exposure), …

Designed to be representative of US population according to census data

Data sets publicly available (http://www.cdc.gov/nchs/nhanes.htm)

Ring *et al*. (2017)

Population simulator for HTTK

Sample
NHANES quantities

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

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)

Generating demographic subgroups

User can specify….	Default if not specified
Age limits	0-79 years
Sex (# males, # females)	NHANES proportions
Race/ethnicity (5 NHANES categories)	NHANES proportions
BMI/weight categories	NHANES proportions

- NHANES quantities sampled from appropriate *conditional* distribution (given specifications)
  - Physiological parameters predicted accordingly

Ring *et al*. (2017)

NHANES Demographic Examples

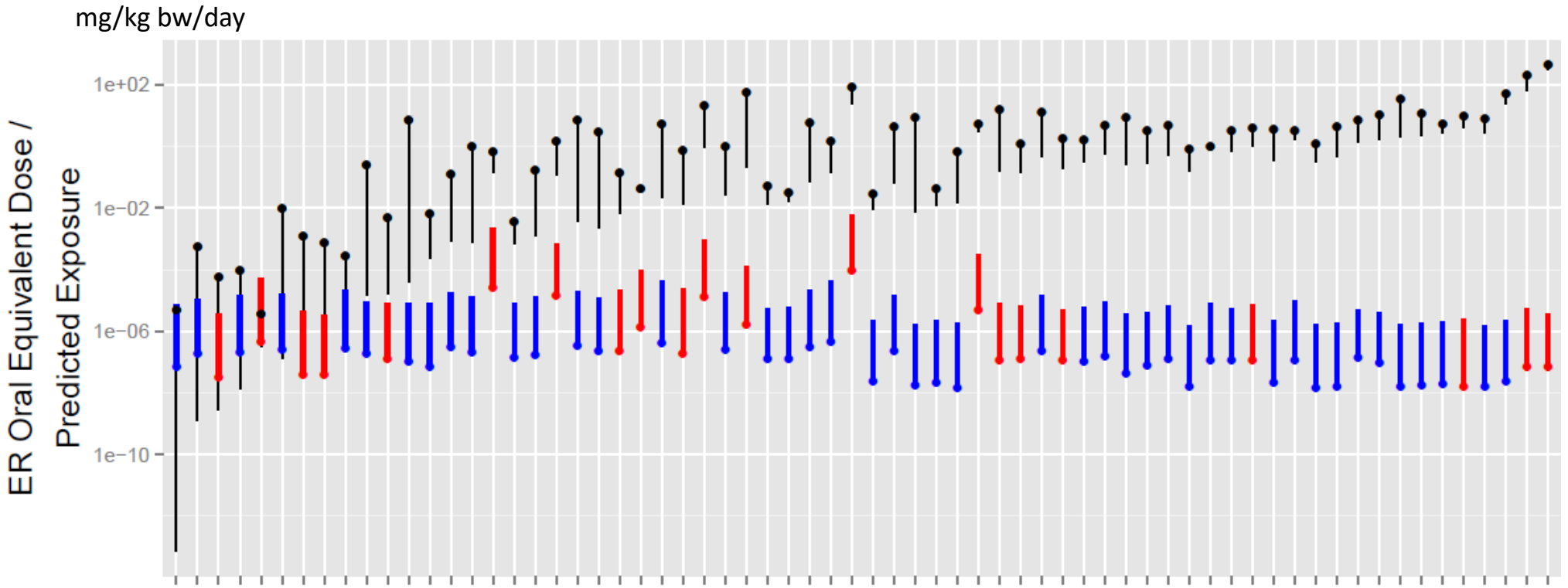
```
library(httk)

# Oral equivalent (mg/kg/day) for in vitro activity of 1 µM for Acetochlor
calc_mc_oral_equiv(1,chem.cas="34256-82-1")

# Oral equivalent (mg/kg/day) for NHANES “Mexican American” Population
calc_mc_oral_equiv(1,chem.cas="34256-82-1", reths = "Mexican American")

# Oral equivalent (mg/kg/day) for NHANES “Mexican American” Population aged 18-25 years
calc_mc_oral_equiv(1,chem.cas="34256-82-1",agelim_years=c(18,25),reths = "Mexican
American")

# Probably too few individuals in NHANES for direct resampling (“dr”) so use virtual
individuals (“vi”) resampling method:
calc_mc_oral_equiv(1,chem.cas="34256-82-1",method="vi",agelim_years=c(18,25),reths =
"Mexican American")
```

