Toxicokinetics in the high throughput arena

John Wambaugh
National Center for Computational Toxicology
Office of Research and Development
U.S. Environmental Protection agency
wambaugh.john@epa.gov

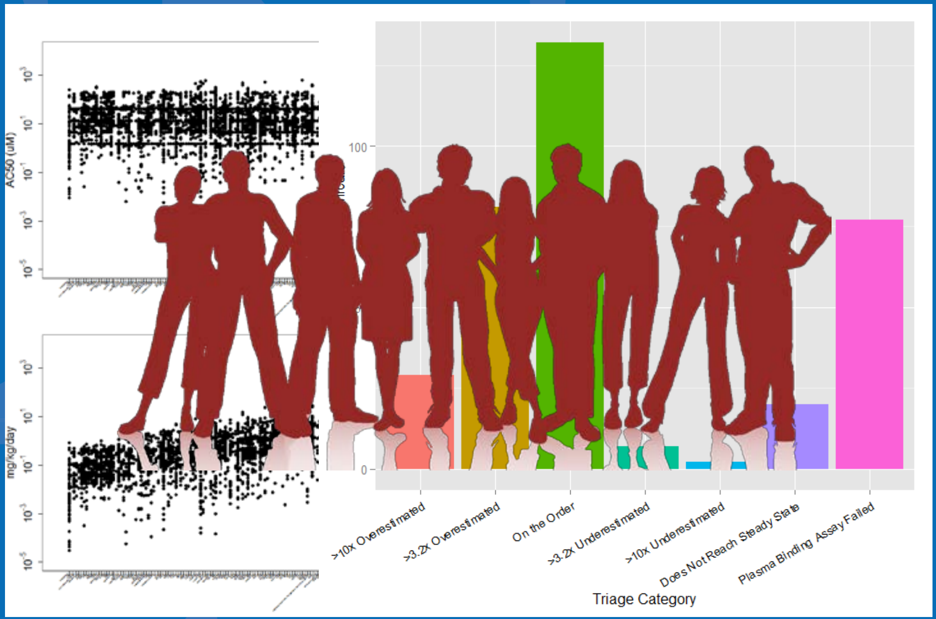

Figure includes image from Thinkstock

"From Cell Cultures to Humans: Modeling Kinetics – Understanding Human Relevance"

CropLifeAmerica & RISE
Science Speaks
Spring Conference
Arlington, VA
April 24, 2015

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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)

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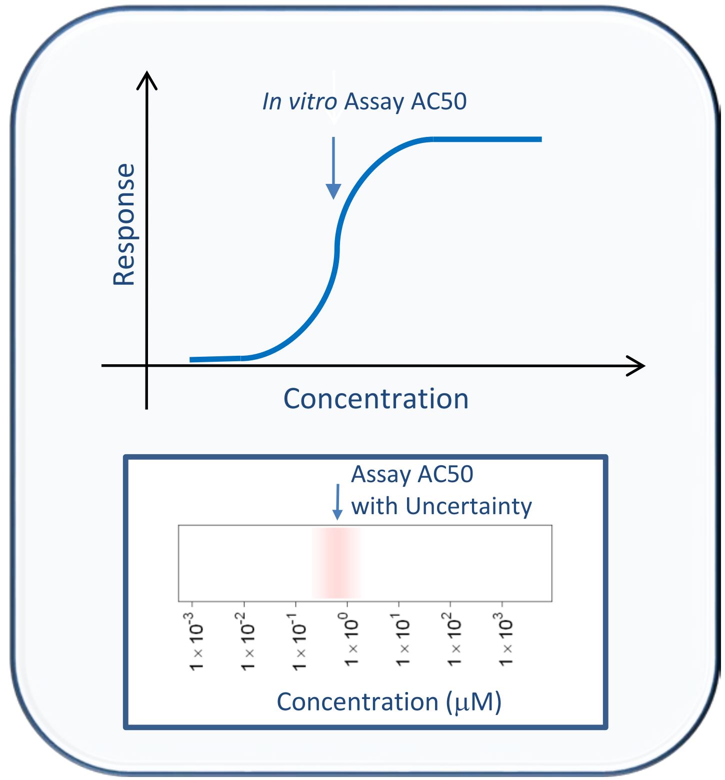
High-Throughput Bioactivity

- **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

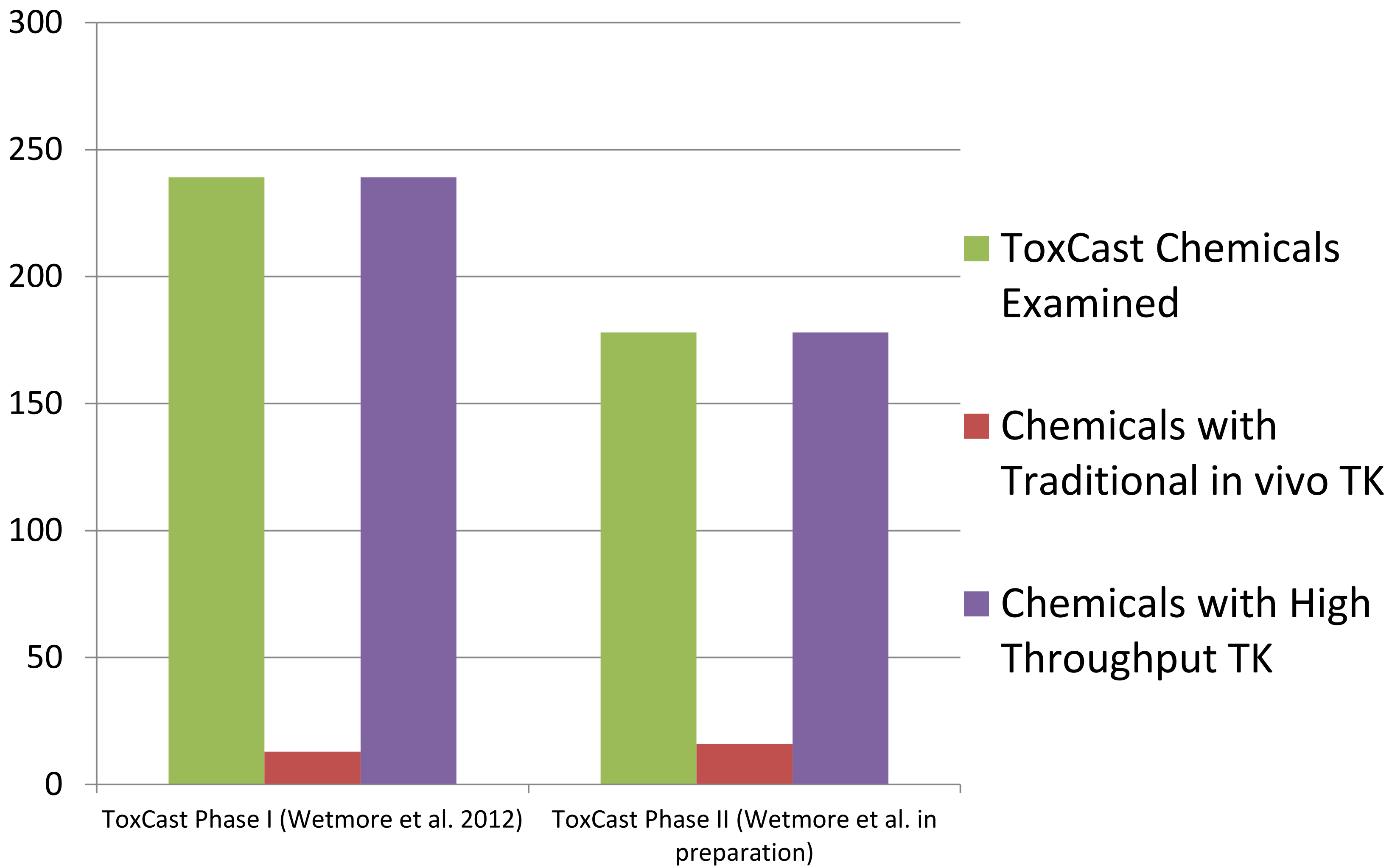
Comparison of HTTK predicted oral equivalent doses (box and whisker plots in mg/kg/day) with doses for no effect and low effect groups in animal studies

