Developing Reverse Toxicokinetic Models to Correlate *In Vitro* and *In Vivo* Activity

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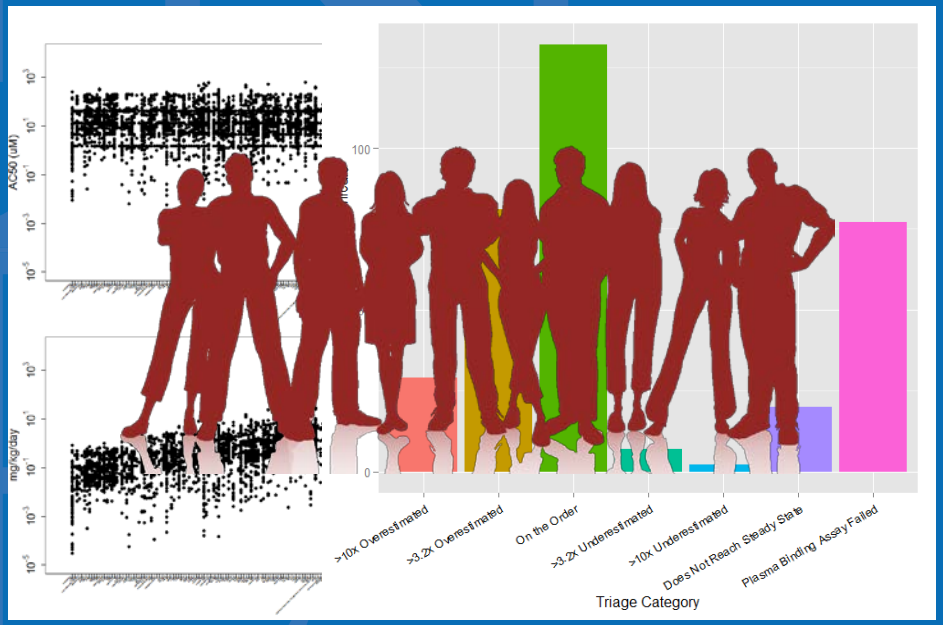

Figure includes image from Thinkstock

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Introduction

- High throughput screening (HTS) methods exist for identifying chemical concentrations that may induce changes to human biology *(e.g.,* Tox21, ToxCast*)*
- High throughput exposure (HTE) methods exist for predicting potential human exposure for arbitrary chemicals (*e.g.,* ExpoCast – Wambaugh *et al.*, 2014)
- Toxicokinetics (TK) provides a bridge between HTS and HTE by predicting tissue concentrations due to exposure

Introduction

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

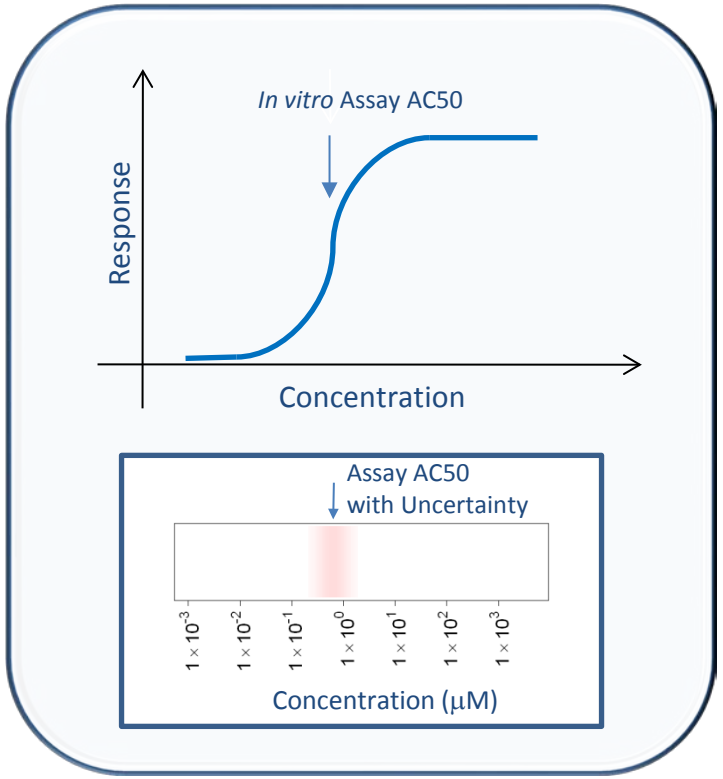
- Relatively high throughput TK (HTTK) methods have been used by the pharmaceutical industry to determine range of efficacious doses and to prospectively evaluate success of planned clinical trials (Jamei, *et al.*, 2009; Wang, 2010)

  - A key application of HTTK has been “reverse dosimetry” (also called Reverse TK or RTK)

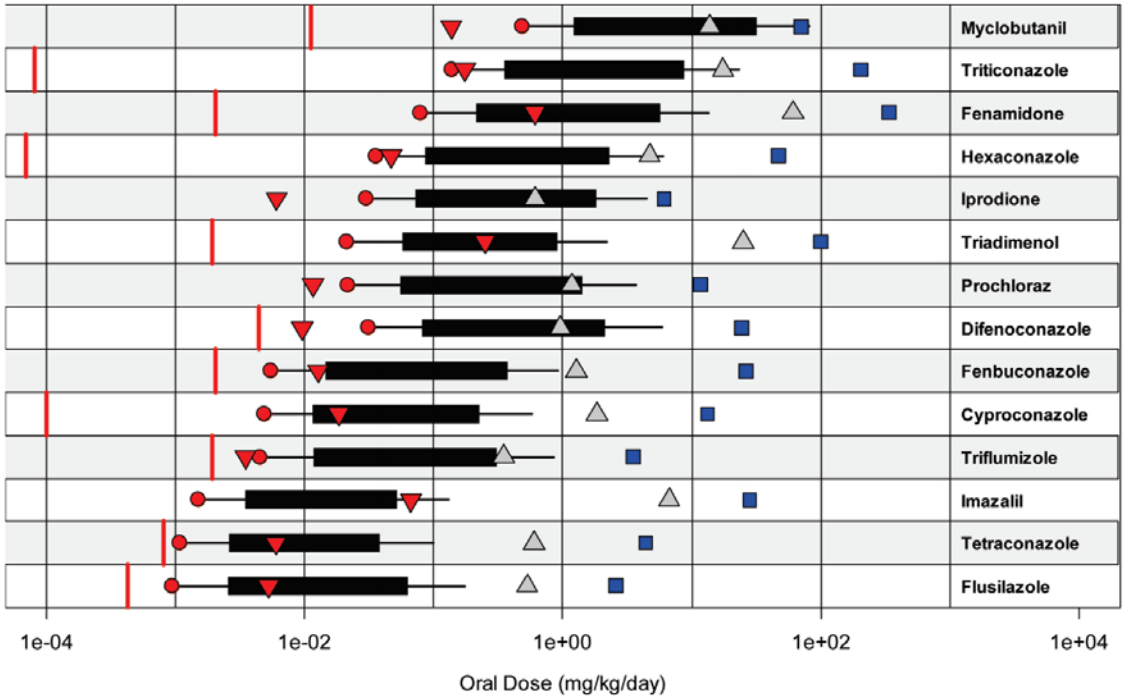
  - RTK can approximately convert *in vitro* HTS results to daily doses needed to produce similar levels in a human for comparison to exposure data (Wetmore, *et al.,* 2012)

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, RTK, and *in Vivo* Toxic Doses



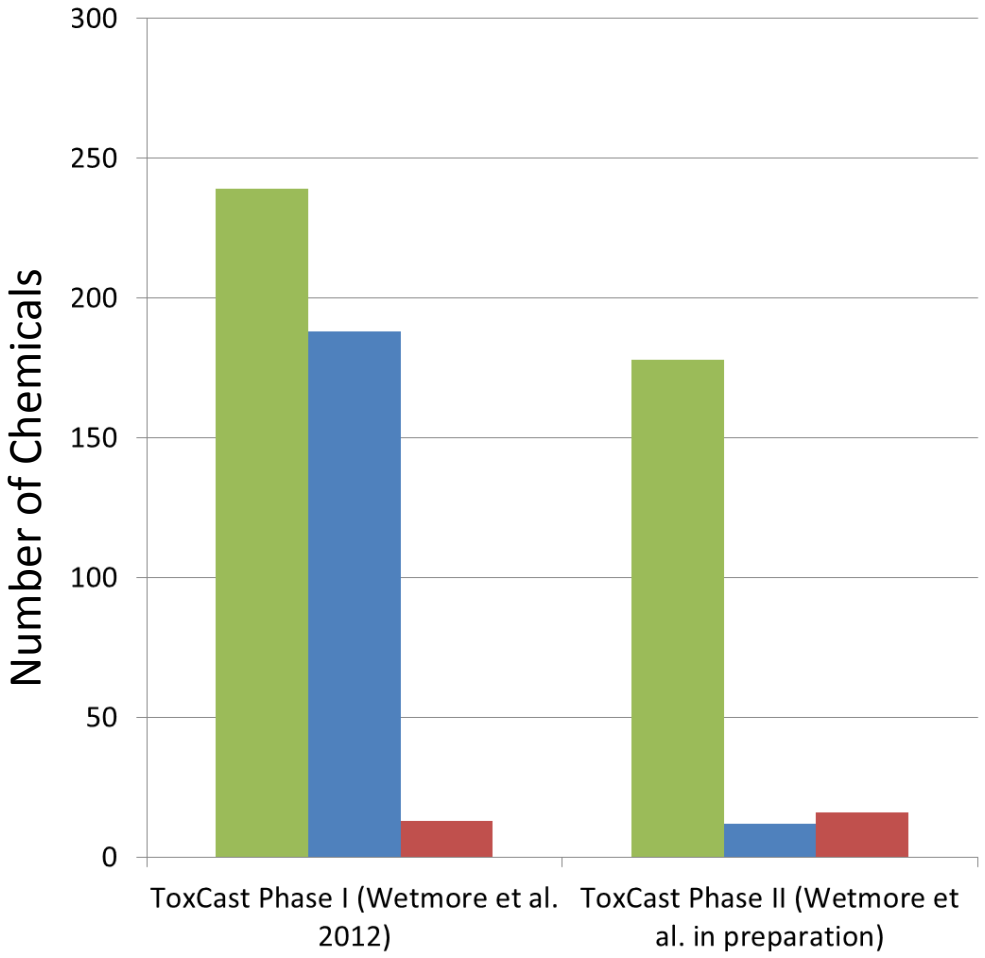
Judson *et al*. (2011)

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.

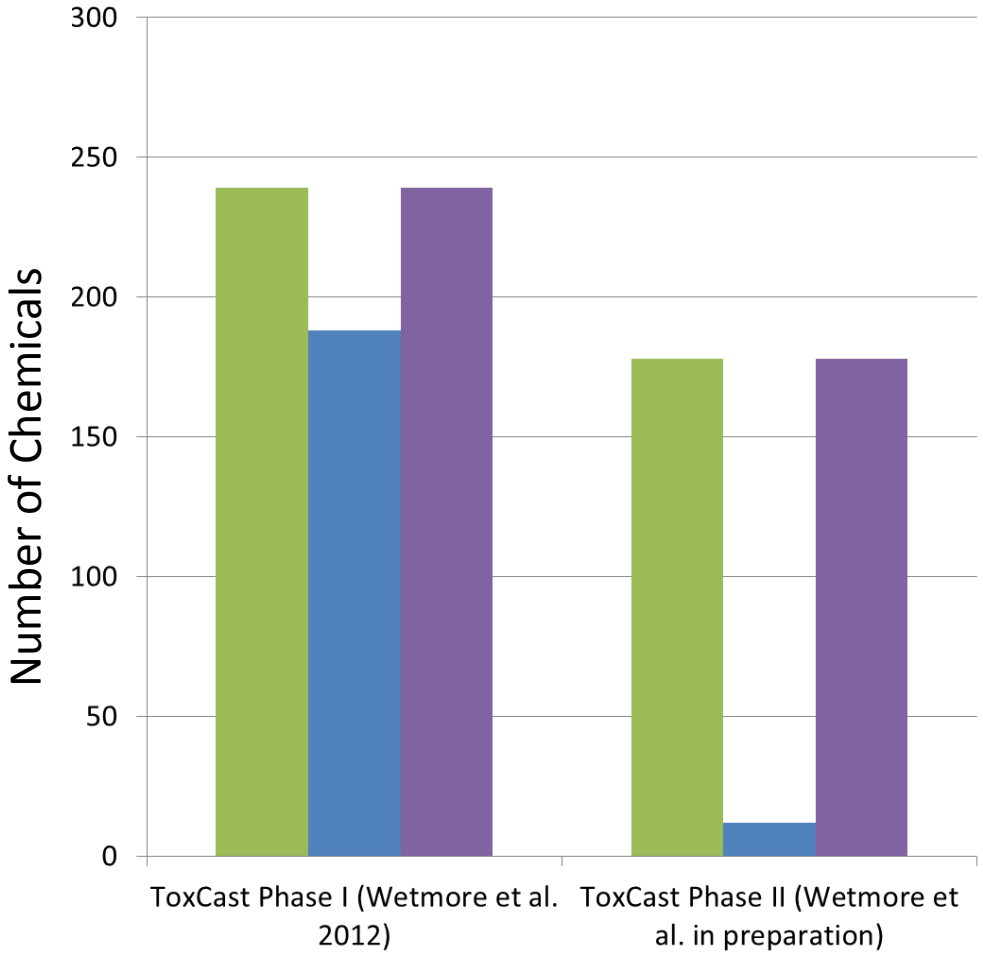
In Vitro Bioactivity, *In Vivo* Toxicokinetics, and Human Exposure



Egeghy *et al.* (2012):