Can also specify gender, weight categories, and kidney function

Ring *et al*. (2017)

High Throughput Risk Prioritization for the Total Population

High throughput toxicokinetics bridges high throughput screening and exposure estimates



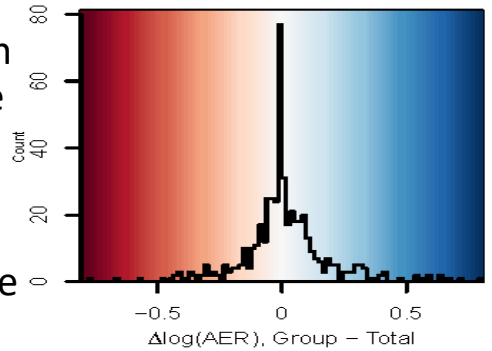
ToxCast Chemicals

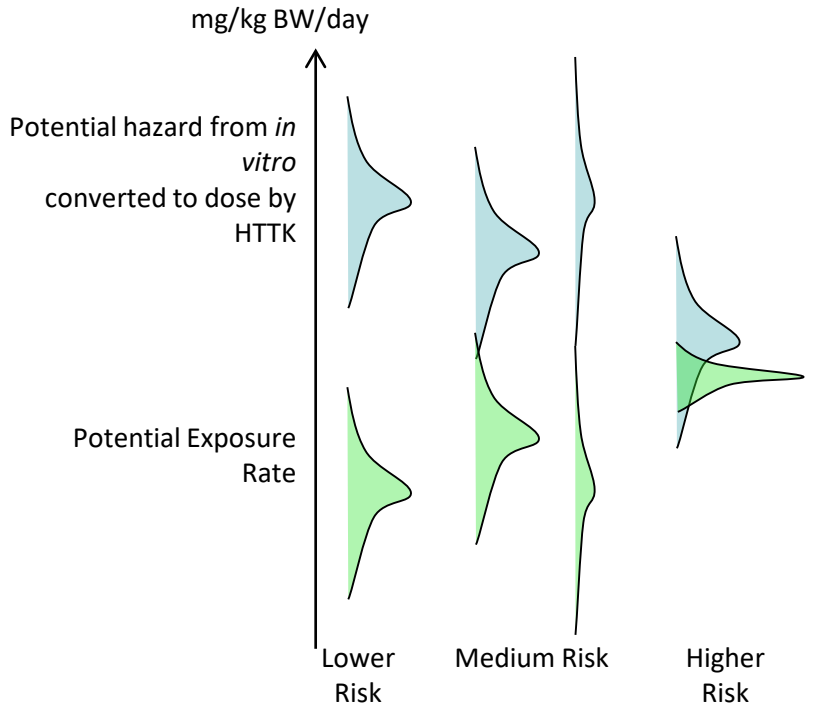
December, 2014 Panel:
"Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening"