- **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.

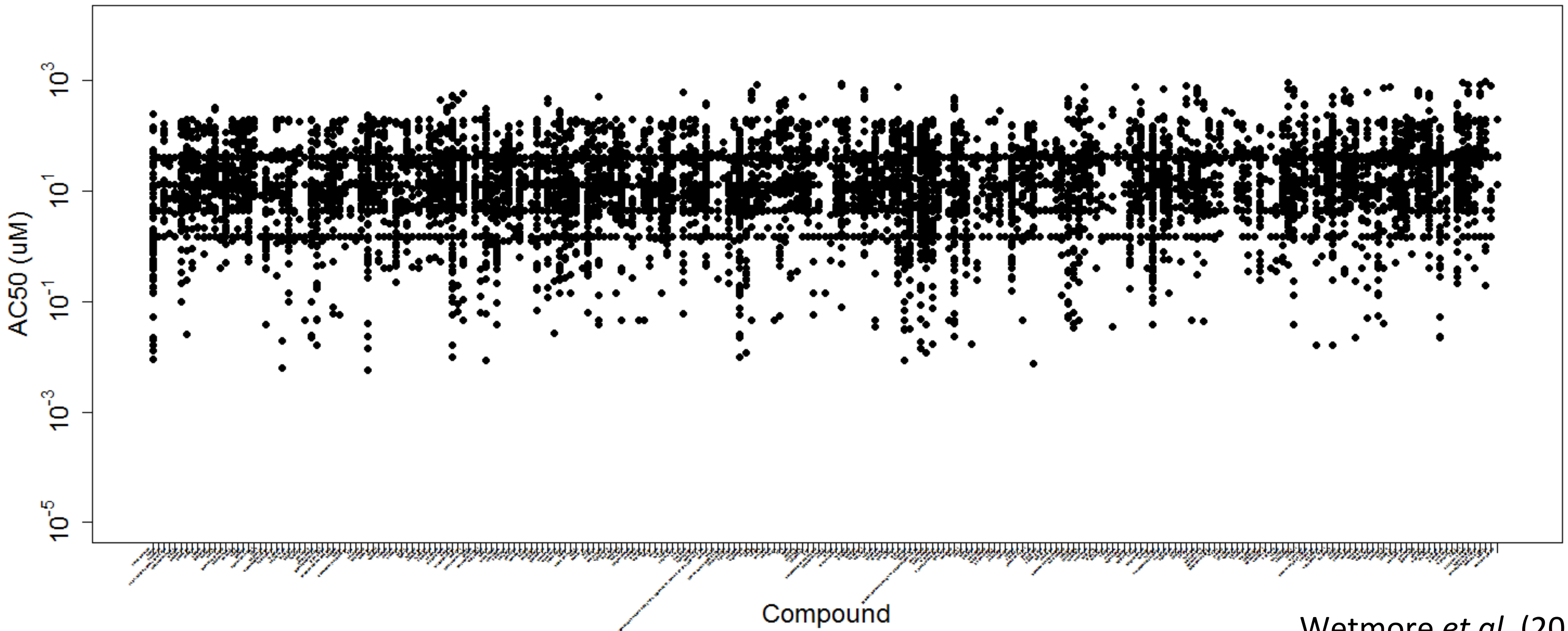
Judson *et al*. (2011)

The Need for *In Vitro* Toxicokinetics



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

ToxCast *in vitro* Bioactive Concentrations

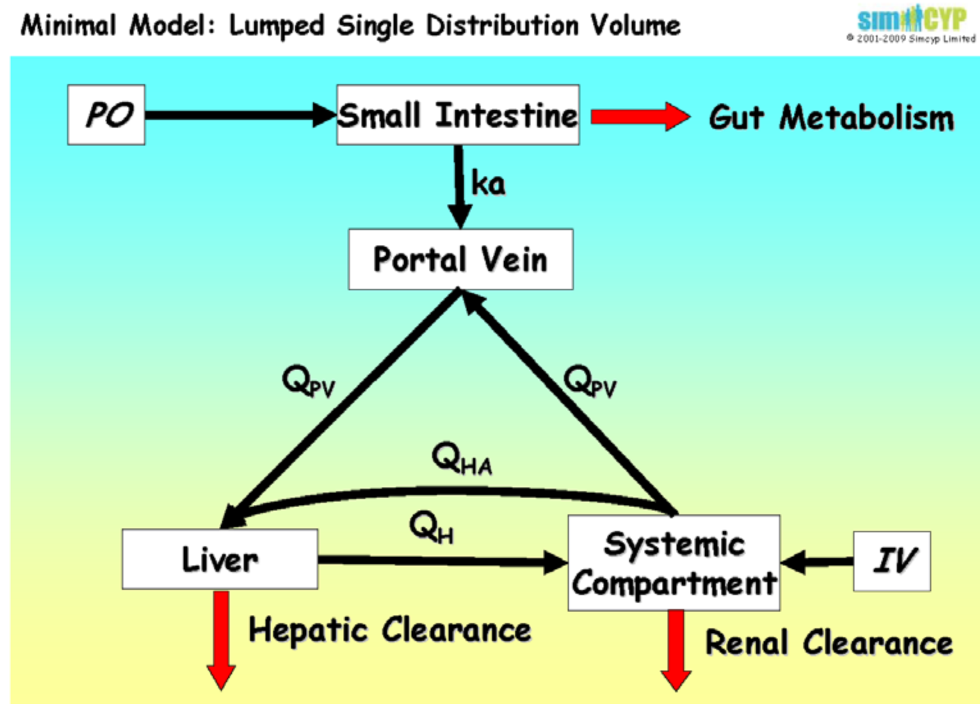


Wetmore *et al.* (2012)

- 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?