There is a paucity of data for providing exposure context to HTS data

In Vitro Bioactivity, *In Vitro* Toxicokinetics, and Human Exposure

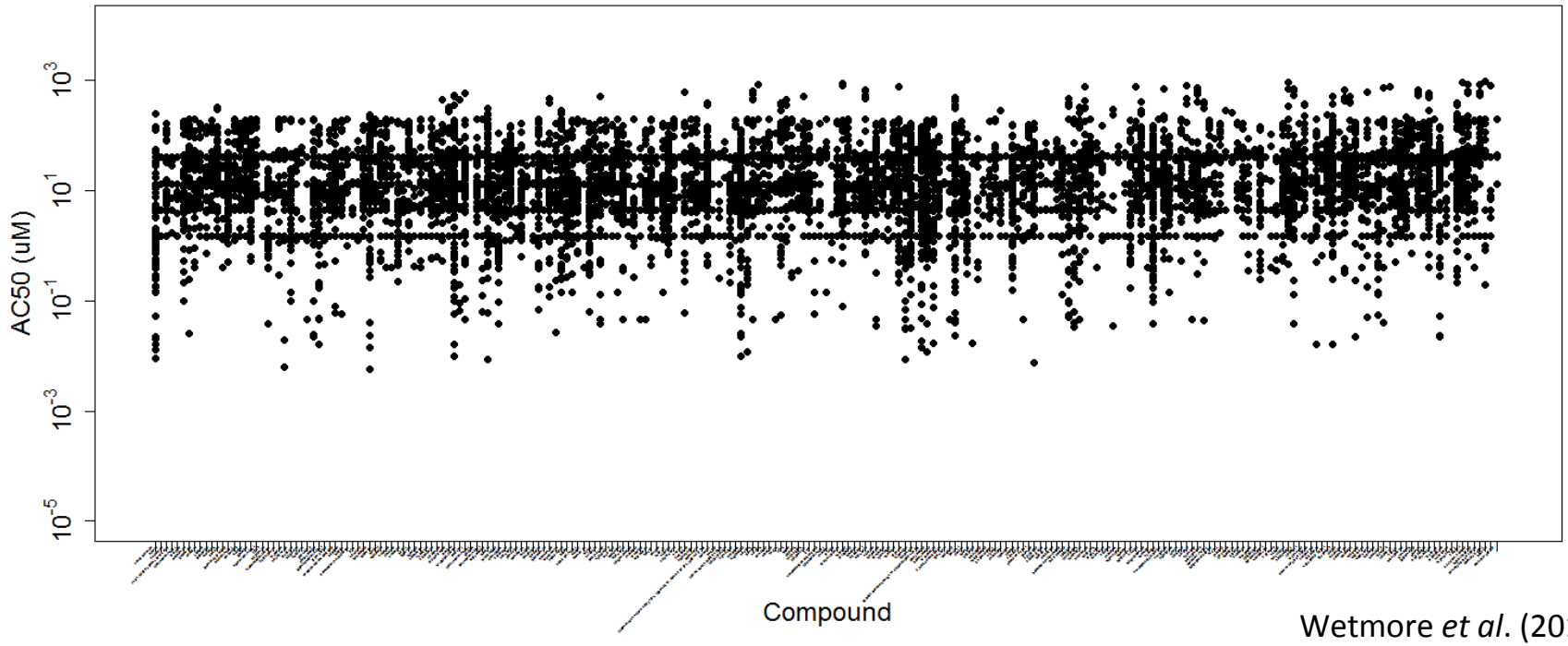


Egeghy *et al.* (2012):

There is a paucity of data for providing exposure context to HTS data

HTTK studies like Wetmore *et al.* (2012), can address the need for toxicokinetic data

ToxCast *in vitro* Bioactive Concentrations



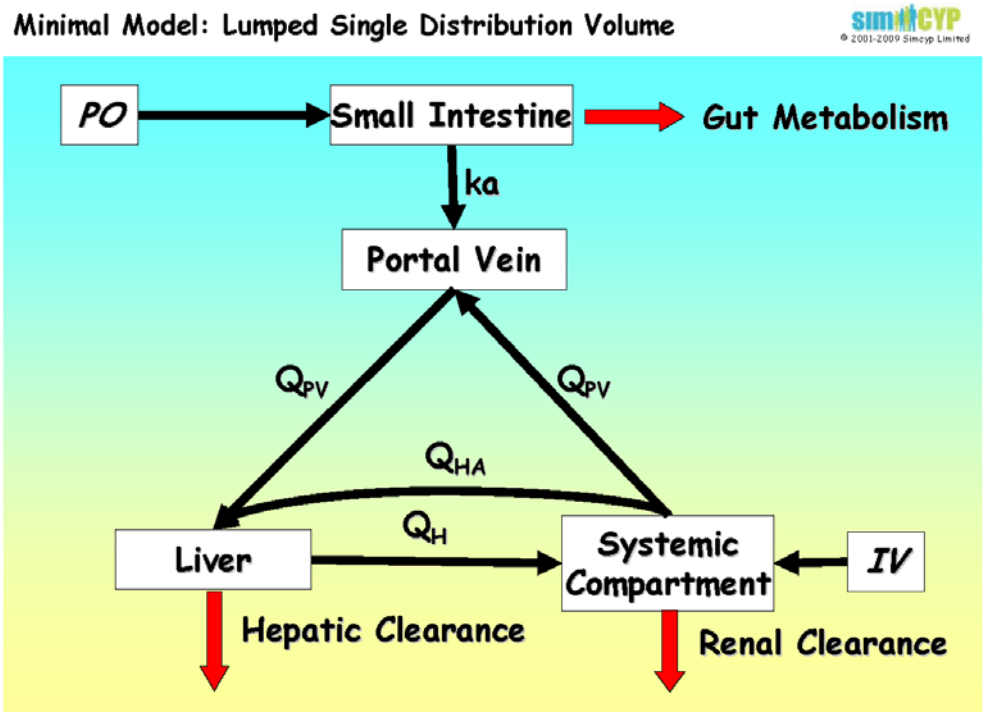
Wetmore *et al*. (2012)

- One point for each chemical-*in vitro* assay combination with a systematic (Hill function) concentration response curve

Predicting Steady-State Plasma Concentration

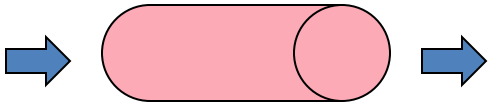
Jamei *et al.* (2009)

- *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
- No oral absorption/ bioavailability included



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

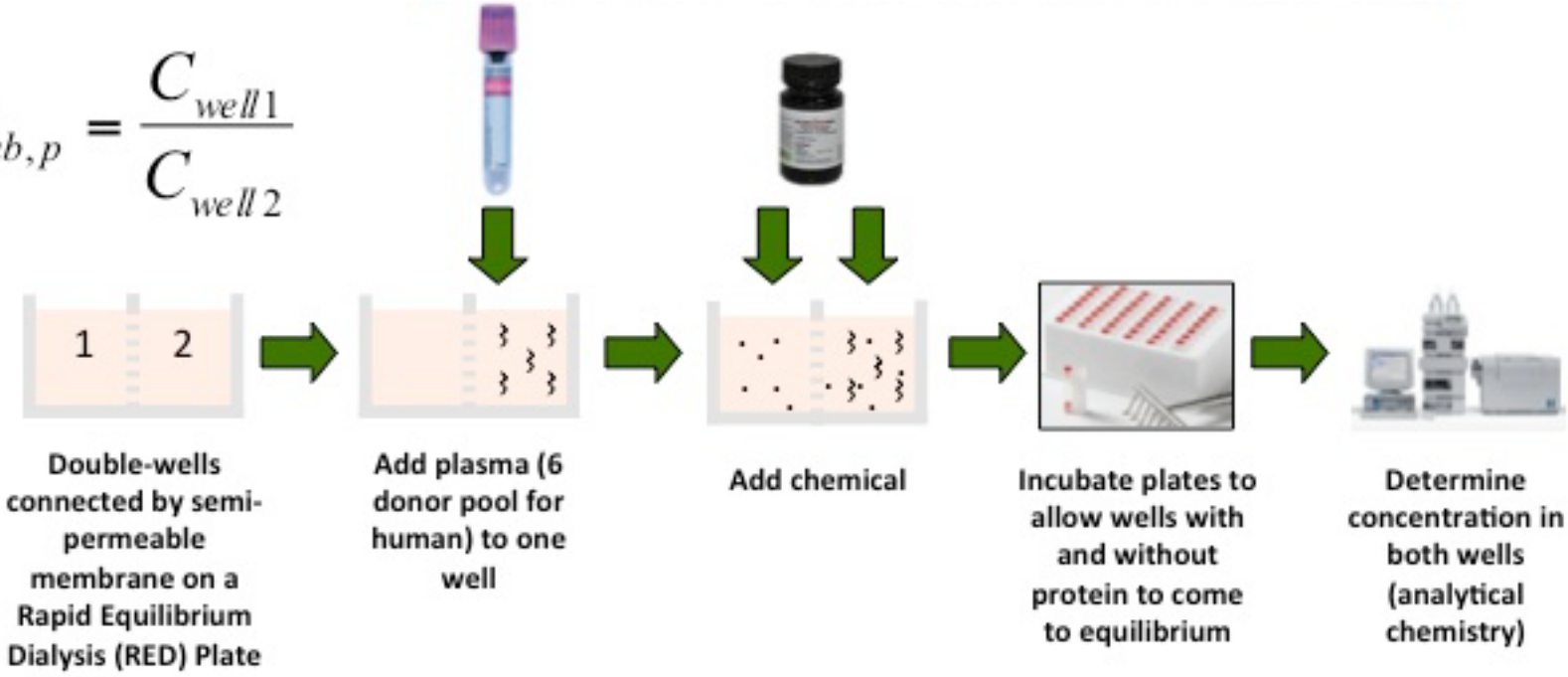
Oral dose in (mg/kg/day)



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

Plasma Protein Binding (Fraction Unbound in Plasma)

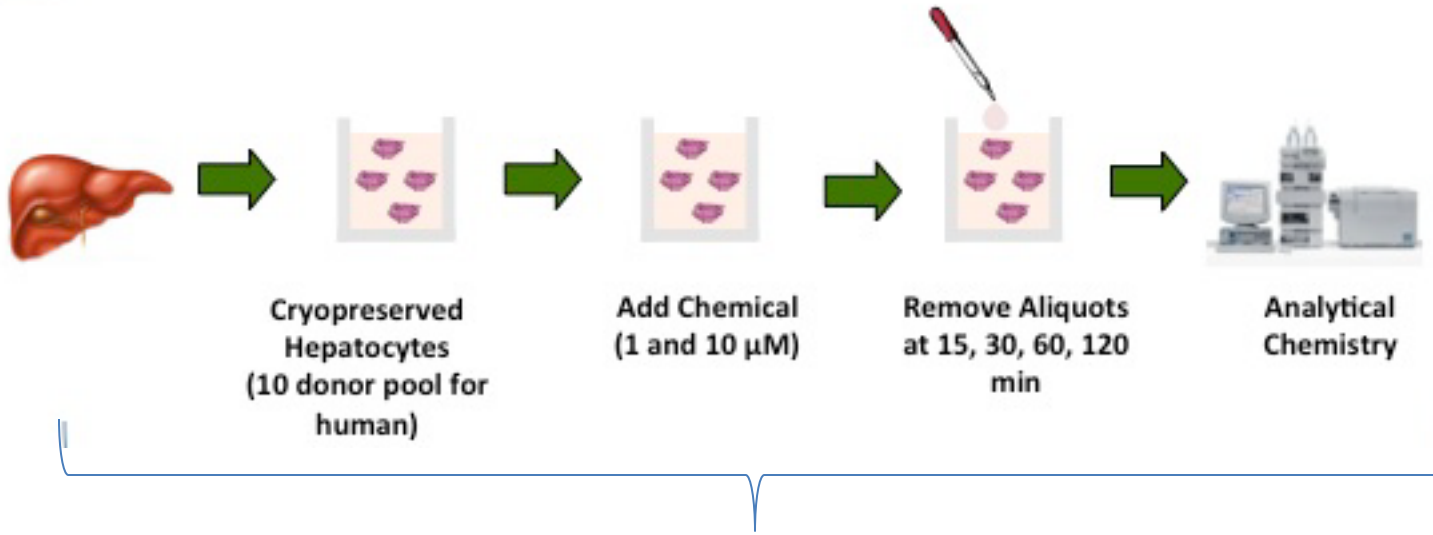
$$F_{ub,p} = \frac{C_{well\,1}}{C_{well\,2}}$$



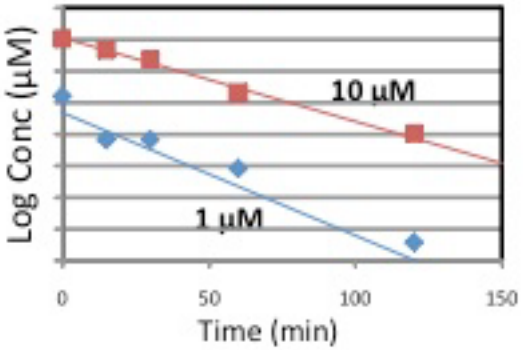
RED Method:
Waters *et al.* (2008)

- 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. (2014) – Assessed variability in metabolism for a dozen ToxCast compounds

Intrinsic Hepatic Clearance



The rate of disappearance of parent compound (slope of line) is the **hepatic clearance** (µL/min/10^6 hepatocytes)



1 and 10 µM to check for saturation of metabolizing enzymes.