Life-stage and Demographic Specific Predictions

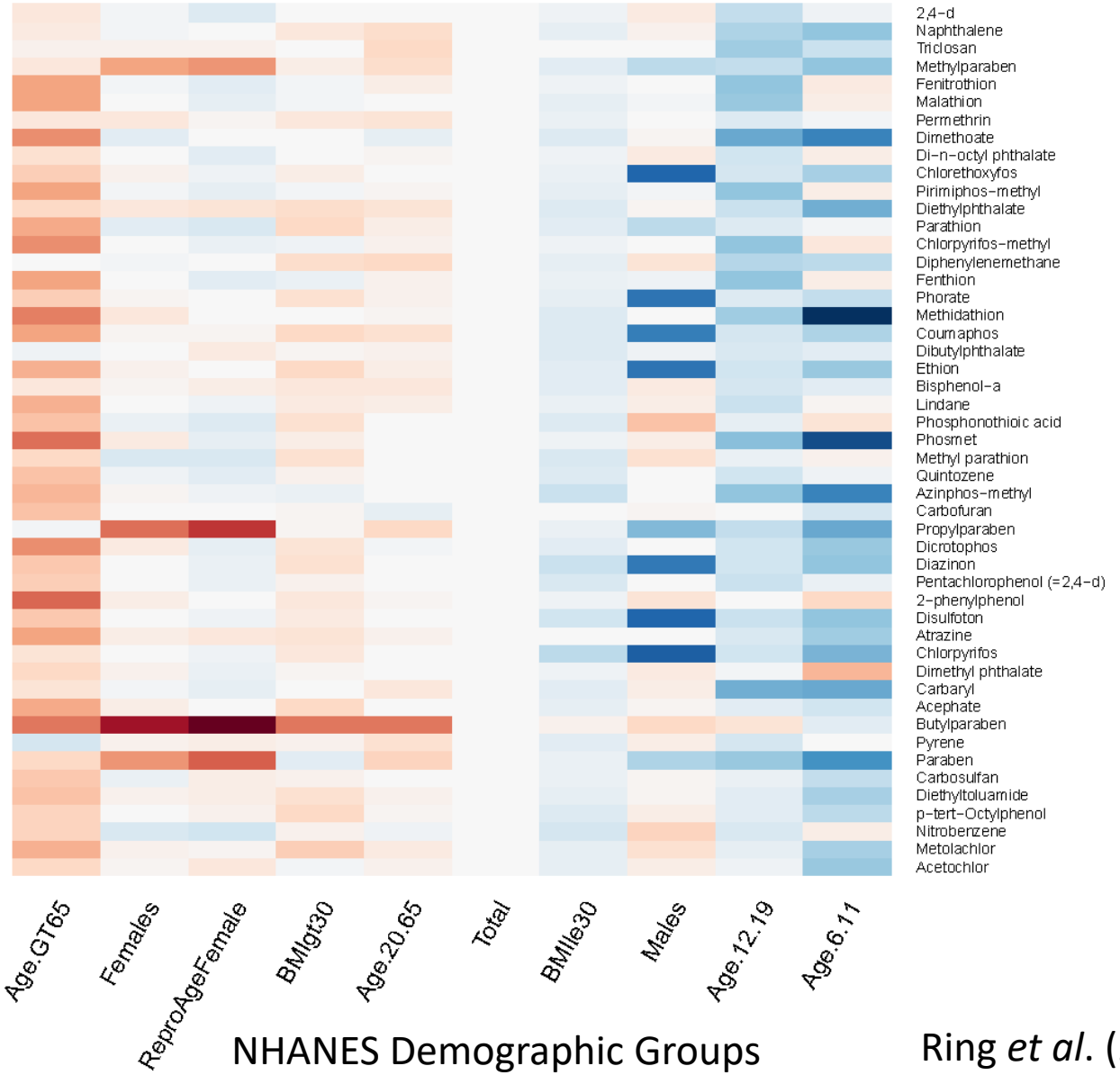
- We use HTTK to calculate margin between bioactivity and exposure for specific populations

- Most NHANES chemicals do not have traditional PK models (Strope et al., 2017)





Change in Activity : Exposure Ratio



NHANES Chemicals

NHANES Demographic Groups

Ring *et al.* (2017)

Version history for "httk"