High Throughput Toxicokinetics (HTTK)

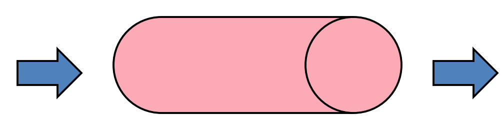
Jamei *et al*. (2009)



- *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

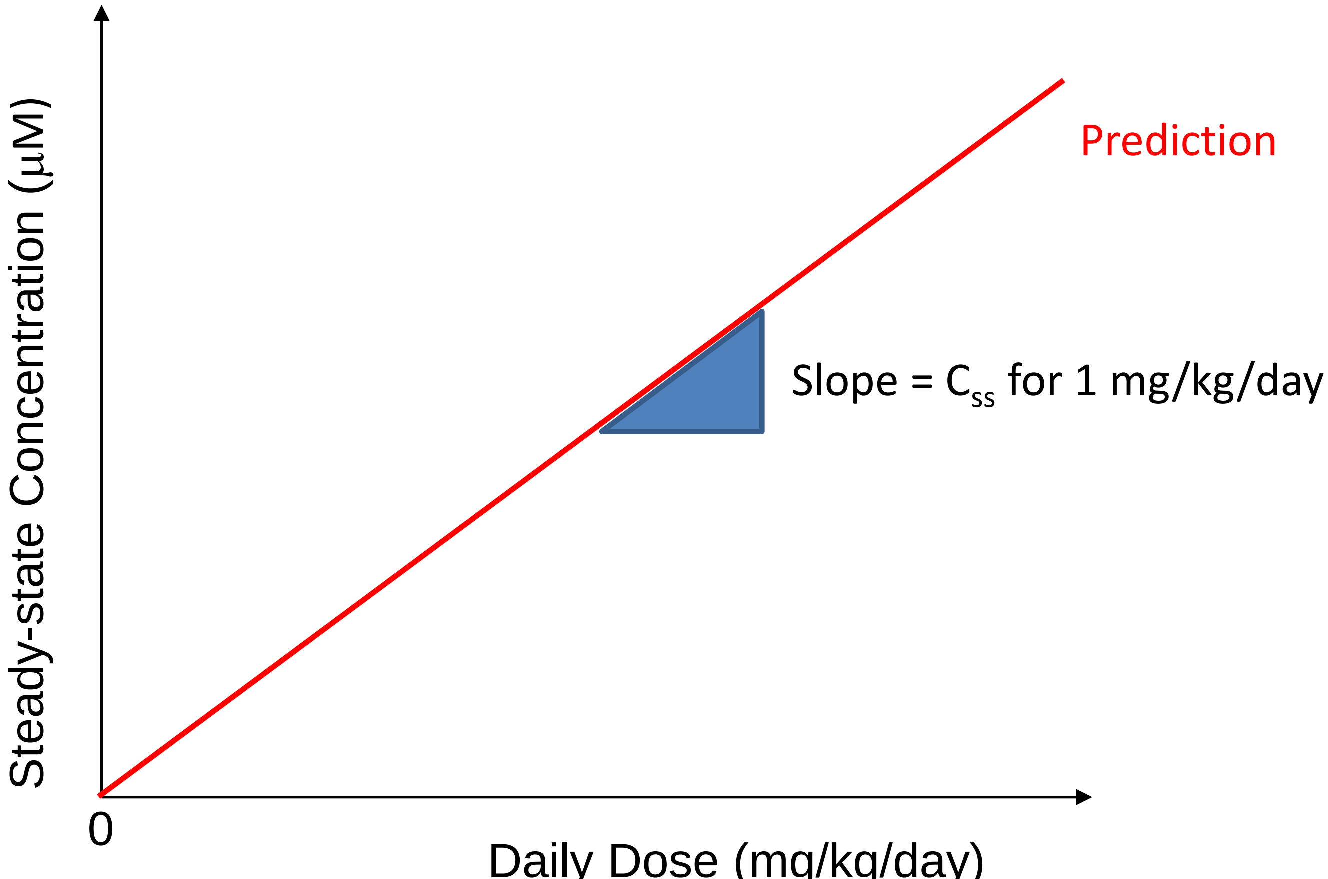
$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{\text{ub}}) + \left(\text{Q}_{\text{l}} * \text{F}_{\text{ub}} * \frac{Cl_{\text{int}}}{\text{Q}_{\text{l}} + \text{F}_{\text{ub}} * Cl_{\text{int}}}\right)}$$

Oral dose in (mg/kg/day)