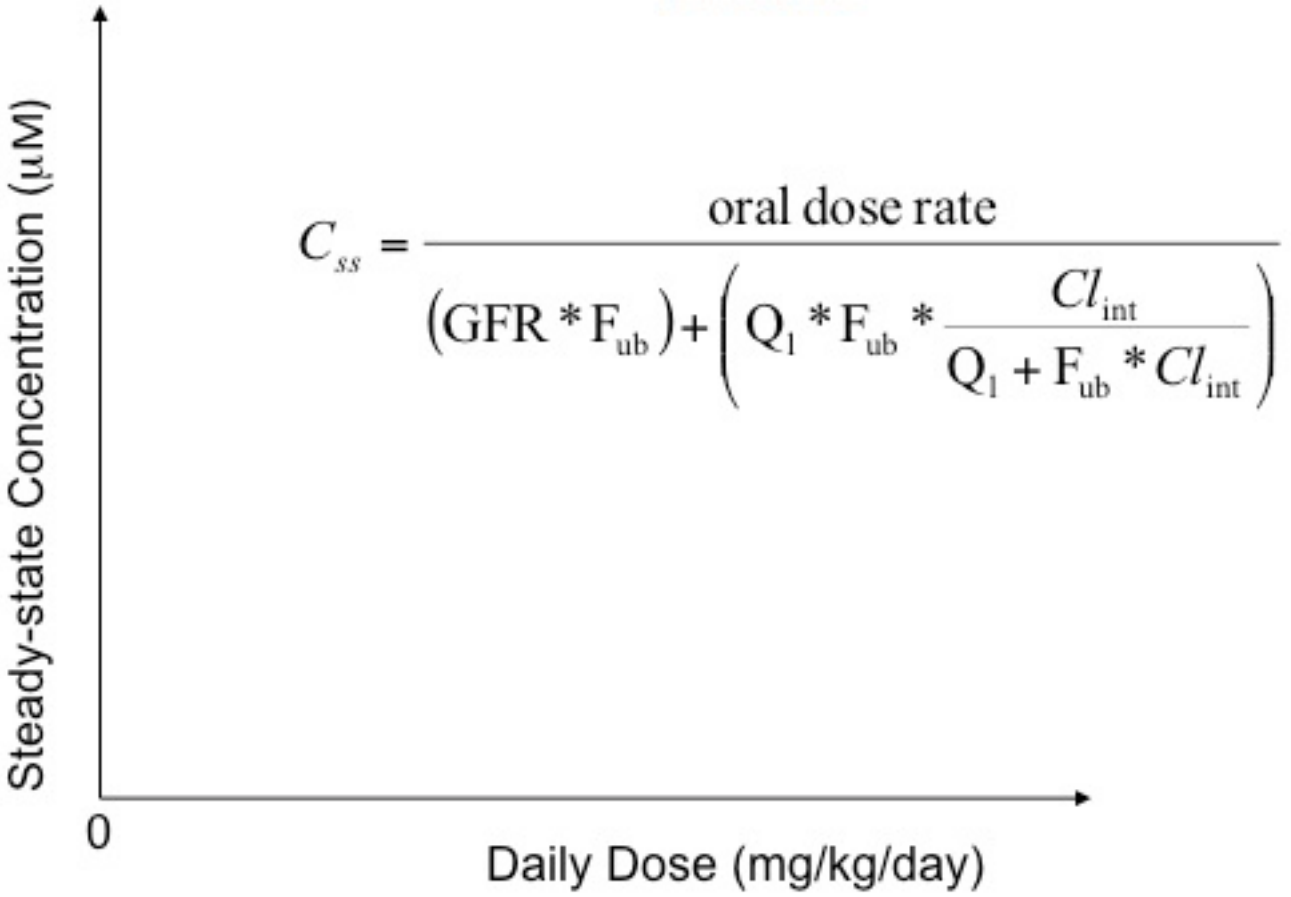
We perform the assay at

Cryopreserved hepatocyte

Method: Shibata *et al.* (2002)

- Data on ToxCast chemicals initially collected at Hamner Institutes

Steady-State is Linear with Dose

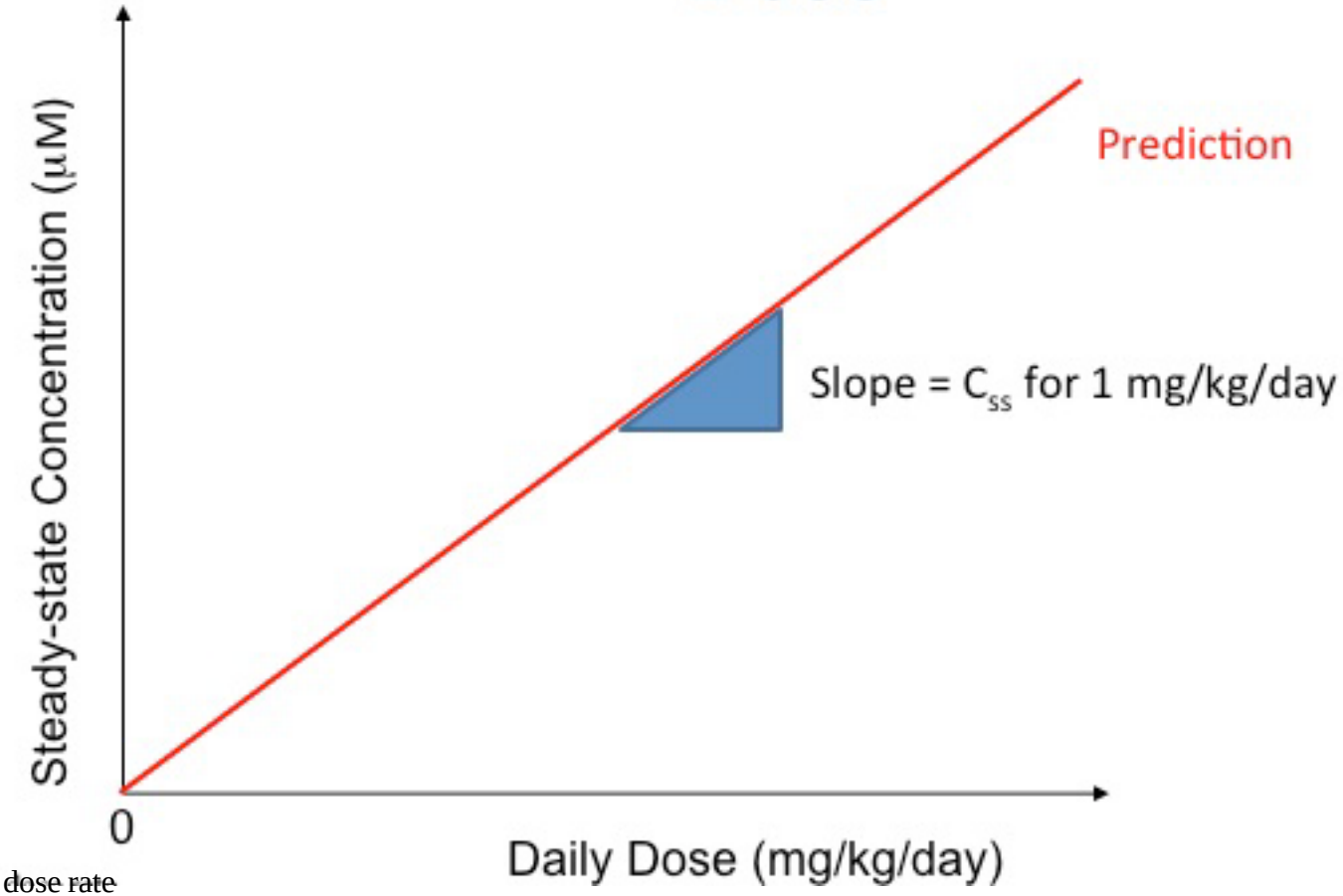


$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

- 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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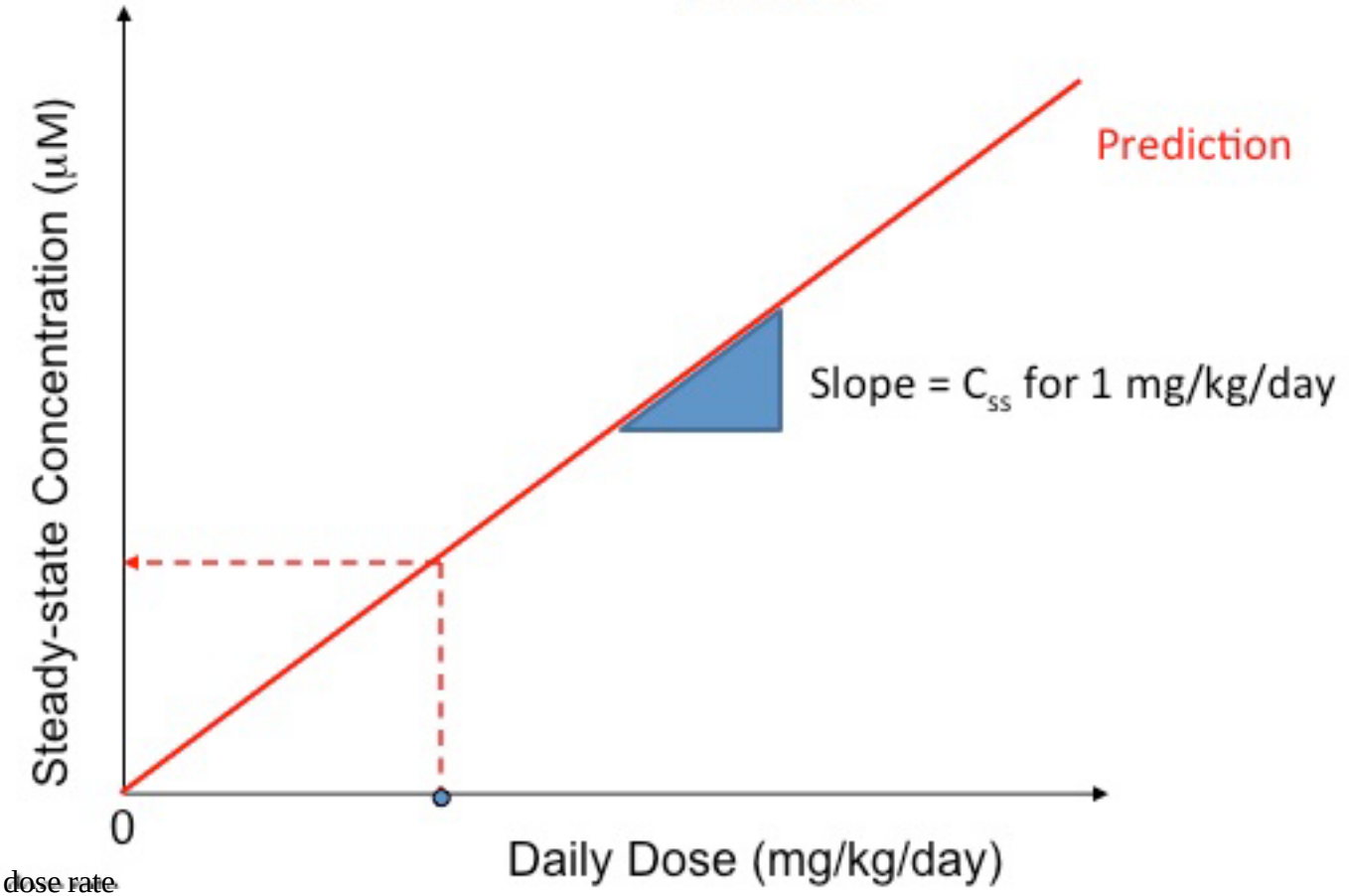

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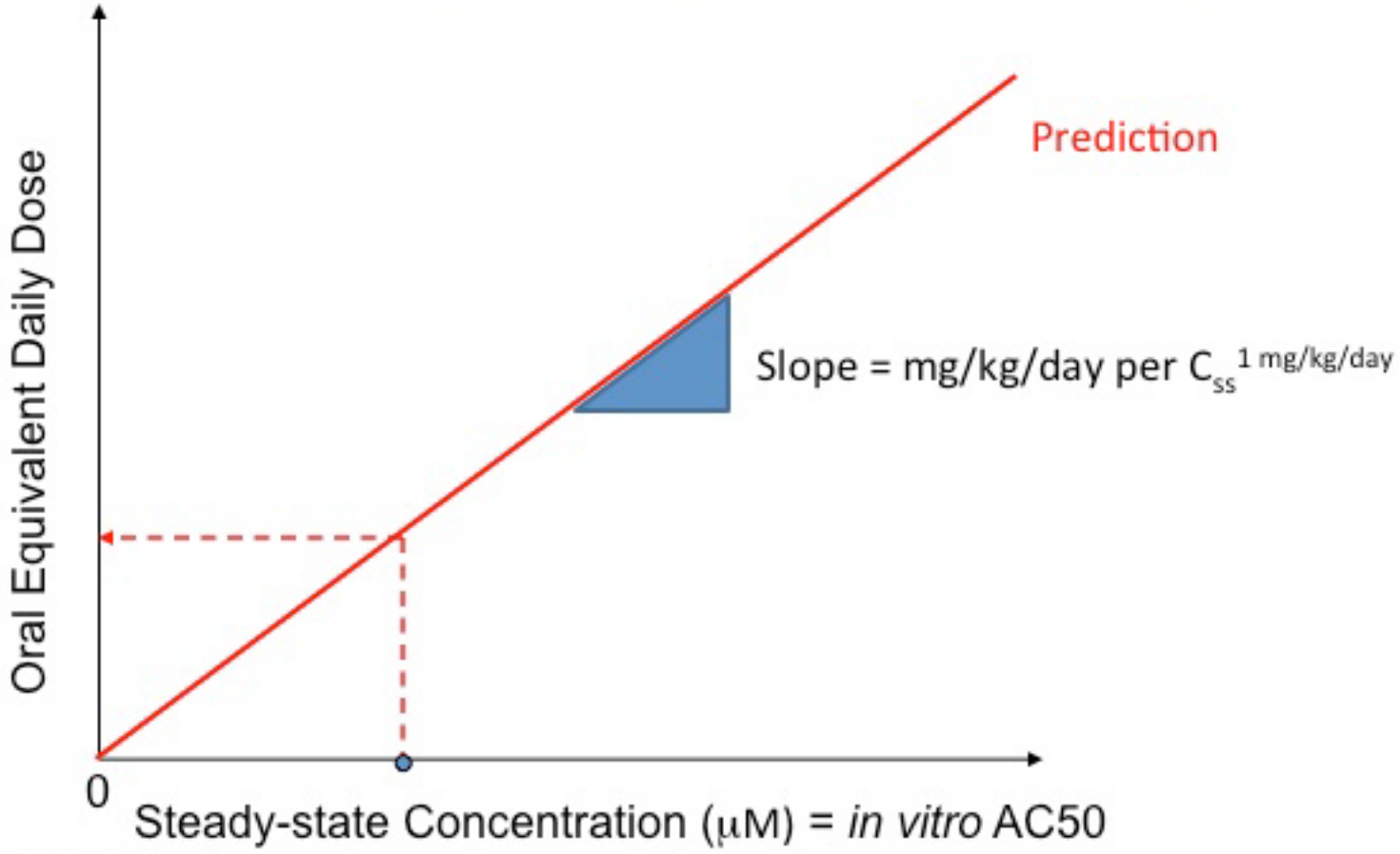
- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al*. (2012)