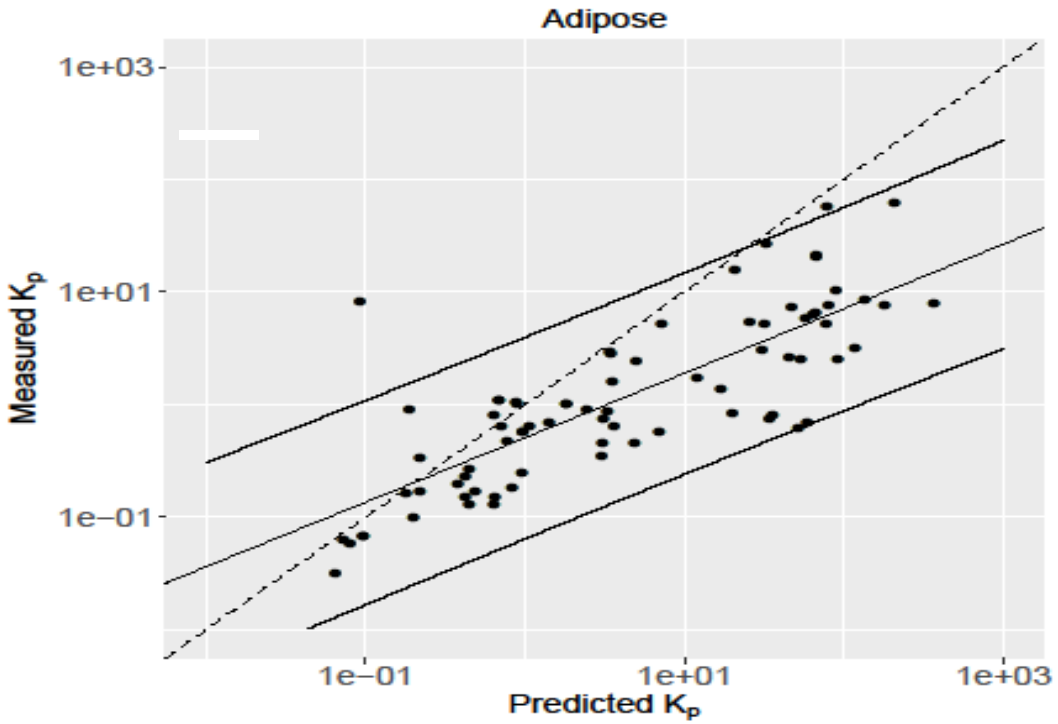
The publicly available R package contains code and data that has been part of peer-reviewed publications (Old versions are archived)

- Version 1.1 accompanied "Toxicokinetic Triage for Environmental Chemicals" Wambaugh et al. (2015) Tox. Sci.
- Version 1.2 accompanied submission of "httk: R Package for High-Throughput Toxicokinetics" Pearce et al., Journal of Statistical Software (2017a)
- Version 1.3 accompanied "Incorporating High-Throughput Exposure Predictions with Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing" Wetmore et al., Toxicological Sciences (2015).
- Version 1.4 addressed comments for revision of Pearce et al., Journal of Statistical Software (2017)
- Version 1.5 accompanied "Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability," Ring et al. Environment International (2017)
- Version 1.6 accompanied "Evaluation and Calibration of High-Throughput Predictions of Chemical Distribution to Tissues," Pearce et al. (2017) submission to Journal of Pharmacokinetics and Pharmacodynamics
- Version 1.7 accompanied publication of Pearce et al., Journal of Statistical Software (2017)

- Subsequent version numbers will be assigned as papers are accepted on:
  - New in vivo data (Wambaugh)
  - In silico HTTK parameter predictions (Sipes)
  - Gestational model (Kapraun)
  - Inhalation exposure (Evans and Pearce)
  - New human data from Cyprotex (Wambaugh and Wetmore)
  - New rat data and revised IVIVE model (Honda)
  - More flexible PBPK model (Pearce)