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

Steady-State is Linear with Dose

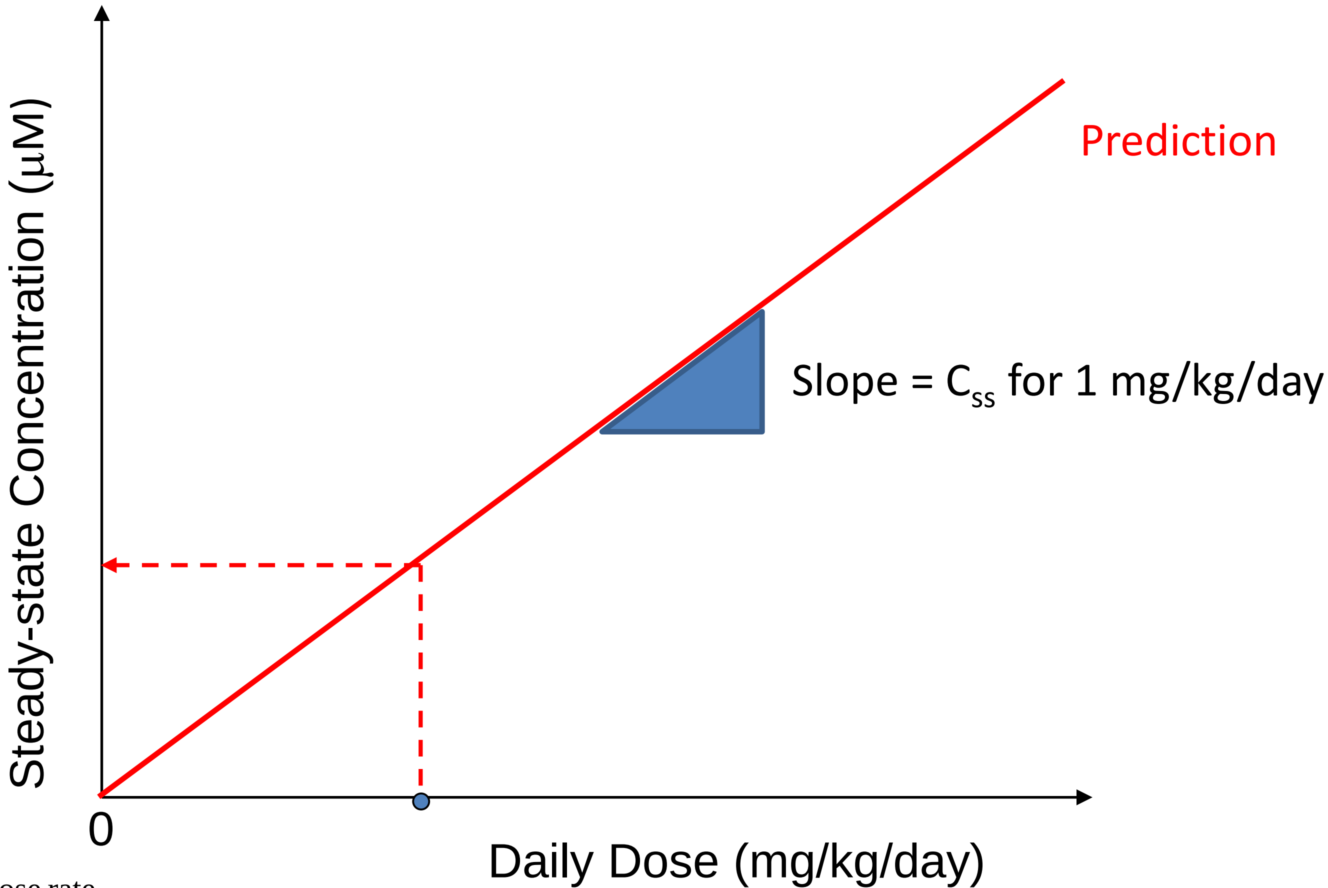


$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{\text{ub}}) + \left(\text{Q}_{\text{l}} * \text{F}_{\text{ub}} * \frac{Cl_{\text{int}}}{\text{Q}_{\text{l}} + \text{F}_{\text{ub}} * Cl_{\text{int}}}\right)}$$

Wetmore *et al*. (2012)

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

Steady-State is Linear with Dose

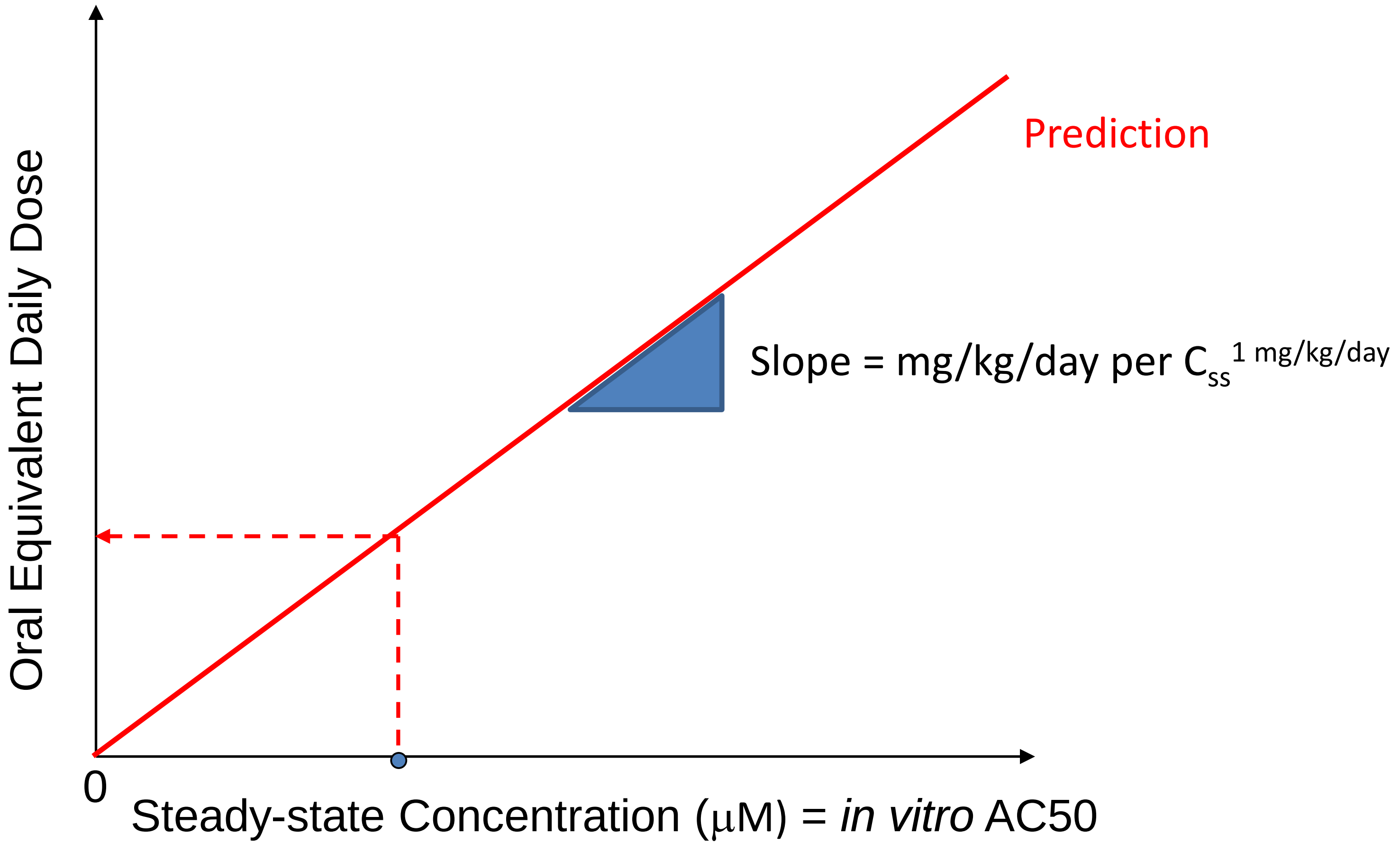


$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{\text{ub}}) + \left(\text{Q}_{\text{l}} * \text{F}_{\text{ub}} * \frac{Cl_{\text{int}}}{\text{Q}_{\text{l}} + \text{F}_{\text{ub}} * Cl_{\text{int}}}\right)}$$

- 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)