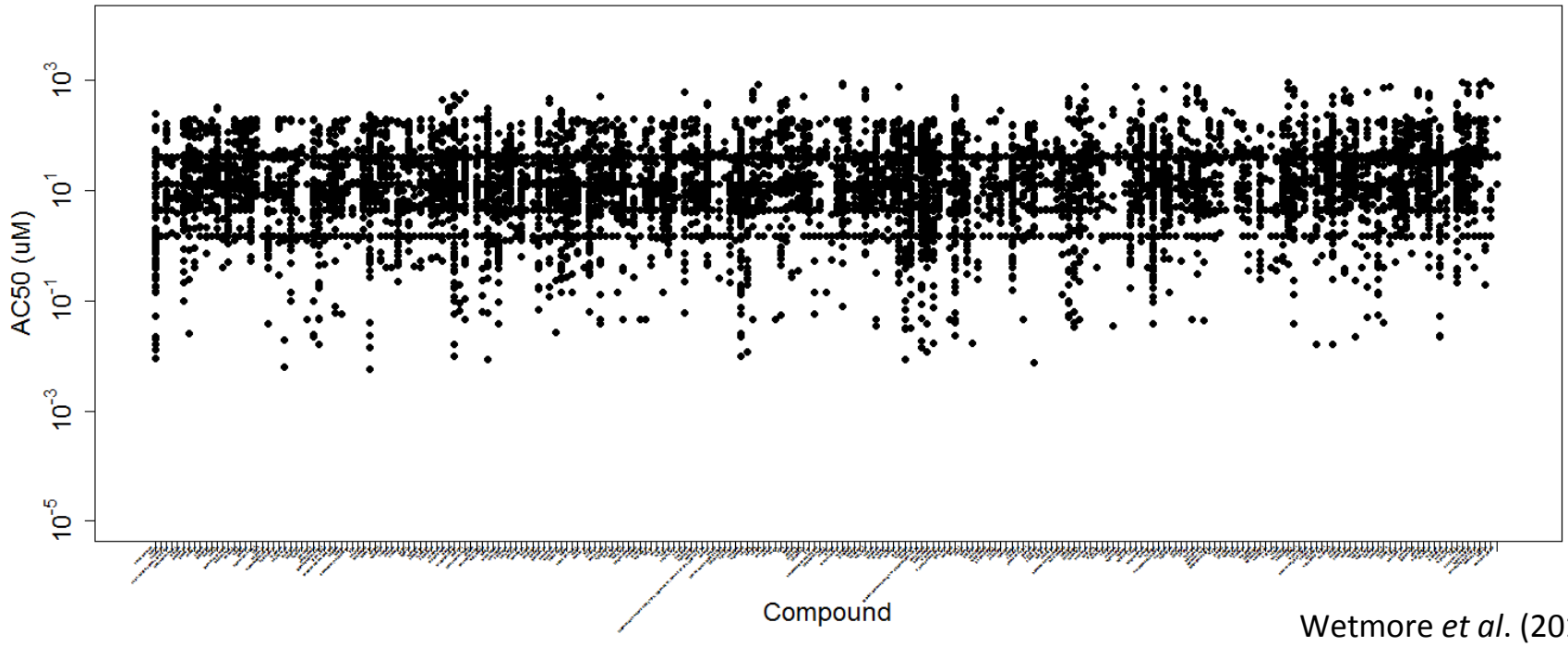
HTTK Allows Steady-State *In Vitro-In Vivo* Extrapolation (IVIVE)



- 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)

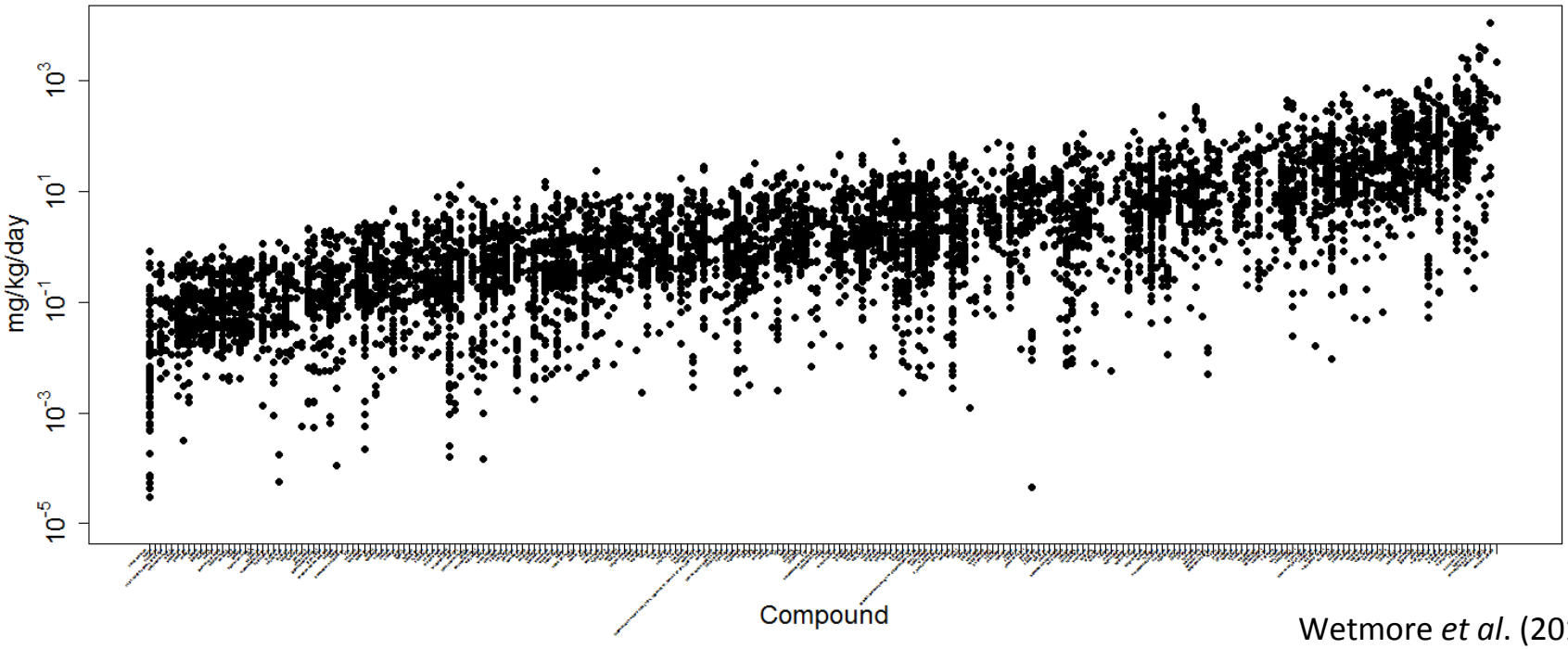
ToxCast *in vitro* Bioactive Concentrations



Wetmore *et al*. (2012)

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

RTK 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

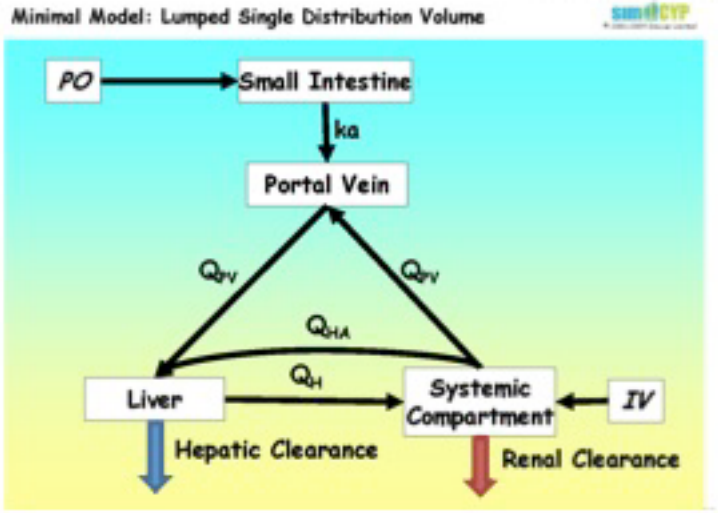
Combination of higher exposure and sensitivities

Populations that are More Sensitive

Images from Thinkstock

Variability in Steady-State TK Model

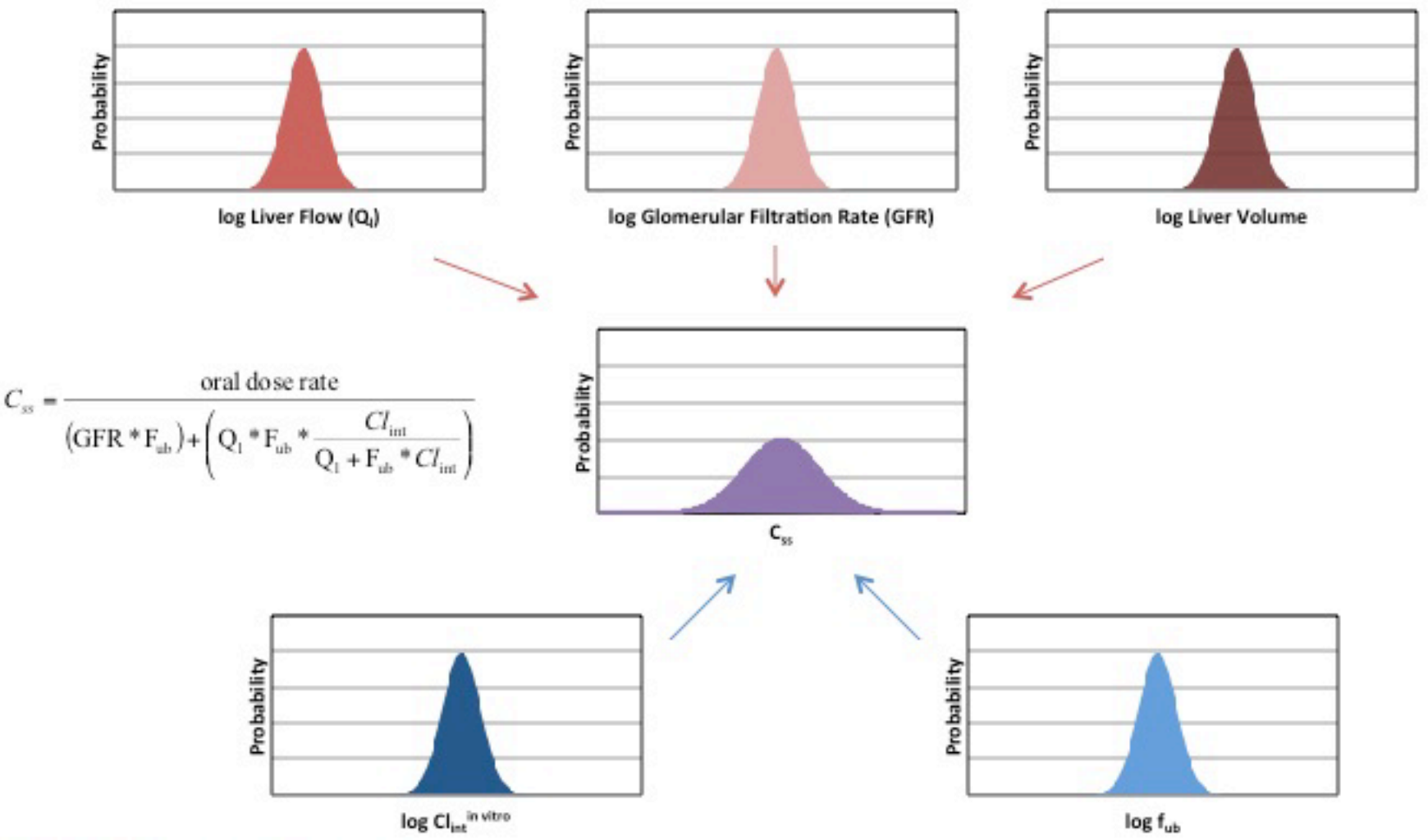
Jamei *et al*. (2009)



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

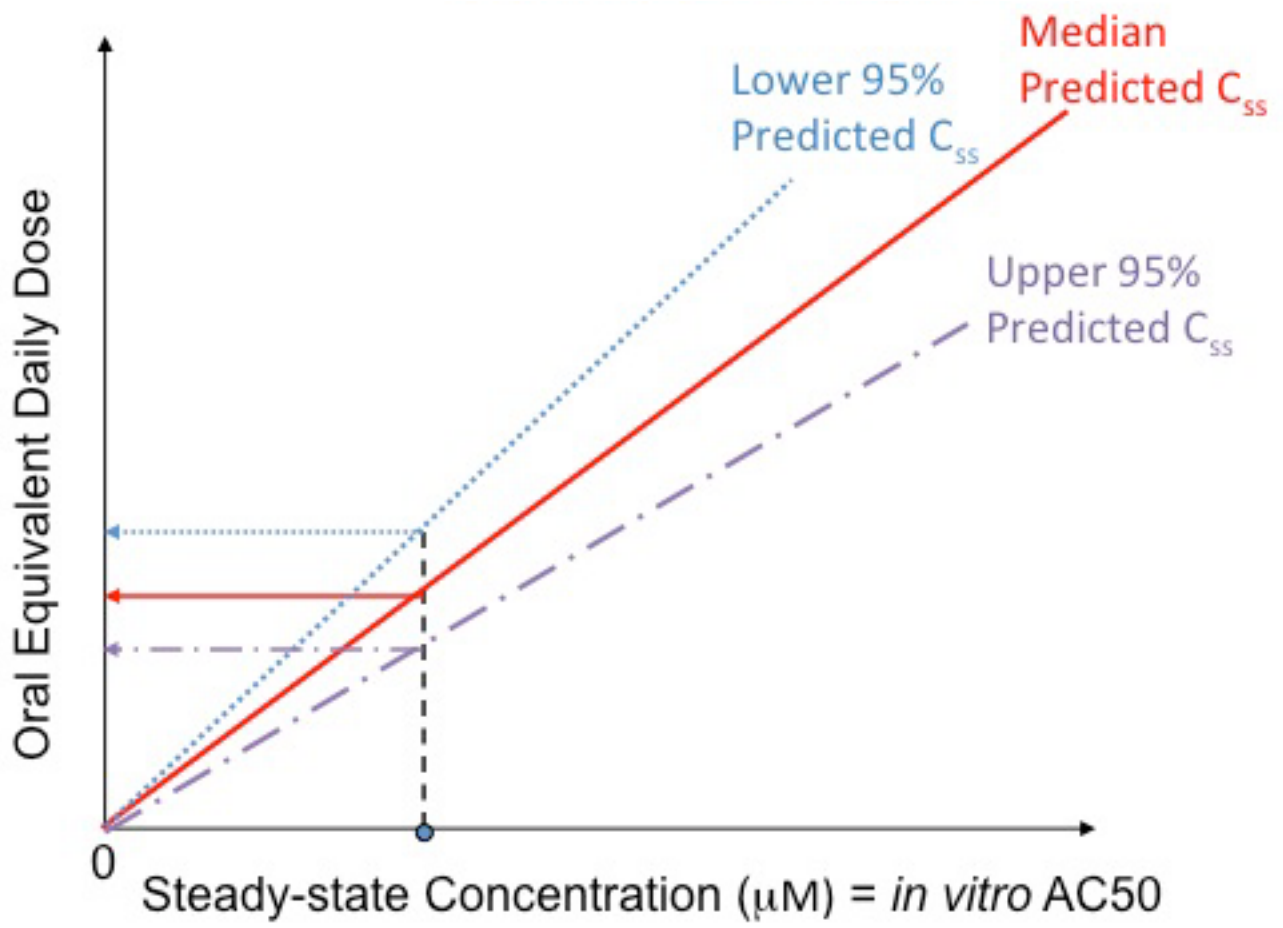
- *In vitro* clearance (µL/min/10^6 hepatocytes) is scaled to a whole organ clearance using the density of hepatocytes per gram of liver and the volume of the liver (which varies between individuals)
- Glomerular filtration rate (GFR) and blood flow to the liver (Q_l) both vary from individual to individual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