Lead programmer Robert Pearce

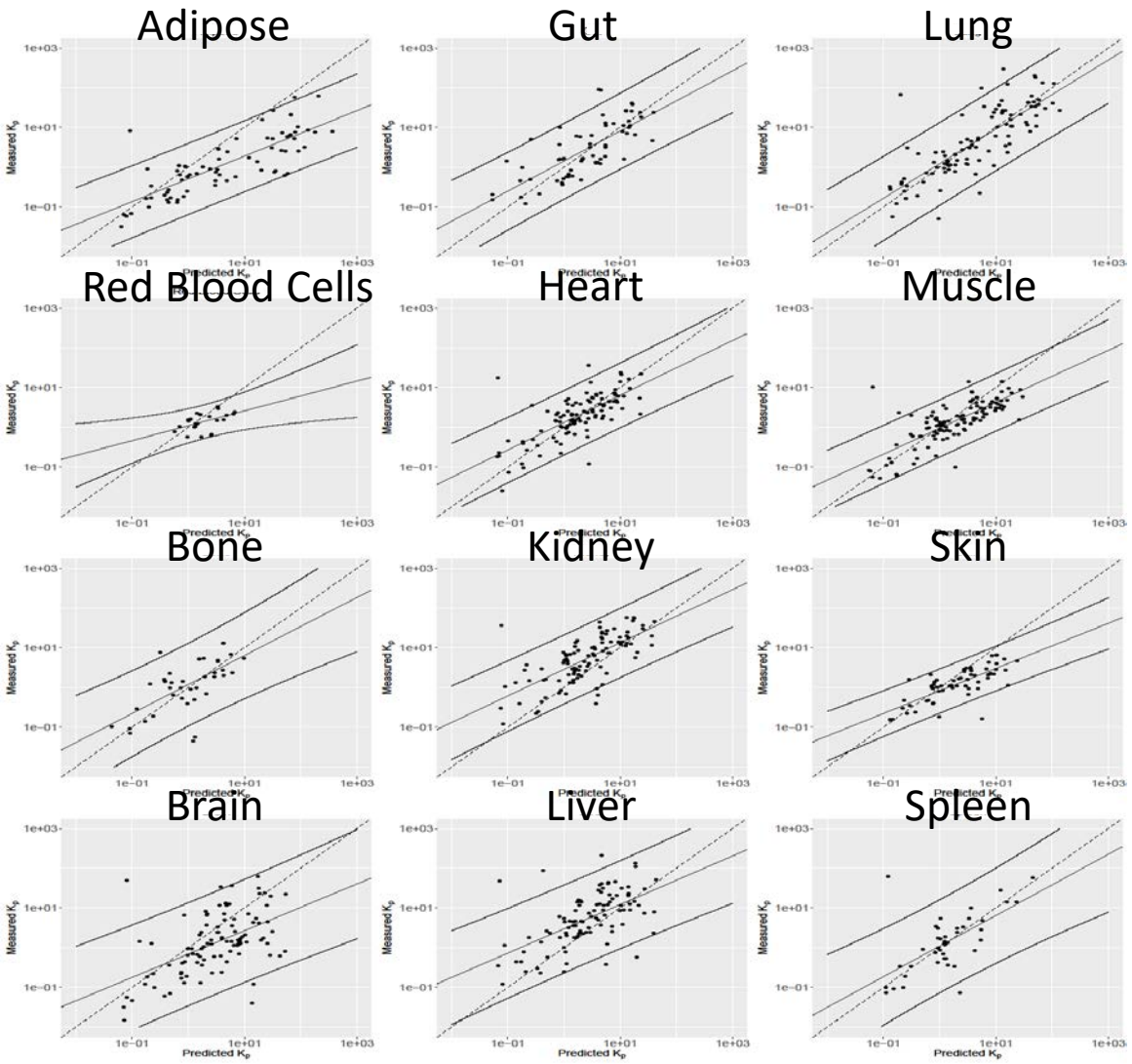
Pearce et al. (2017) Available On-line



- Analyzed literature measurements of chemical-specific partition coefficients (PC) in rat
  - 945 tissue-specific PC
  - 137 unique chemicals
  - Mostly pharmaceuticals
- Calibrating *in silico* predictors (Schmitt, 2008) to actual performance
- Evaluated with human measured volumes of distribution for 498 chemicals from Obach (2008)
  - All pharmaceuticals

Journal of Pharmacokinetics and Pharmacodynamics.