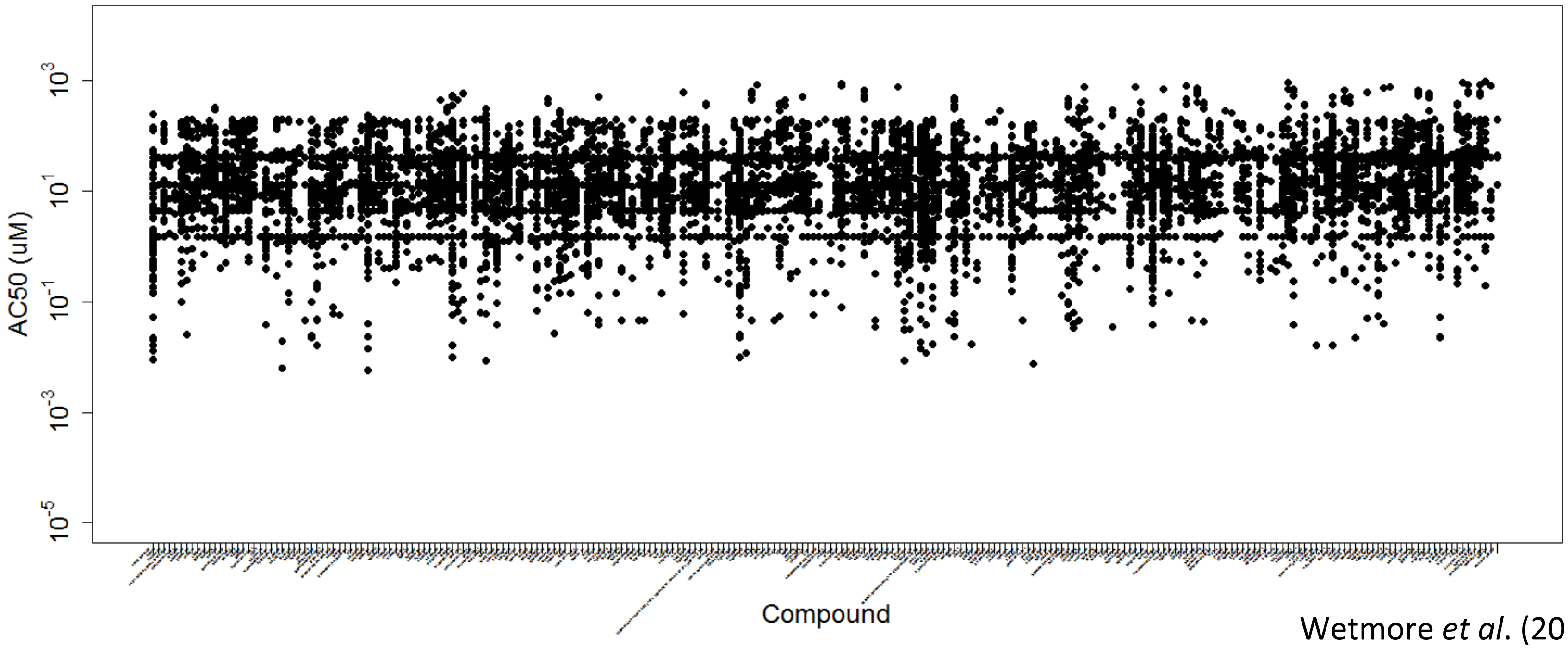
HTTK Allows Steady-State *In Vitro*-*In Vivo* Extrapolation (IVIVE)



- 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

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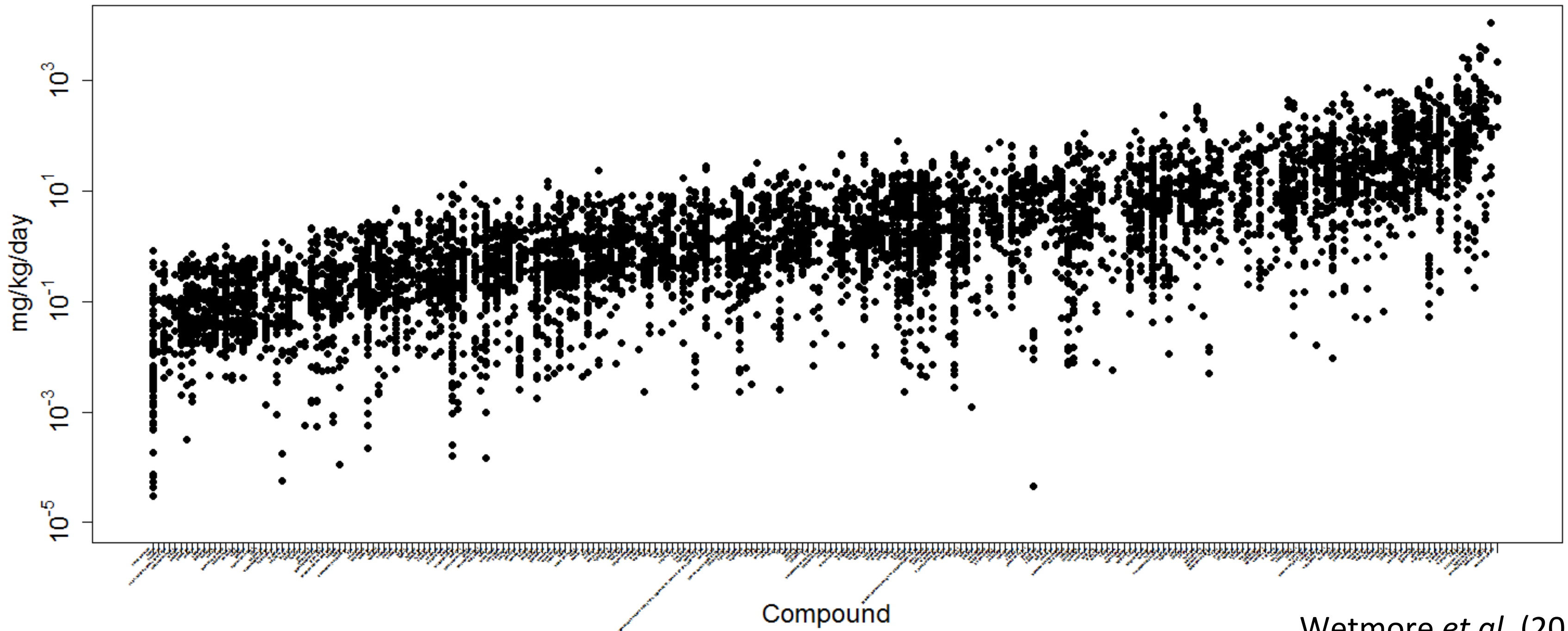
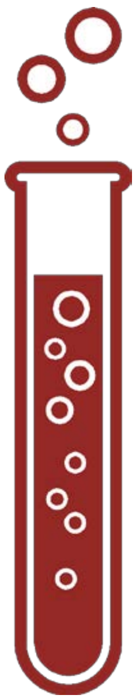
HTTK Oral Equivalents