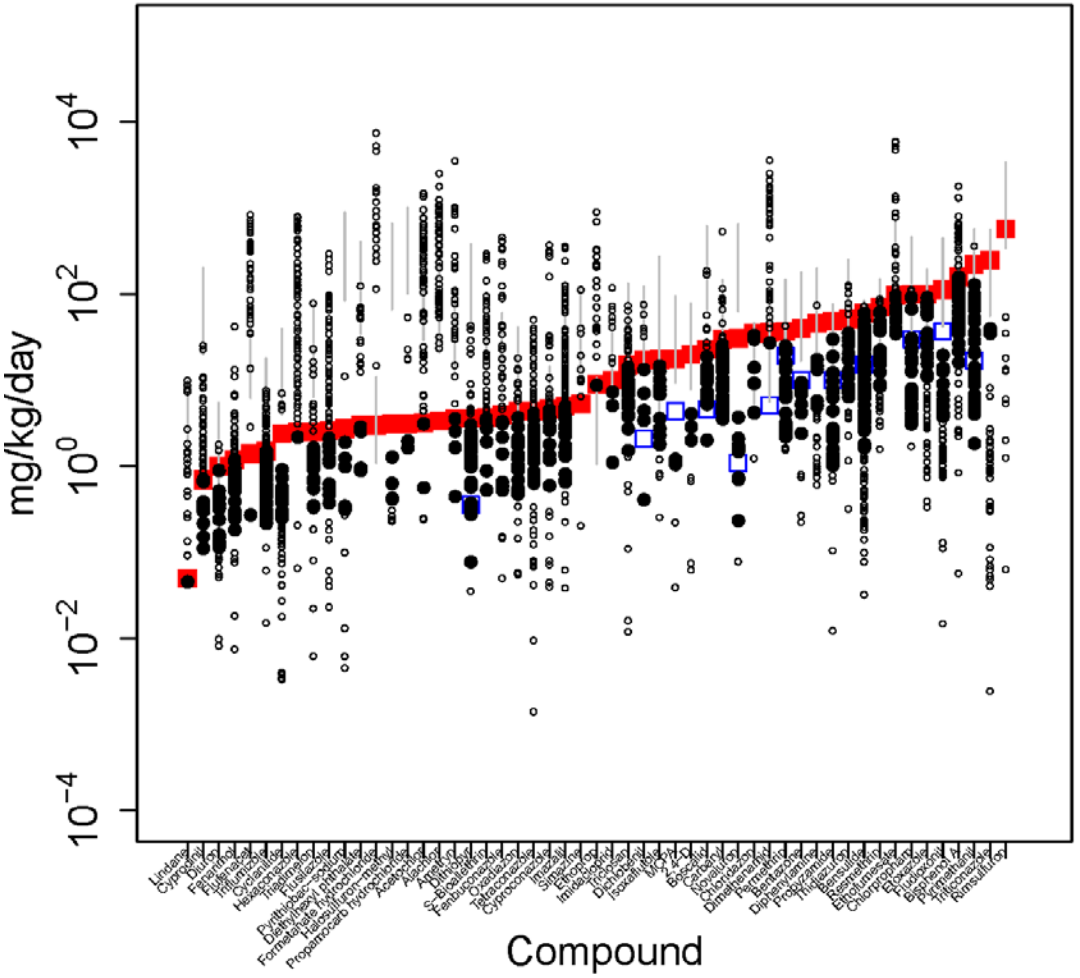
- The higher the predicted C_{ss}, the lower the oral equivalent dose, so the upper 95% predicted C_{ss} from the MC has a lower oral equivalent dose

Human HTS With Rat HTTK

- Concordance of steady state oral equivalent doses and *in vivo* No Observed (□) and Lowest Observed (■) dose levels provides an additional method for evaluation

- Wetmore *et al.* (2013) chose 53 chemicals with good coverage of *in vivo* endpoints in the Toxicity Reference Database (ToxRefDB – Martin *et al.*, 2009)

http://actor.epa.gov/toxrefdb/

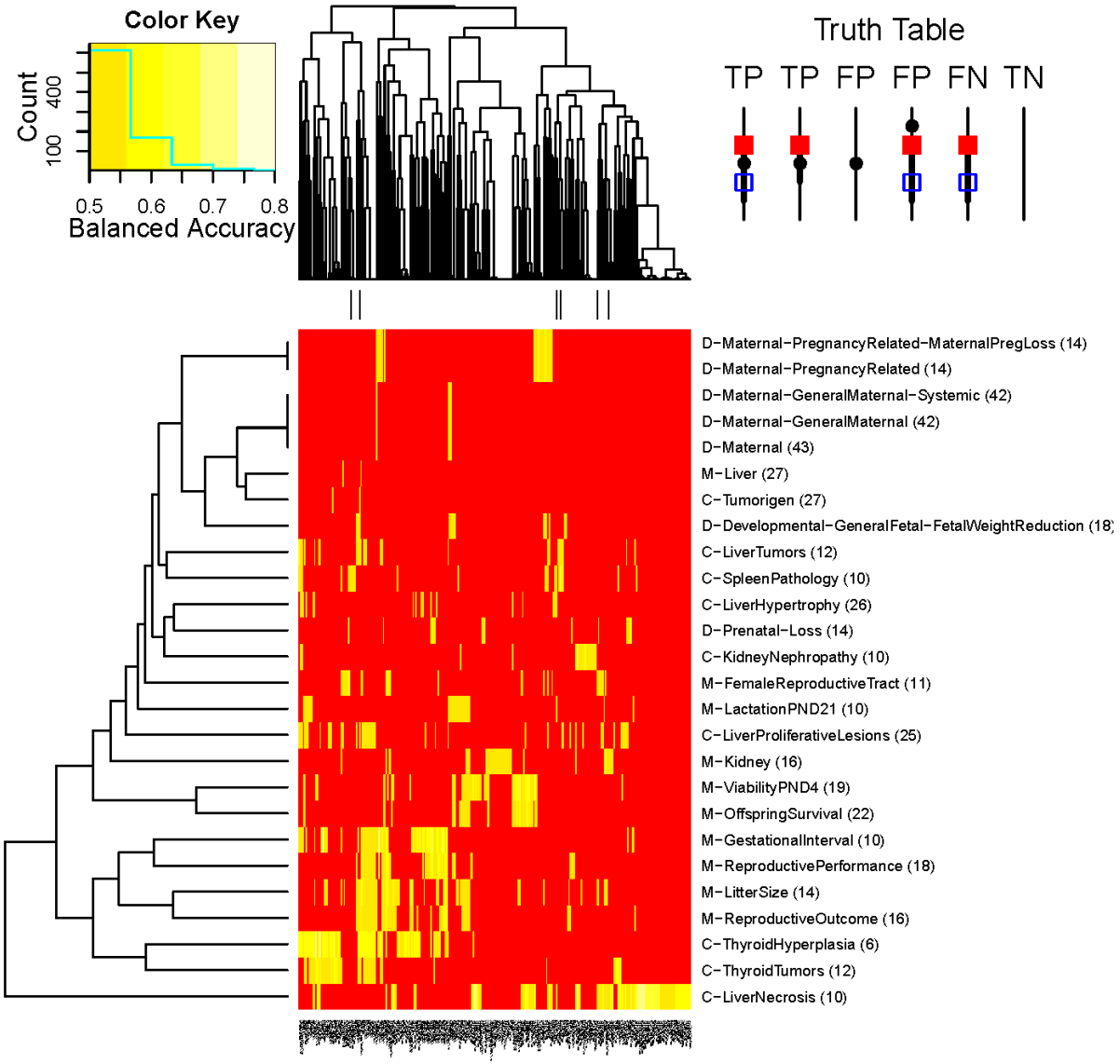


Wetmore, *et al.* (2013)

Correlating Human *in vitro* and Rat *in vivo*

United States Environmental Protection Agency

- Can find statistical associations with individ ToxCast *in vitro* assays a ToxRefDB endpoints
- Correlations are weak – the *n* for any given endpoint-assay combination is too low
  - You still need to know biology, *i.e.*, must have biological hypothesis
- Only looking at steady-state doses



ToxCast Assays

Wetmore, *et al.* (2013)

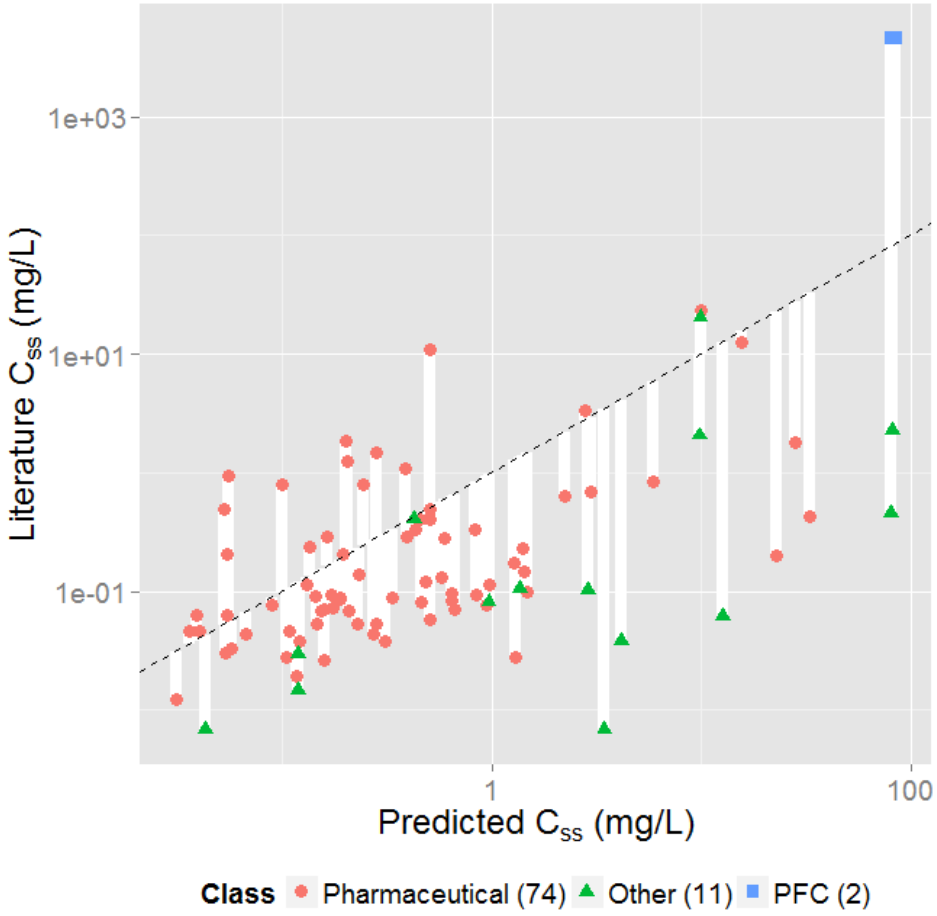
In vivo Predictive Ability and Domain of Applicability

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

- For environmental compounds, there will be no clinical trials

- Uncertainty must be well characterized ideally with rigorous statistical methodology
  - We will use direct comparison to *in vivo* data in order to get an empirical estimate of our uncertainty
  - Any approximations, omissions, or mistakes should work to increase the estimated uncertainty when evaluated systematically across chemicals

Using *in vivo* Data to Evaluate 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)

Predicting When RTK Will Work

- We can use computer algorithms to analyze chemical descriptors to try to predict when the residual will be small
- Factors included are:
  - Physico-chemical properties
    - Log(Kow), molecular weight, acid/base association constants (pKa), general pharmaceutical or perfluorinated compound classification
  - *In vitro* HTTK data
    - Plasma protein binding (F_{ub}) and hepatic clearance
  - Active chemical transport
    - Use quantitative structure activity relationships (QSARs) to predict likelihood each compound is a substrate for 17 different transporters (e.g, Sedykh et al, 2013)