Pearce et al. (2017) Available On-line



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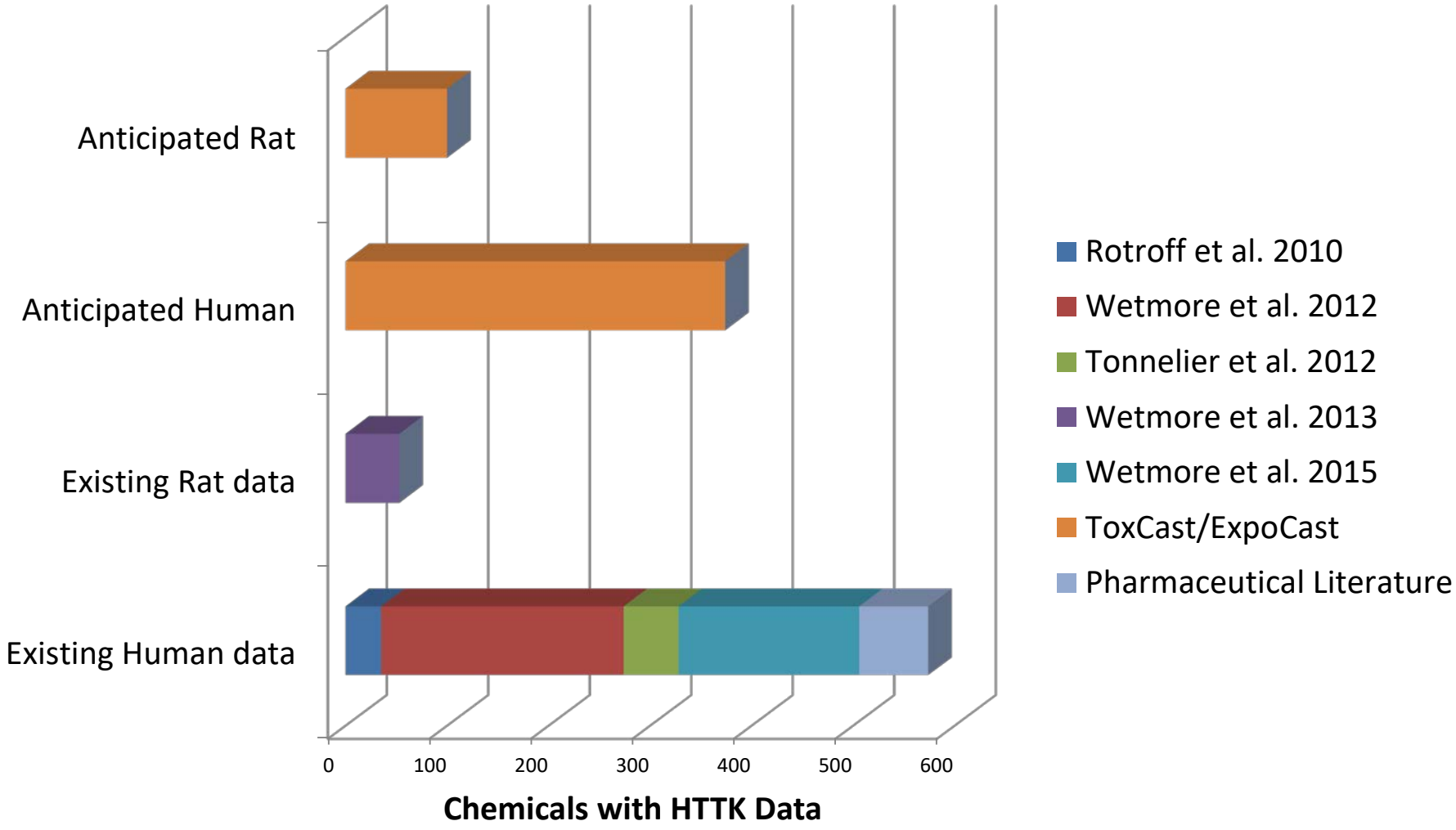
Journal of Pharmacokinetics and Pharmacodynamics.

Chemicals with HTTK Data



Does My Chemical Have HTTK Data?