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

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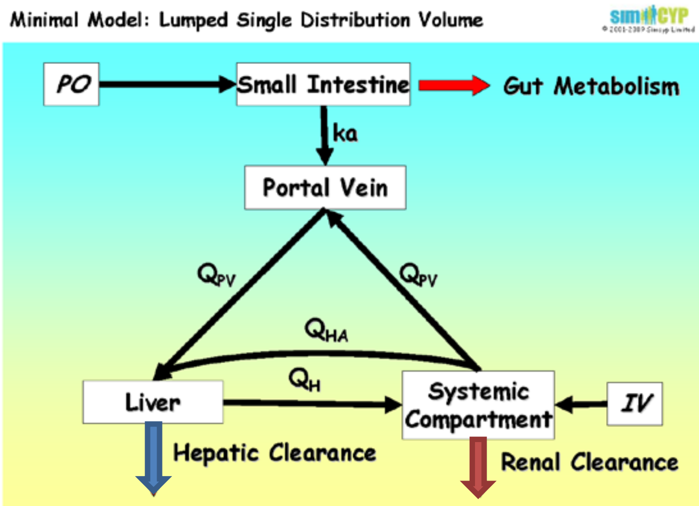
Variability in this Steady-State TK Model

Jamei *et al*. (2009)



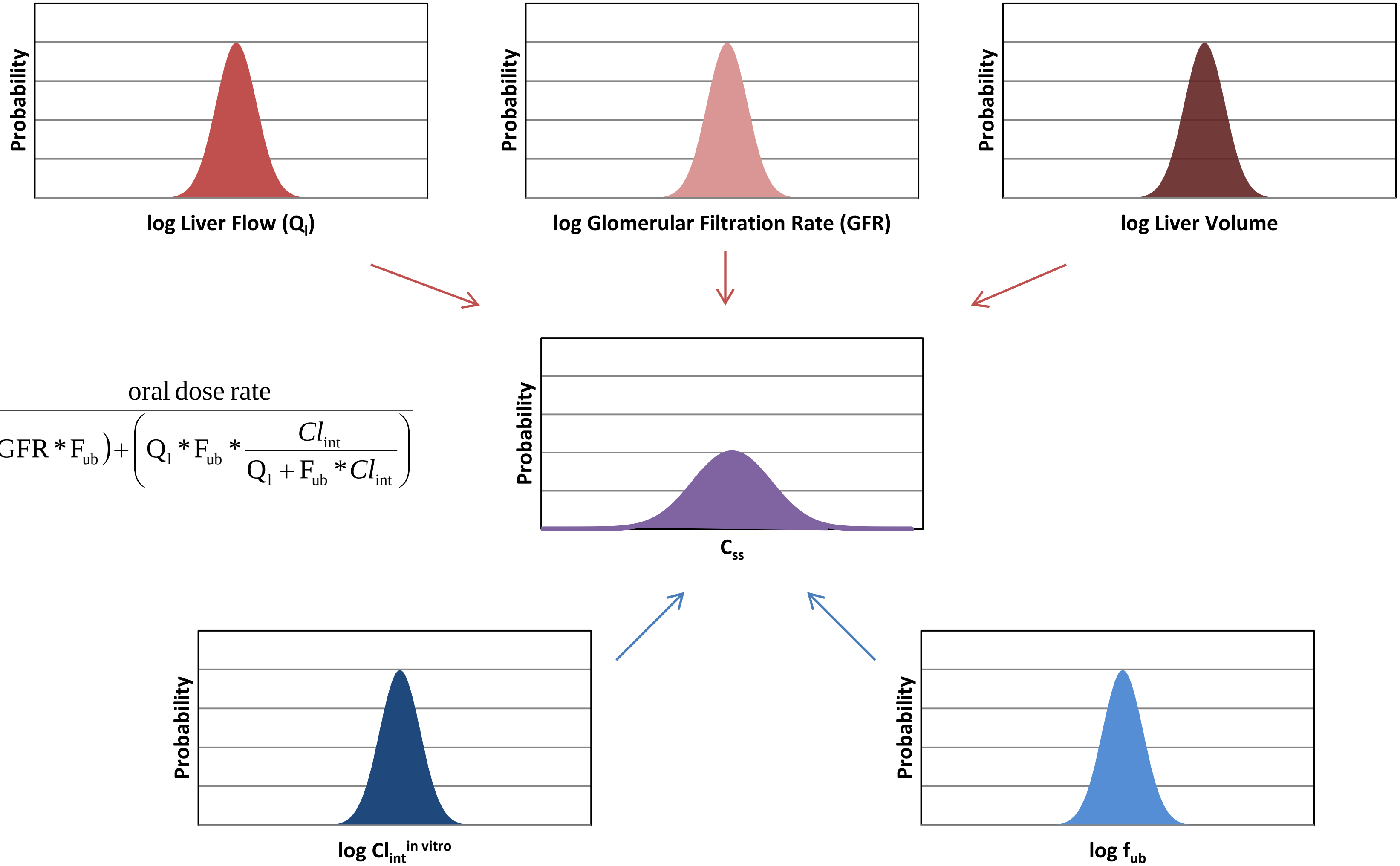
$$C_{ss} = \frac{\text{oral dose rate}}{\left(\mathrm{GFR} * \mathrm{F_{ub}}\right) + \left(\mathrm{Q_l} * \mathrm{F_{ub}} * \frac{Cl_{int}}{\mathrm{Q_l} + \mathrm{F_{ub}} * Cl_{int}}\right)}$$

(Passive) Renal Clearance — 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

$$C_{ss} = \frac{\text{oral dose rate}}{(GFR * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}$$



Wetmore *et al.* (2012)