Transporter QSAR Predictions

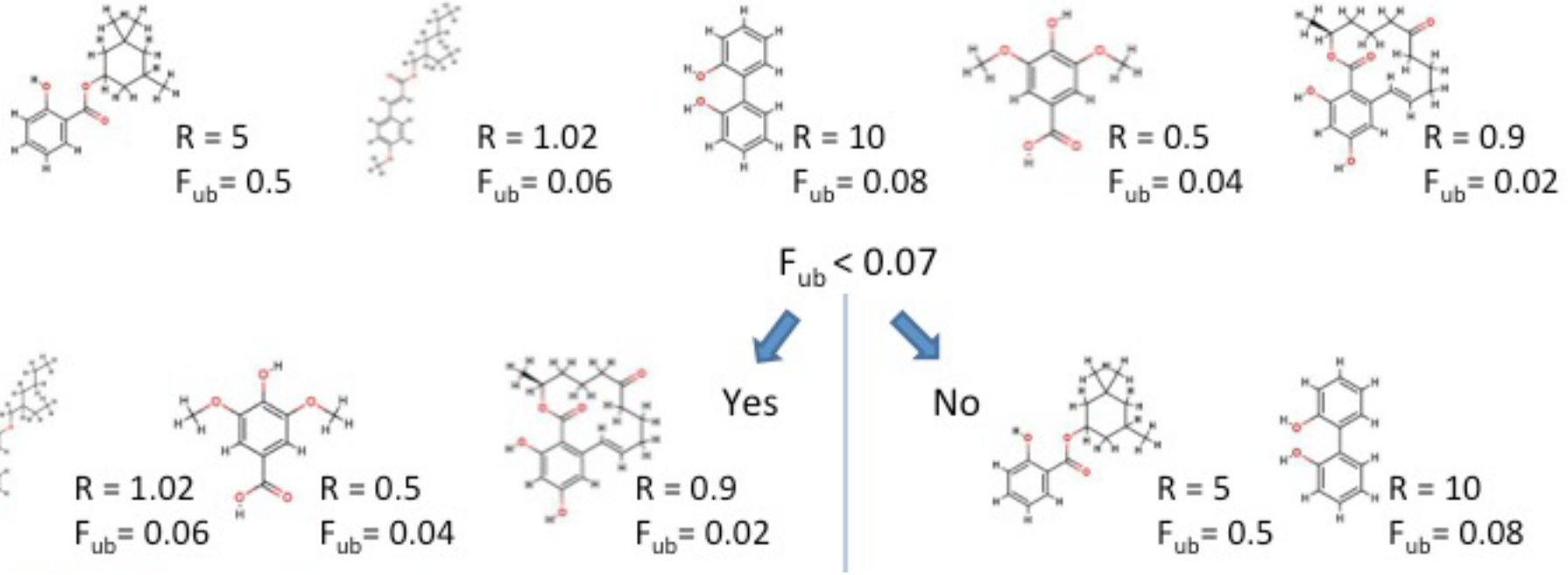
United States Environmental Protection Agency

From Alexander Sedykh and Alex Tropsha (UNC) and Sieto Bosgra (TNO)

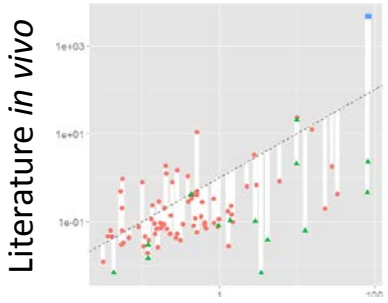
Compound	ASBT_pKm	ASBTx	BCRP	BSEP	MCT1	MDR1	MDR1_pKm	MRP1	MRP2	MRP3x	MRP4x	MRP5x	NTCPx	OATP2B1	OCT1x	PEPT1_pKm	PEPT1x
2,2-Bis(4-hydroxyphenyl)-1,1,1-trichloroethane (HPTE)	NA	0.006	0.482	0.394	0.564	0.163	4.425	0.45	0.301	0.36	0.224	0.464	0.341	0.532	NA	4.142	0.071
2,4-D	NA	0.012	0.405	0.378	0.584	0.082	4.389	0.388	0.233	0.252	0.213	0.387	0.259	0.283	0.748	3.765	0.121
2,5-Hexanedione	NA	0.031	0.288	NA	0.71	0.246	4.5	0.368	0.174	0.126	0.437	0.185	0.197	0.105	0.832	3.253	0.193
2-Phenylphenol	NA	0.007	0.451	0.456	0.744	0.168	4.638	0.097	0.244	0.192	0.2	0.443	0.192	0.283	0.957	3.969	0.116
4-(2,4-Dichlorophenoxy)butyric acid	NA	0.007	0.439	0.394	0.438	0.086	4.454	0.413	0.249	0.23	0.351	0.264	0.278	0.312	0.701	3.551	0.097
6-Desisopropylatrazine	NA	0.024	0.414	0.447	0.603	0.178	4.408	0.34	0.16	0.145	0.589	0.384	0.18	0.207	0.887	3.595	0.141
Abamectin	NA	0.167	0.388	0.45	NA	0.93	5.185	0.916	0.698	0.924	0.29	NA	NA	0.076	NA	NA	NA
Abamectin	NA	0.167	0.388	0.45	NA	0.93	5.185	0.916	0.698	0.924	0.29	NA	NA	0.076	NA	NA	NA
Acephate	NA	0.015	0.266	NA	0.626	0.129	4.444	0.585	0.187	0.216	0.546	0.17	0.203	0.143	0.648	3.23	0.124
Acetaminophen	NA	0.016	0.247	0.479	0.699	0.058	4.477	0.254	0.183	0.188	0.062	0.279	0.18	0.218	0.796	3.352	0.237
Acetamiprid	NA	0.011	0.6	0.34	0.545	0.162	4.326	0.415	0.169	0.143	0.431	0.29	0.159	0.276	0.797	3.572	0.208
Acetochlor	NA	0.013	0.327	0.54	0.403	0.162	4.511	0.456	0.233	0.296	0.538	0.177	0.142	0.31	0.528	3.831	0.153
Acetylsalicylic acid	NA	0.005	0.194	0.596	0.466	0.055	4.524	0.366	0.238	0.235	0.08	0.318	0.182	0.229	0.747	3.553	0.286
Acifluorfen	NA	NA	0.641	0.348	0.364	0.309	4.328	0.338	0.542	0.485	0.208	NA	0.37	0.711	0.169	NA	NA
Acrylamide	NA	NA	0.331	NA	0.913	0.268	4.639	0.298	0.162	0.126	0.22	0.206	NA	0.096	0.885	3.132	0.138
Aflatoxin	NA	0.012	0.537	0.626	0.468	0.468	4.565	0.783	0.386	0.23	0.179	0.509	0.144	0.224	0.49	NA	0.082
Alachlor	NA	0.012	0.326	0.537	0.413	0.19	4.522	0.451	0.211	0.236	0.538	0.179	0.137	0.299	0.574	3.872	0.143
Aldicarb	NA	0.02	0.27	0.51	0.495	0.064	4.463	0.553	0.178	0.239	0.569	0.156	NA	0.173	0.587	3.597	0.157

Recursive Partitioning Tree for Residuals

- The higher the C_{ss}, the lower the oral equivalent dose
- Ideally the residuals (difference between the literature value and the prediction) are small or $R \equiv C_{ss}^{lit.}/C_{ss}^{pred.} \approx 1$
- If a residual is large, we would prefer to over-predict C_{ss} to be conservative, *i.e.* $R < 1$

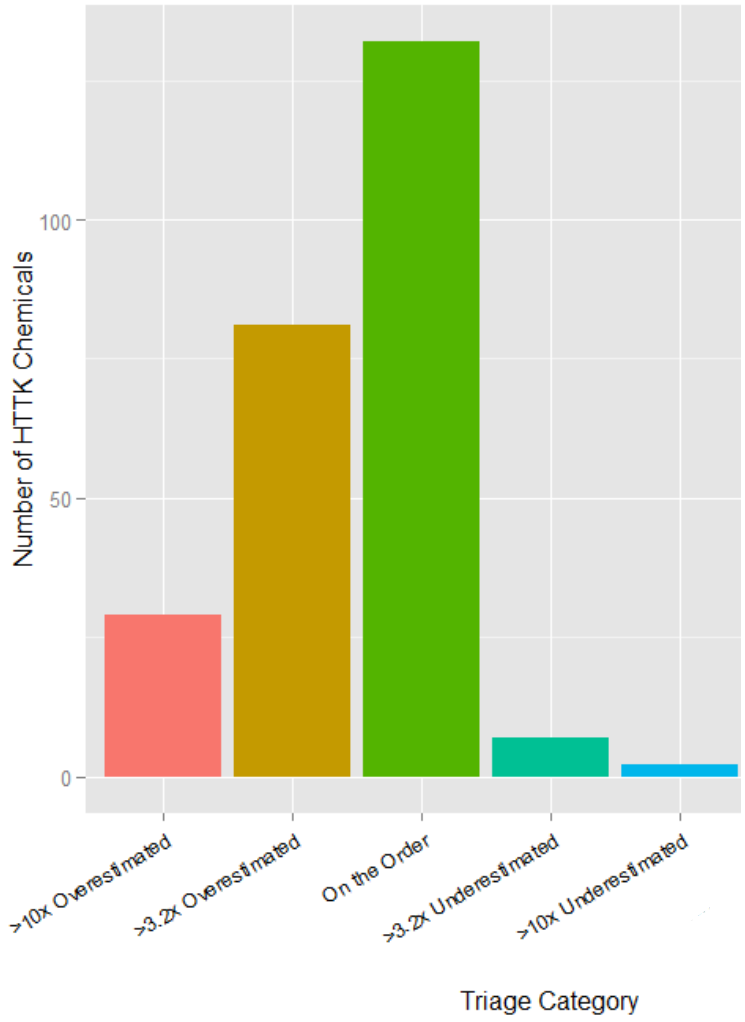


Recursive Partitioning Tree for



- Regression tree predicts expect
  residual based on physico-
  chemical properties, transporte
  QSARs, and *in vitro* HTTK data
- We can use this predicted error
  as a chemical-specific estimate
  of the accuracy of HTTK predict
- If the predicted C_{ss} underestima
  value, the necessary exposure p
  will be higher

Evaluation of HTTK Performance and Domain of Applicability



- Through comparison to *in vivo* data, a cross-validated predictor (random forest, using 50,000 classifier trees) of success or failure of HTTK has been constructed
- The largest single class of chemicals fall into the category of "On the order" (within approximately a factor of three)
- More likely to overestimate (conservative error) than underestimate plasma concentrations from an exposure

RTK Assumptions

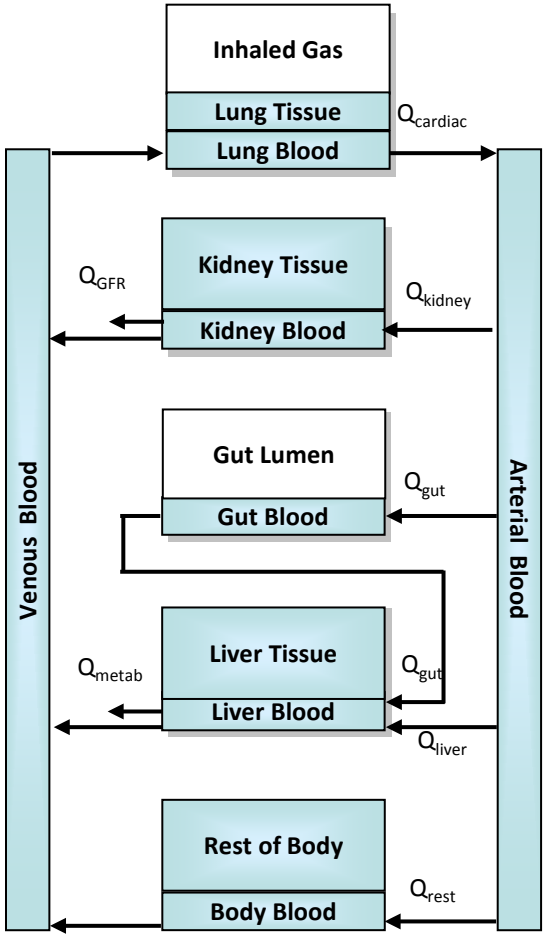
- To date, the TK models used for environmental chemicals have been relatively simple, making three key assumptions:
  1) Whole body is at the same concentration (*i.e.*, plasma)
  2) Environmental exposure is constant and uniform (*i.e.*, constant infusion)
  3) Enough time has passed that the plasma concentration is at steady-state with respect to the environment
- QSARs for tissue-specific properties address the first
- We can test the second two assumptions using dynamic simulation (*e.g.*, more realistic, sporadic dosing) of physiologically-based toxicokinetic (PBTK) models

High Throughput Physiologically-based Toxicokinetic Models (HTPBTK)

- PBTK models typically require more data than simpler models like we used for C_{ss}, but we can build generic, high throughput PBTK (HTPBTK) models parameterized with:
  - the same *in vitro* HTTK data used for RTK, **plus**
  - QSARs for tissue-specific properties
  - Assumptions about unknown dynamic processes, such as absorption

- We use these HTPBTK models perform both simulation experiments and compare model predictions from *in vitro* data with human and rat *in vivo* measurements

Physiologically-based Toxicokinetic (PBTK) Model



- Some tissues (*e.g.*, arterial blood) are simple compartments, while others (*e.g.*, kidney) are compound compartments consisting of separate blood and tissue sections.
- 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.
  - Partition coefficients from Schmitt (2008a and b)
  - Describe a specific species using chemical-independent physiology (Davies and Morris, 1993)
- Chemical enters the body primarily through oral absorption, but we don't know absorption rate and bioavailability (assume "fast", *i.e.* 1/h and 100%)
- The only way chemicals "leave" the body are through metabolism (change into a metabolite) in the liver or excretion by glomerular filtration into the proximal tubules of the kidney and out of the body

Evaluating RTK Assumptions

- Can use HTPBTK simulations to evaluate appropriateness of RTK assumptions
- Can use HTPBTK to predict traditional TK metrics, such as peak concentration (C_{max}) and time integrated area under the curve (AUC) for various tissues
- Below we show approach to “steady-state” due to three simulated daily doses