Is a chemical available?

```
> "80-05-7" %in% get_cheminfo()
[1] TRUE
```

```
> library(httk)
> get_cheminfo()
  [1] "2971-36-0"   "94-75-7"     "94-82-6"     "90-43-7"     "1007-28-9"
  [6] "71751-41-2"  "30560-19-1"  "135410-20-7" "34256-82-1"  "50594-66-6"
 [11] "15972-60-8"  "116-06-3"    "834-12-8"    "33089-61-1"  "101-05-3"
 [16] "1912-24-9"   "86-50-0"     "131860-33-8" "22781-23-3"  "1861-40-1" …
> get_cheminfo(info="all")
```

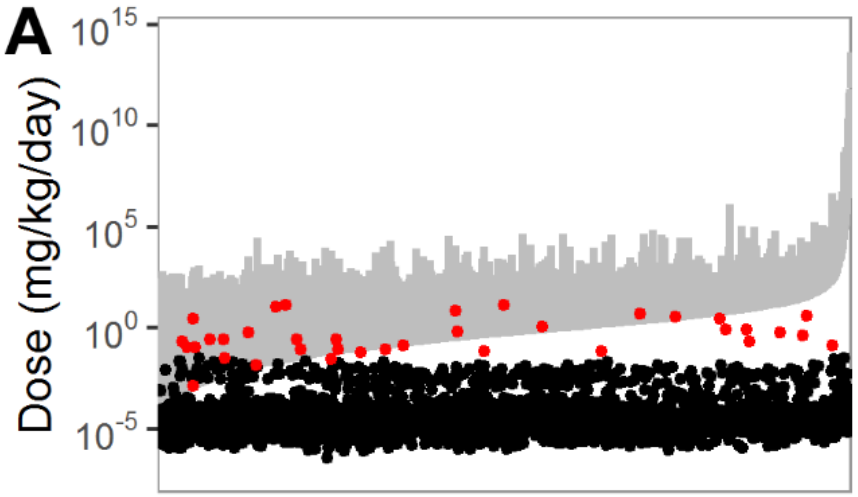
All data on chemicals A, B, C

```
subset(get_cheminfo(info="all"),Compound%in%c("A","B","C"))
```

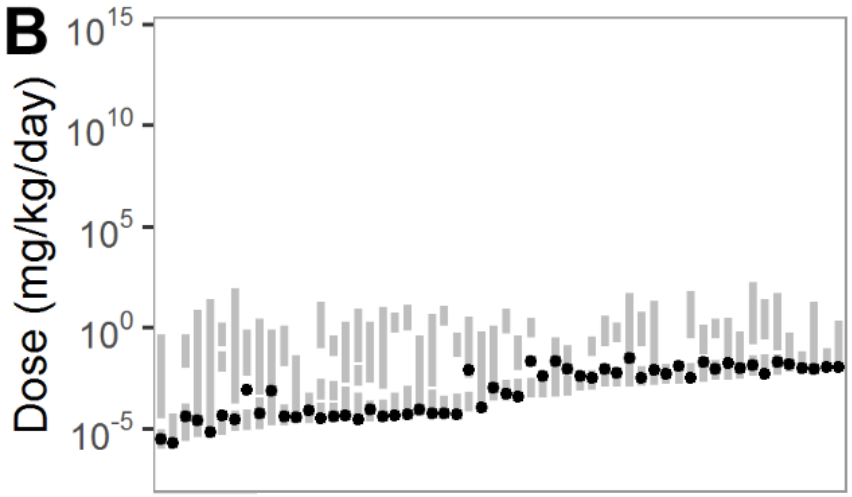
Compound	CAS	logP	pKa_Accept	pKa_Donor	MW	Human.Clint	Human.Clint.pValue	Human.Funbound.plasma	DSSTox_Substance_Id	Structure_Formula	Substance_Type	
2,4-d	94-75-7	2.81	<NA>	2.81	221.03	0	0.149	0.04	DTXSID0020442	C8H6Cl2O3	Single	Compound
2,4-db	94-82-6	3.53	<NA>	4.5	249.09	0	0.104	0.01	DTXSID7024035	C10H10Cl2O3	Single	Compound
2-phenylphenol	90-43-7	3.09	<NA>	10.6	170.211	2.08	0.164	0.04	DTXSID2021151	C12H10O	Single	Compound
6-desisopropylatrazine	1007-28-9	1.15	1.59	<NA>	173.6	0	0.539	0.46	DTXSID0037495	C5H8ClN5	Single	Compound