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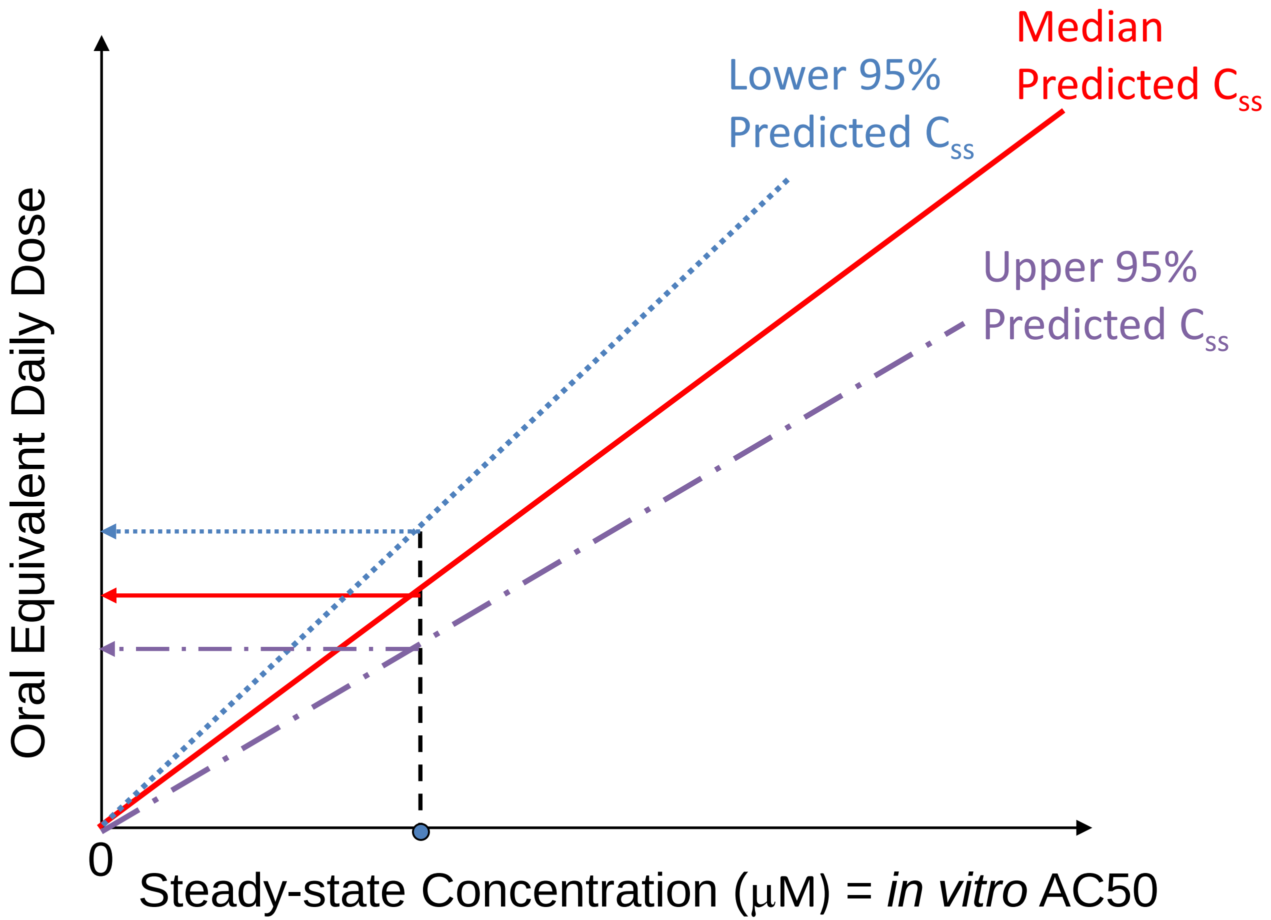
Steady-State In Vitro-In Vivo Extrapolation (IVIVE)



- 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

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Dosimetry and Exposure Provides Context for HTS

Endocrine disruption AOP *(Judson et al., in prep.)*

ToxCast Bioactivity Converted to mg/kg/day with HTTK (Wetmore et al., 2012)

ExpoCast Exposure Predictions (Wambaugh *et al.*, 2014)

ToxCast Chemicals

December, 2015 Panel:
“Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening“

DOCKET NUMBER: EPA–HQ–OPP–2014–0614

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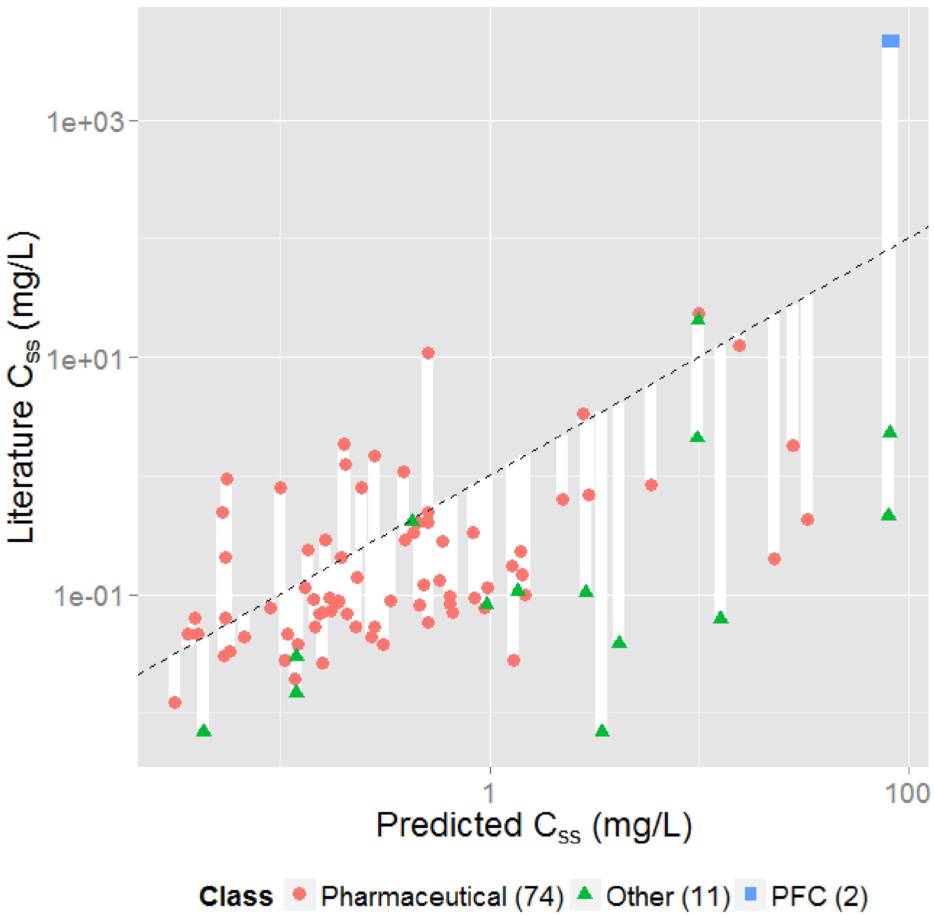
In vivo Predictive Ability and Domain of Applicability

- 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)

Wambaugh *et al.,* (submitted)

Predicting When RTK Will Work

- 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
- 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
    - *In vitro* HTTK data
    - Active chemical transport predictions

Wambaugh *et al.,* (submitted)