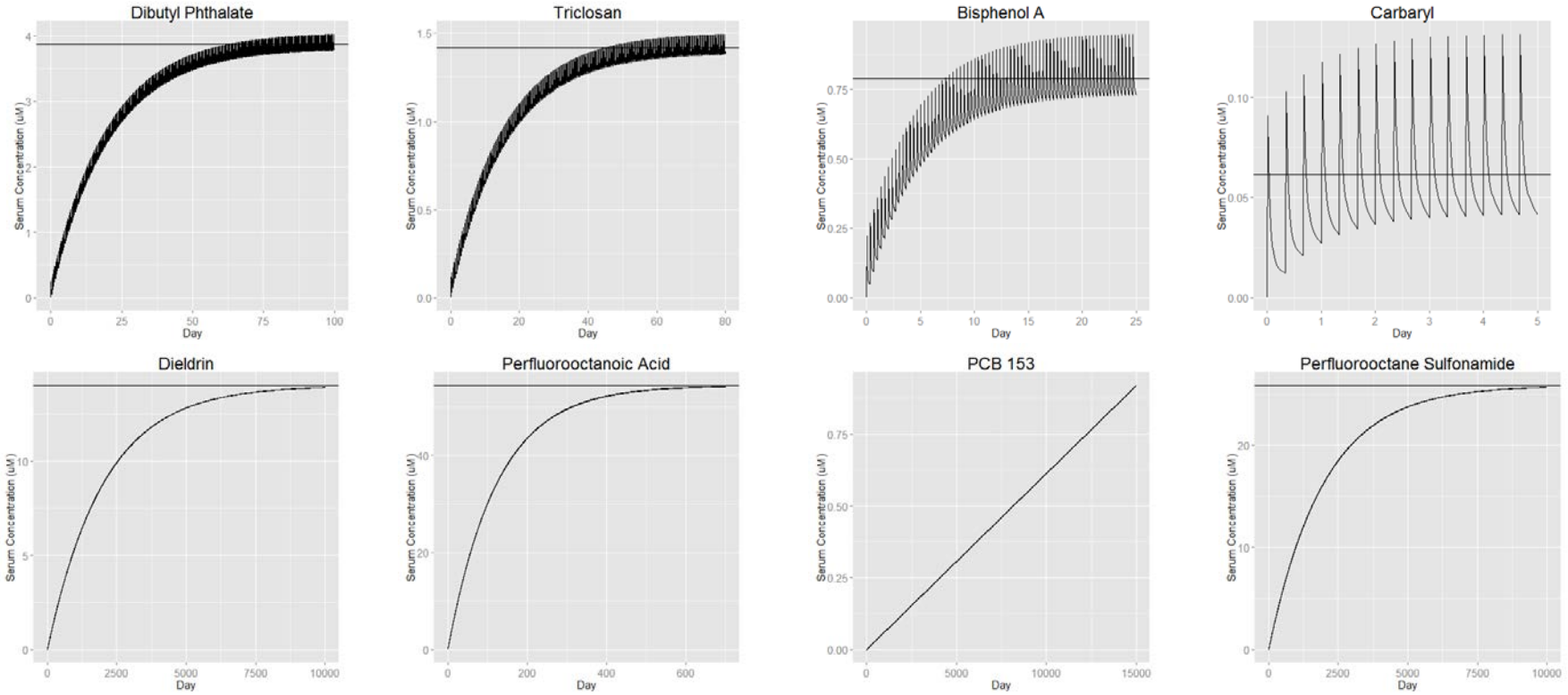
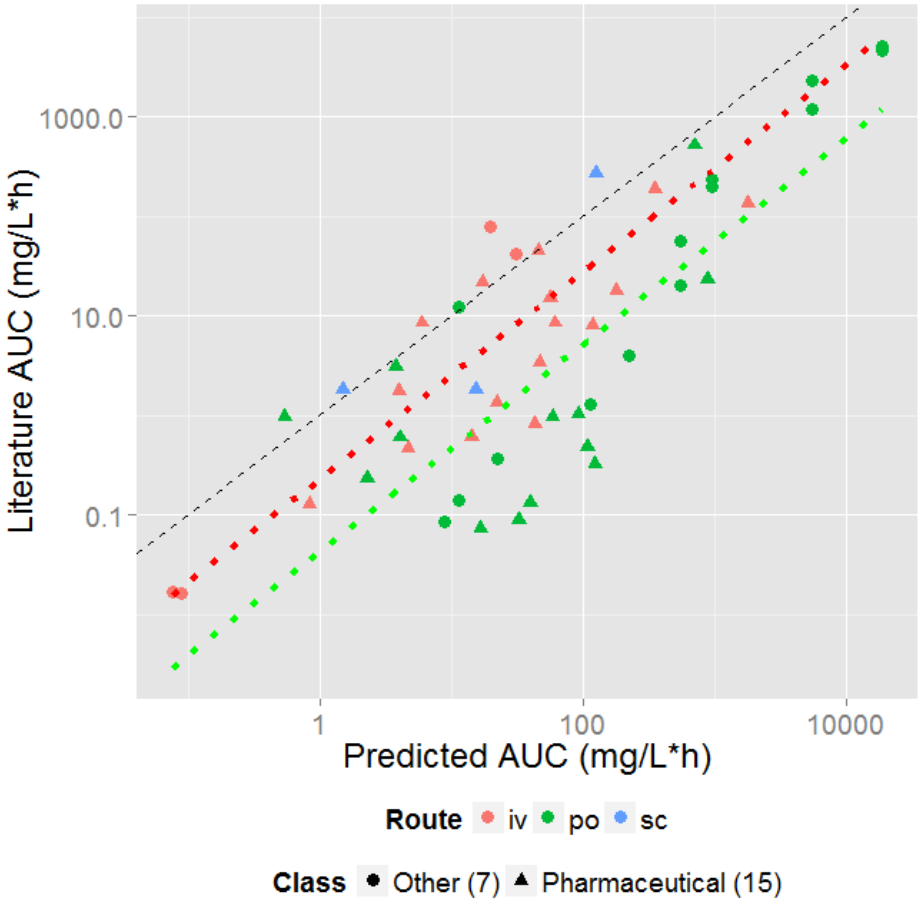


Figure from Robert Pearce

Evaluation Data for HTTK

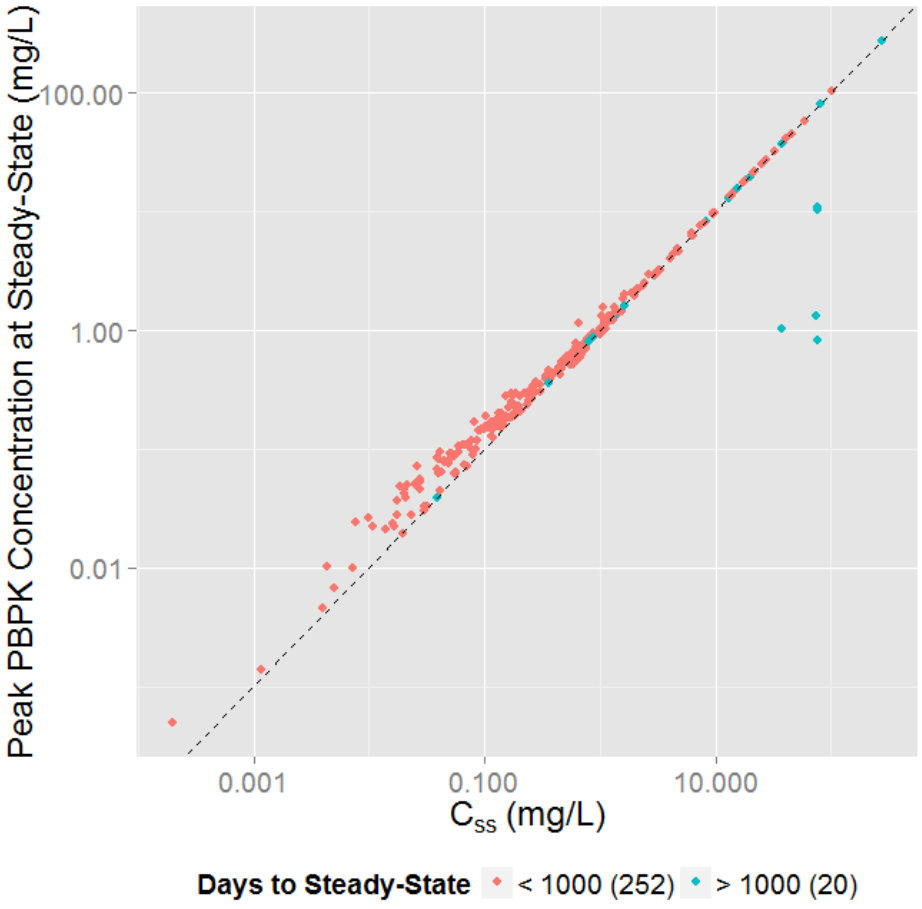
- For most non-therapeutic compounds, it is unlikely that there will ever be controlled human TK data
- Though some *in vivo* TK data exists to allow statistical assessment of HTTK predictions, these data are predominantly for pharmaceutical compounds
- Wetmore *et al.*, (2013): Rat HTTK data for 53 chemicals
- Compile and collect *in vivo* TK data for some or all of the rat HTTK compounds:
  - Allows evaluation of predictions based on *in vitro* and QSAR (*e.g.*, clearance and volume of distribution)
  - Allows measurement of other key processes (*e.g.*, absorption rates, extra-hepatic metabolism).

Evaluating HTPBTK Predictions from *In Vitro* Data



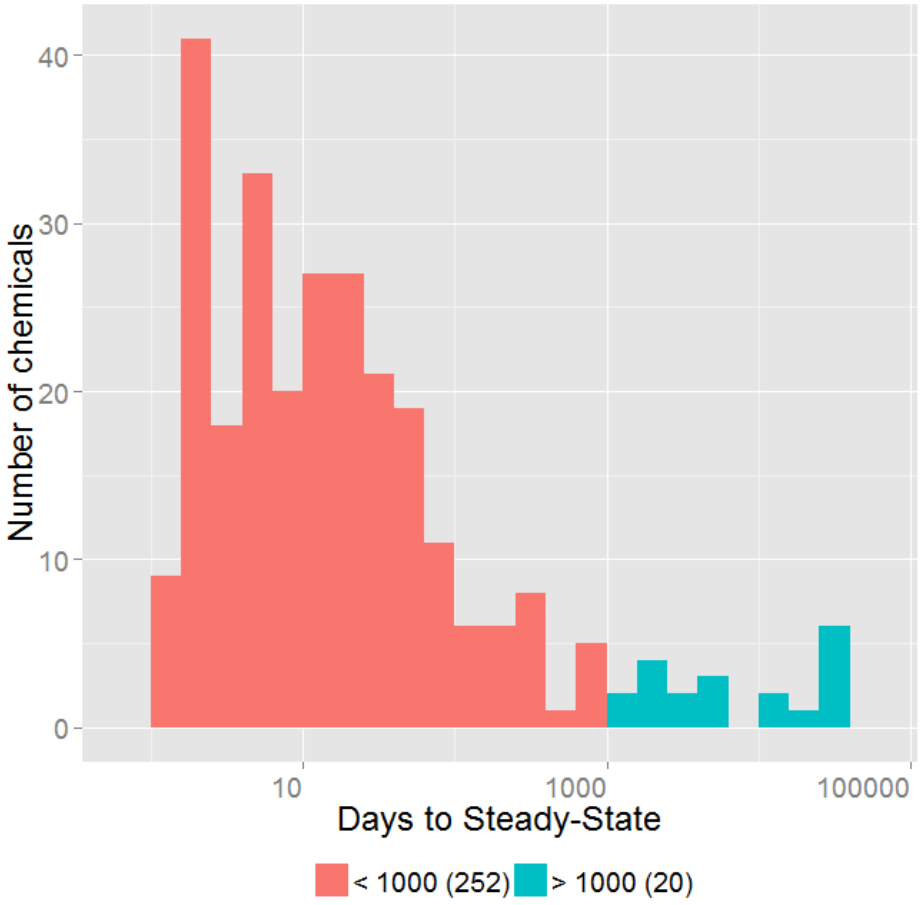
- HTPBTK predictions for the AUC (time integrated plasma concentration or Area Under the Curve)
- *in vivo* measurements from the literature for various treatments (dose and route) of rat (R^2 ~ 0.69)
- Predictions are generally conservative – *i.e.*, predicted AUC higher than measured
- Oral dose AUC ~5.4x higher than intravenous dose AUC

Peak Concentration vs. C_{ss}



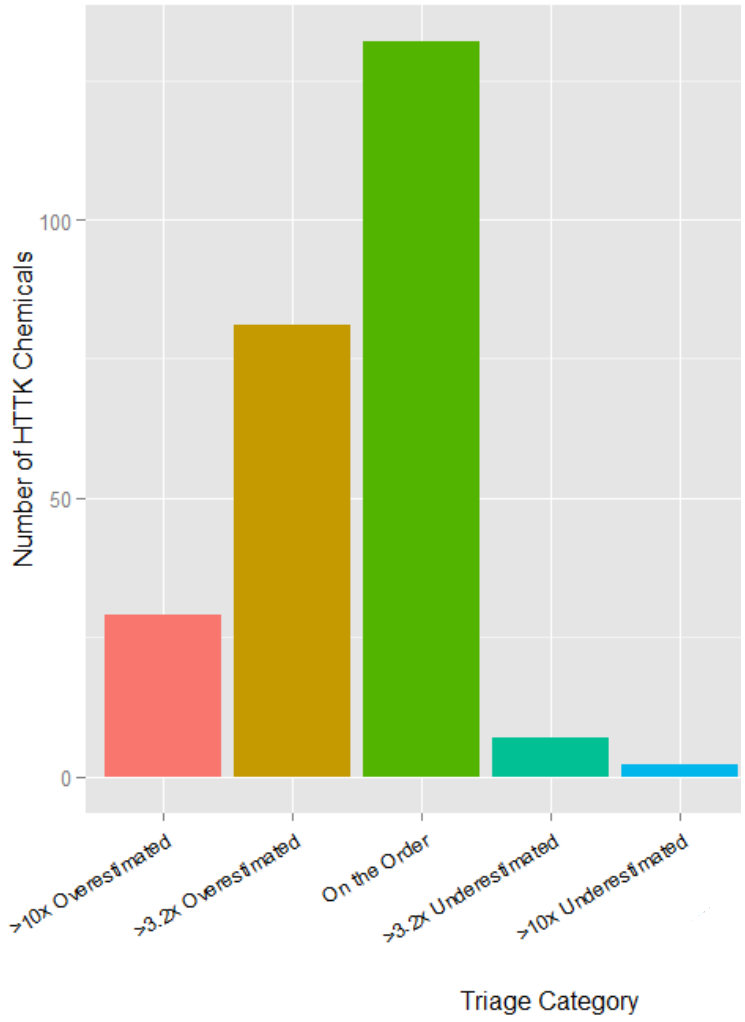
- Peak serum concentrations from the HTPBTK model are compared against the steady-state concentration predicted by the three compartment model for a constant infusion exposure (as in Wetmore *et al.* 2012)
- The dashed, identity (1:1) line indicates that for most compounds the peak concentrations are very similar to C_{ss}