In Silico HTTK Predictions

- Tox21 has screened >8000 chemicals – Sipes *et al.* (2017) wanted to compare *in vitro* active concentrations with HTTK predicted maximum plasma concentrations with high throughput exposure predictions from Wambaugh et al. (2014)
- "httk" package only had 543 chemicals
- Used Simulations Plus ADMet Predictor to predict for entire library (supplemental table) and used add_chemtable() function to add into "httk" package



Dose range for all 3925 Tox21 compounds eliciting a 'possible'-to-'likely' human *in vivo* interaction alongside estimated daily exposure



56 compounds with potential *in vivo* biological interaction at or above estimated environmental exposures

Figure from Sipes *et al.*, (2017)

What you can do with R Package "httk"

- Allows one compartment, three-compartment, and PBTK modeling
- Allows conversion of *in vitro* concentration to *in vivo* doses
- Allows prediction of internal tissue concentrations from dose regimen (oral and intravenous)
- A peer-reviewed paper in the Journal of Statistical software provides a how-to guide (Pearce et al., 2017a)
- You can use the built in chemical library or add more chemical information (examples provided in JSS paper)
- You can predict partition coefficients for novel tissues
- You can use specific demographics from modern U.S. population in the population simulator
  - Gender, age, weight, ethnicity, renal function
- You can load specific (older) versions of the package
- You can control the built-in random number generator to reproduce the same random sequence (function set.seed())

HTTK Limitations (from Ring et al., 2017)

- Oral absorption
  - 100% assumed, but may be very different
  - *In silico* models not necessarily appropriate for environmental chemicals
- Hepatic Clearance (CL_{int})
  - Ten donor pool in suspension for 2-4 h misses variability and low turnover compounds
  - Isozyme abundances and activity: varies with age, ethnicity (at least) (Yasuda et al. 2008, Howgate et al. 2006, Johnson et al. 2006)
  - Parent chemical depletion only
- Isozyme-specific data & modeling (Wetmore et al. 2014)
  - Isozyme-specific metabolism assays not HT
  - *In silico* predictions of isozyme-specific metabolism? Not easy!
    - Existing data is mostly for pharmaceuticals
- Plasma binding assay (F_{up})
  - Assay often fails due to analytical chemistry sensitivity (Wetmore et al., 2012)
  - Plasma protein concentration variability (Johnson et al. 2006, Israili et al. 2001)
  - Albumin or AAG binding? (Routledge 1986)

Summary

- Toxicokinetics (TK) provides a bridge between HTS and HTE by predicting tissue concentrations due to exposure
- High Throughput (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”
- New R package “httk” freely available on CRAN allows statistical analyses to identify strengths and weaknesses
  - All HTTK models and data made public upon peer-reviewed publication
- Includes one compartment, three compartment (e.g., Wetmore et al.) and generic PBTK model
- New bioavailability (CACO2) data being collected and analyzed

EPA United States Environmental Protection Agency

Chemical Safety for Sustainability (CSS) Research Program

Rapid Exposure and Dosimetry (RED) Project

NCCT
Chris Grulke
Greg Honda*
Richard Judson
Andrew McEachran*
Robert Pearce*
Ann Richard
Risa Sayre*
Woody Setzer
Rusty Thomas
John Wambaugh
Antony Williams

NRMRL
Yirui Liang*
Xiaoyu Liu

NHEERL
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Christopher Ecklund
Marina Evans
Mike Hughes
Jane Ellen Simmons

NERL
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Namdi Brandon*
Peter Egeghy
Jarod Grossman*
Hongtai Huang*
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Kristin Isaacs
Sarah Laughlin-Toth*
Seth Newton
Katherine Phillips
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Netherlands Organisation for Applied Scientific Research (TNO)
Sieto Bosgra
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Harvey Clewell
Kamel Mansouri
Chantel Nicolas
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Robin Dodson
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EPA United States Environmental Protection Agency

References

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