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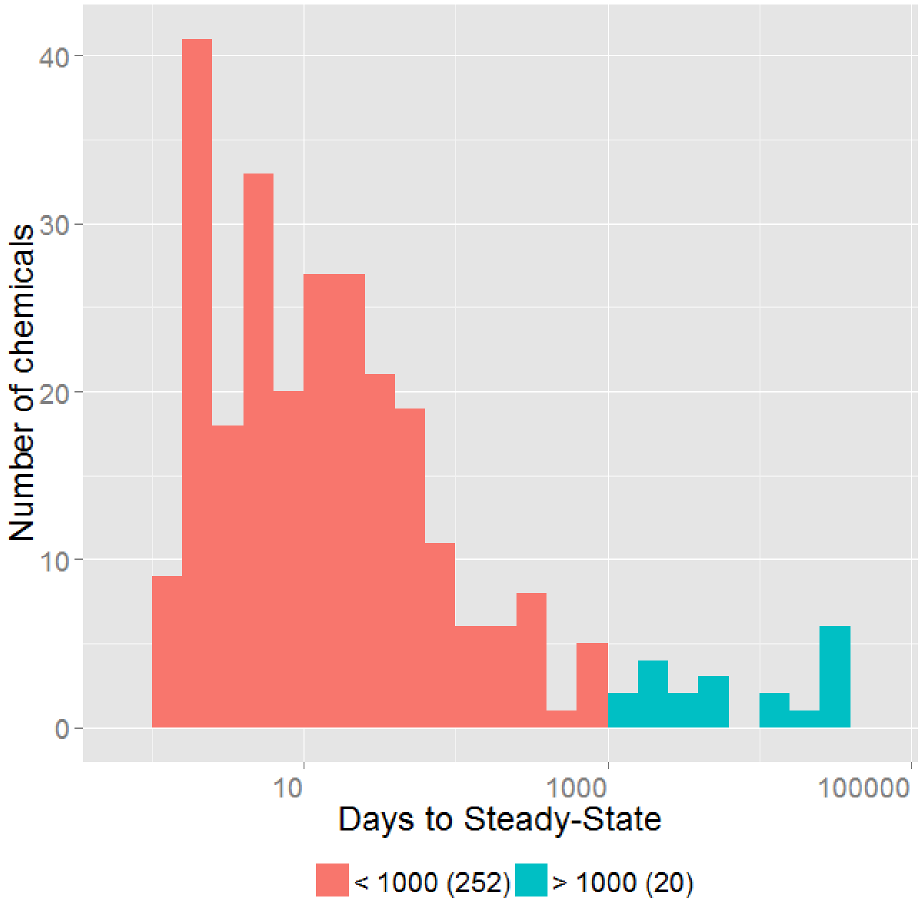
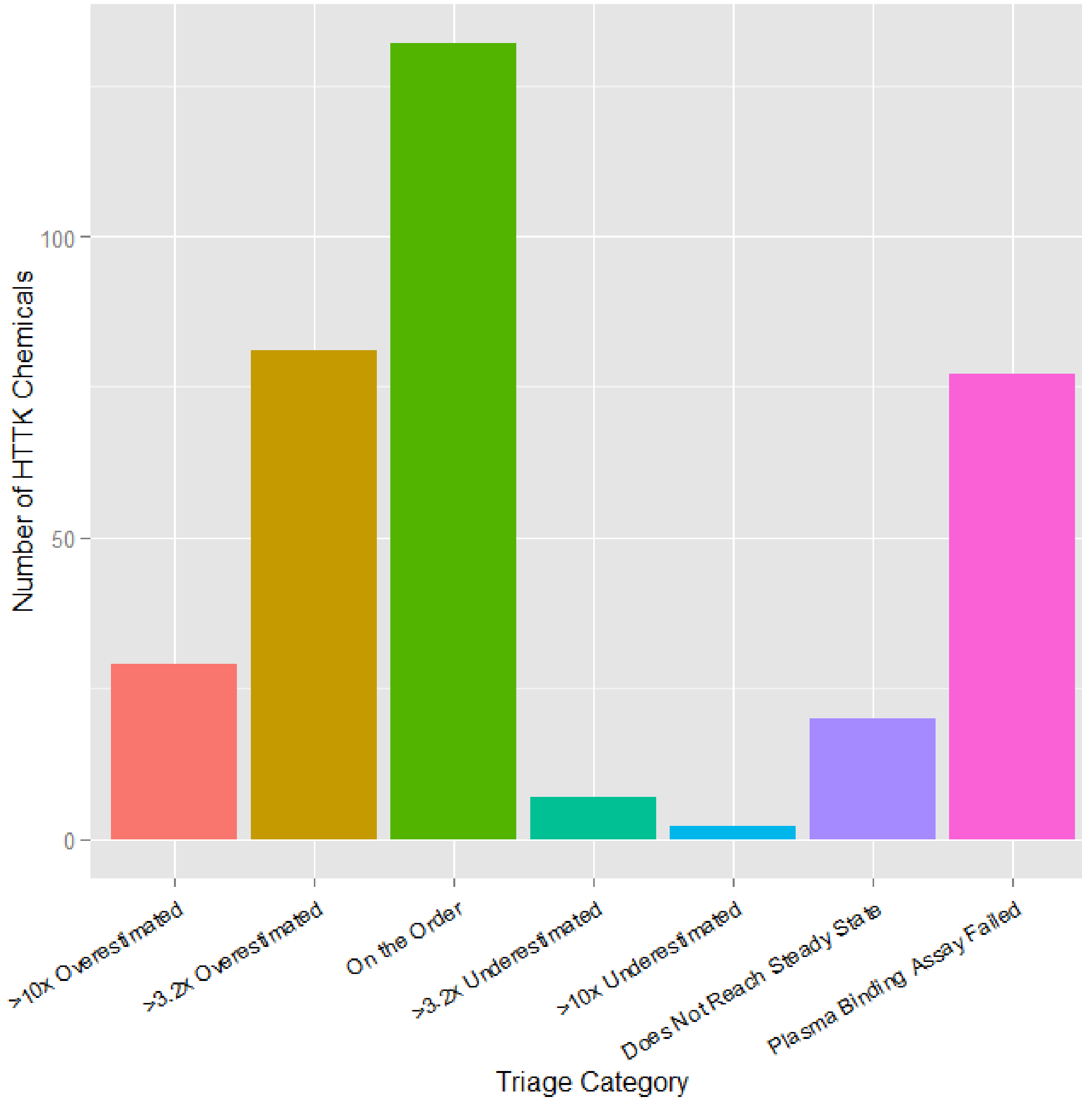
Evaluation of Steady-State Assumption



- 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.

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



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

Collaborators

Arnot Research and Consulting
Jon Arnot
Chemical Computing Group
Rocky Goldsmith
Environmental Protection Agency
Alicia Frame
Hamner Institutes
Barbara Wetmore
Cory Strope
Indiana University
James Sluka
Michigan State University
Jade Mitchell
National Institute for Environmental Health Sciences (NIEHS)
Mike Devito
Nisha Sipes
Kyla Taylor
Kristina Thayer
Netherlands Organisation for Applied Scientific Research (TNO)
Sieto Bosgra
North Carolina State University
Anran Wang
Research Triangle Institute
Timothy Fennell
Silent Spring Institute
Robin Dodson
University of California, Davis
Deborah Bennett
University of Michigan
Olivier Jolliet
University of North Carolina, Chapel Hill
Alexander Sedykh*
Alex Tropsha

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

NCCT
Chris Grulke
Richard Judson
Thomas Knudsen
Chantel Nicolas*
Robert Pearce*
James Rabinowitz
Caroline Ring*
Woody Setzer
Imran Shah
Rusty Thomas

NRMRL
Xiaoyu Liu

NHEERL
Jane Ellen Simmons
Marina Evans
Mike Hughes

***Trainees**

NERL
Craig Barber
Dalizza Colon
Kathie Dionisio*
Peter Egeghy
Kim Gaetz
Kristin Isaacs
Haluk Ozkaynak
Julia Rager*
Mark Strynar
Jon Sobus
Mike Tornero-Velez
Dan Vallero

High Throughput Toxicokinetics Researchers

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