Evaluation of Steady-State Predictions



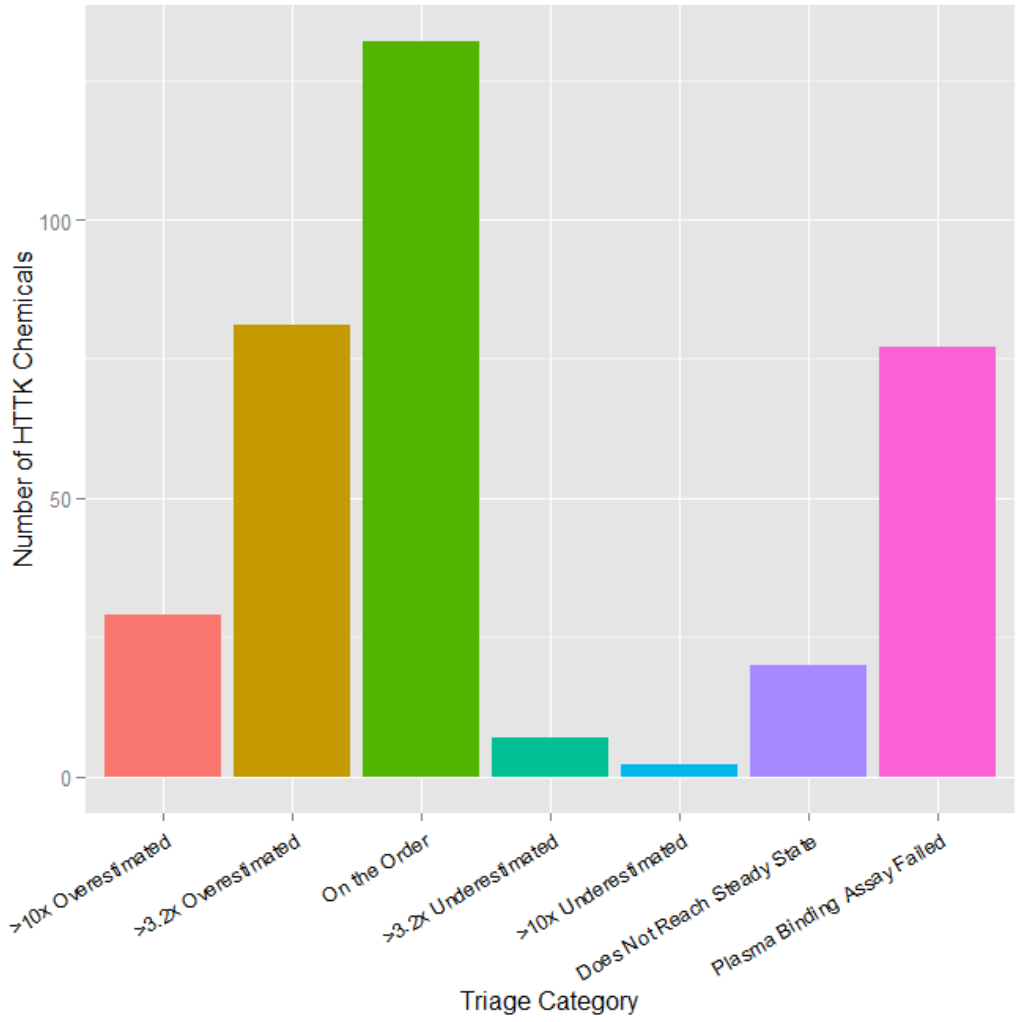
- Using HTPBTK and assuming three daily doses (every eight hours)
- This allows us to evaluate the plausibility of the steady-state dosing assumption.
- We find that the majority of chemicals reach steady state in a few weeks
- A second population of chemicals never reach steady state.

Evaluation of HTTK Performance and Domain of Applicability



- Through comparison to *in vivo* data, a cross-validated (random forest) predictor of success or failure of HTTK has been constructed

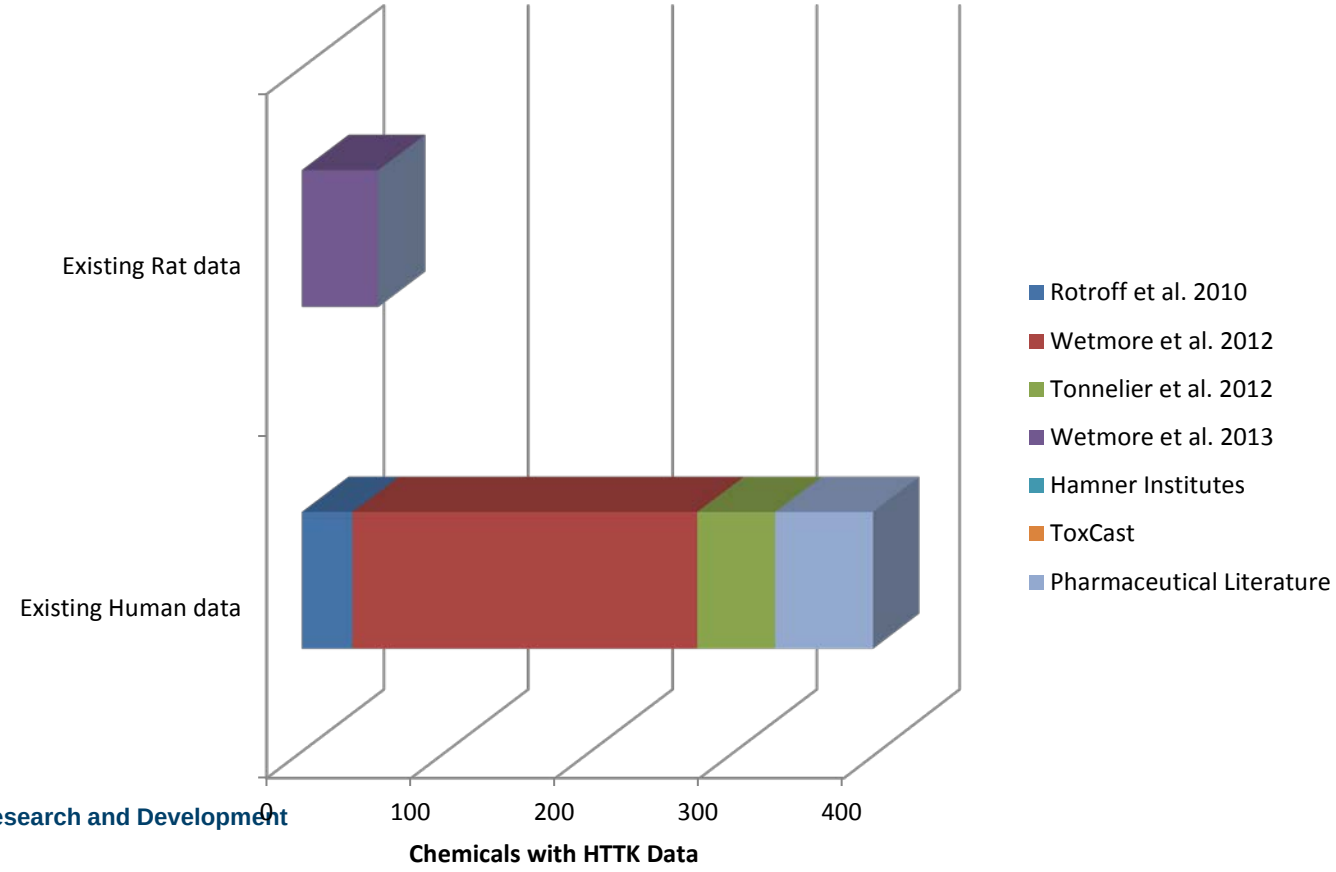
Evaluation of HTTK Performance and Domain of Applicability



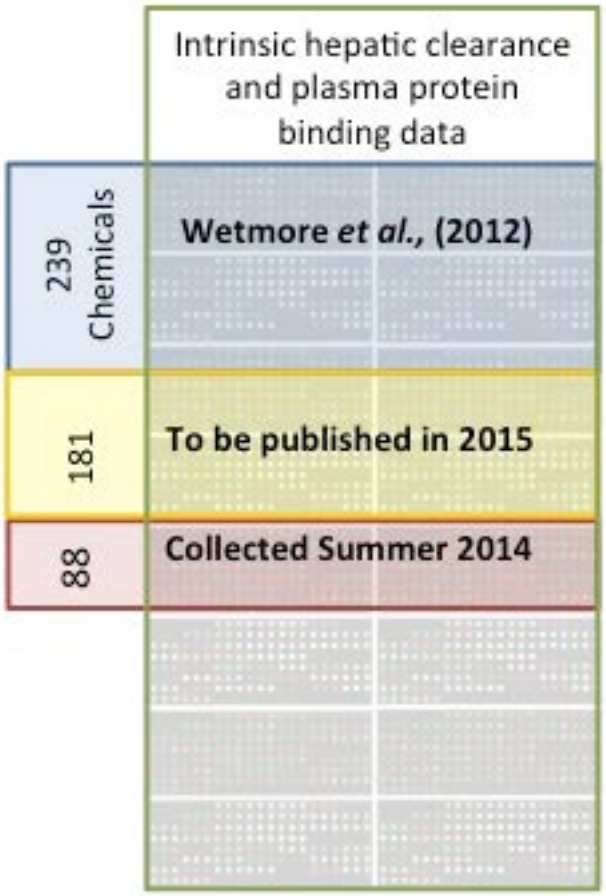
- Through comparison to *in vivo* data, a cross-validated (random forest) predictor of success or failure of HTTK has been constructed
- Add categories for chemicals that do not reach steady-state or for which plasma binding assay fails

Chemicals with HTTK Data

- *In vitro* assays limited by time needed to develop chemical-specific analytical chemistry method

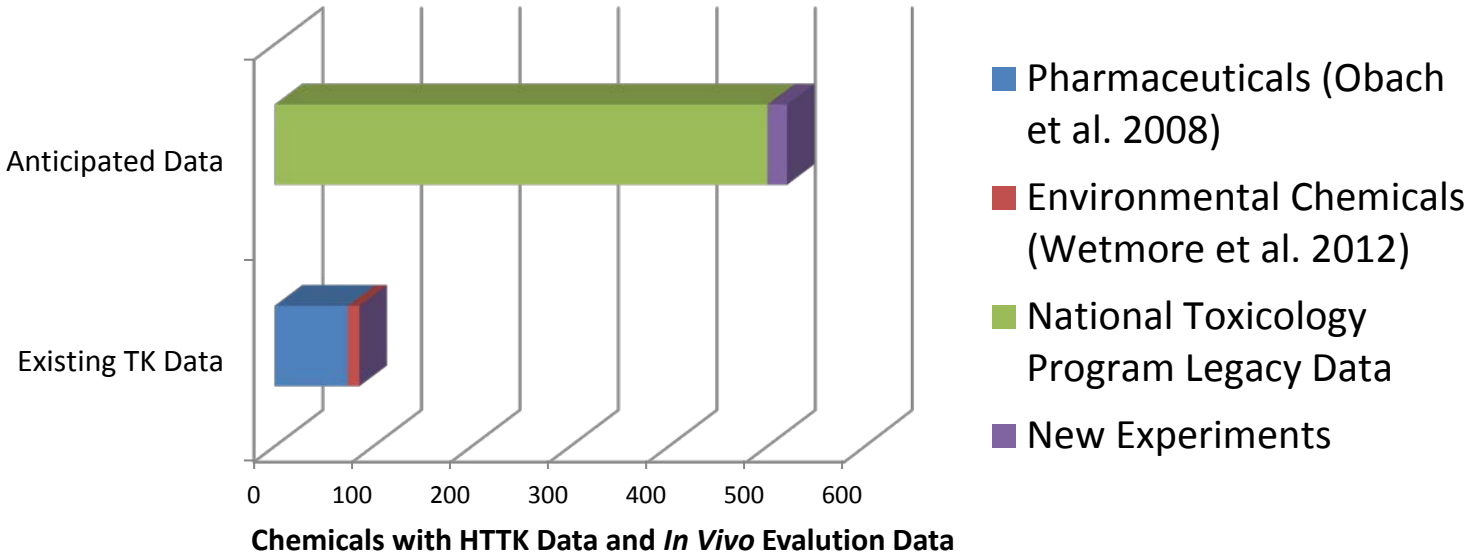


New Data for HTTK



- ToxCast HTTK testing:
  - Measuring metabolism by human hepatocytes
  - Improved assays for measuring binding of chemicals to human plasma protein
  - Obtain data on ToxCast chemicals not investigated by the Hamner Institute studies
  - Reinvestigate chemicals that proved difficult in previous efforts
- This data will eventually allow determination of human oral equivalent doses (mg/kg BW/day) for most ToxCast chemicals.

New Data for Evaluating HTTK Predictions



- Goal of HTTK modeling of pharmaceuticals is to determine efficacious doses for follow-on human clinical trials, the degree of confidence needed is different
- For environmental compounds, their uncertainty must be well characterized ideally with rigorous statistical methodology

HTTK Future Directions

- Working to make all data and models available as R package ("httk")
- Collecting new HTTK data on chemicals using improved (serial dilution of plasma) methodology for measuring plasma binding
- Current MC simulations focus on adults
  - Expanding populations variability analysis to include sensitive population groups to reflect NHANES and beyond (*e.g.*, children <6yo)
- Current *in vivo* data for evaluation of models is from heterogeneous studies. Ongoing efforts to
  - Collect data from limited i*n vivo* studies (EPA/NHEERL and Research Triangle Institute)
  - Organize data from larger, systematic studies (*e.g.*, National Toxicology Program) into computable format
  - Improved evaluation data will allow better assessment of predictive ability and determination of domain of applicability

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

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:**
  - Must consider domain of applicability
  - Chemical-specific analytical chemistry methods make HTTK slower than bioactivity HTS or HTE
- Although we used MC simulation to characterize some aspects of human variability (*e.g.,* body weight of adults), any key determinants of variability that are not included in our simulation have not been be assessed
- **We must carefully characterize the uncertainty in our approach**

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EPA United States Environmental Protection Agency

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