

Higher Throughput Toxicokinetics to Allow Extrapolation

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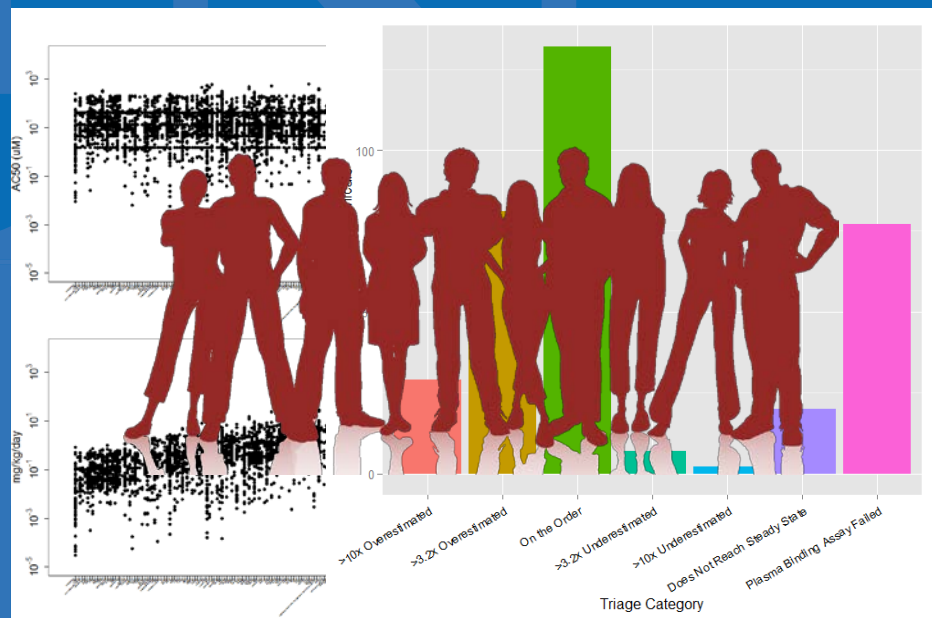
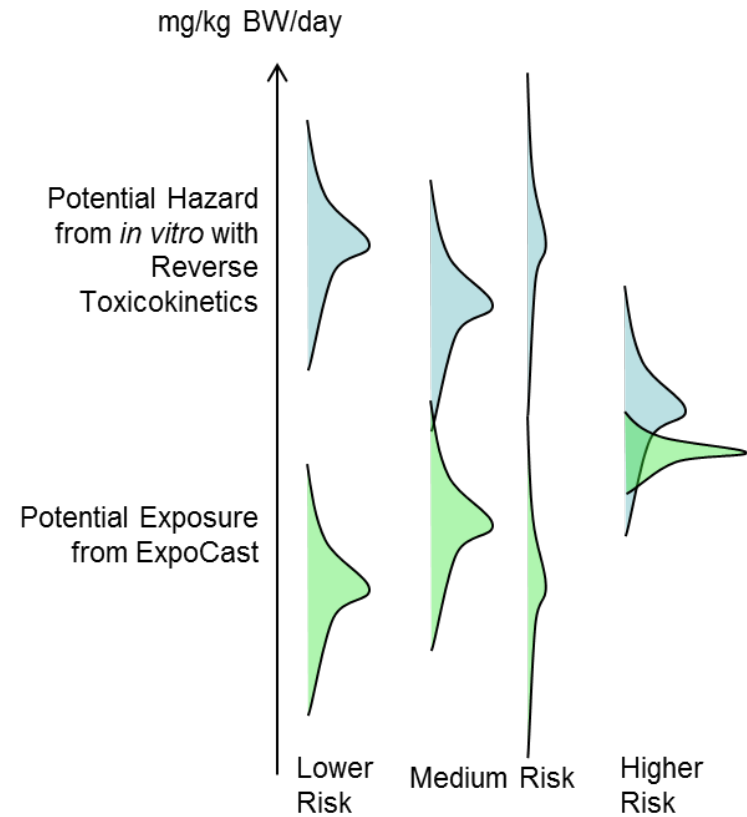


Figure includes image from Thinkstock

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Introduction

- In order to address greater numbers of chemicals we collect *in vitro*, high throughput toxicokinetic (HTTK) data
- The goal of HTTK is to provide a human dose context for *in vitro* concentrations from HTS
 - This allows direct comparisons with exposure
- A key application of HTTK has been reverse dosimetry
 - Allows *in vitro* – *in vivo* extrapolation (What dose causes a bioactive concentration?)
 - Allows exposure reconstruction (What dose is consistent with a biomarker?)



The Need for *In Vitro* Toxicokinetics



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

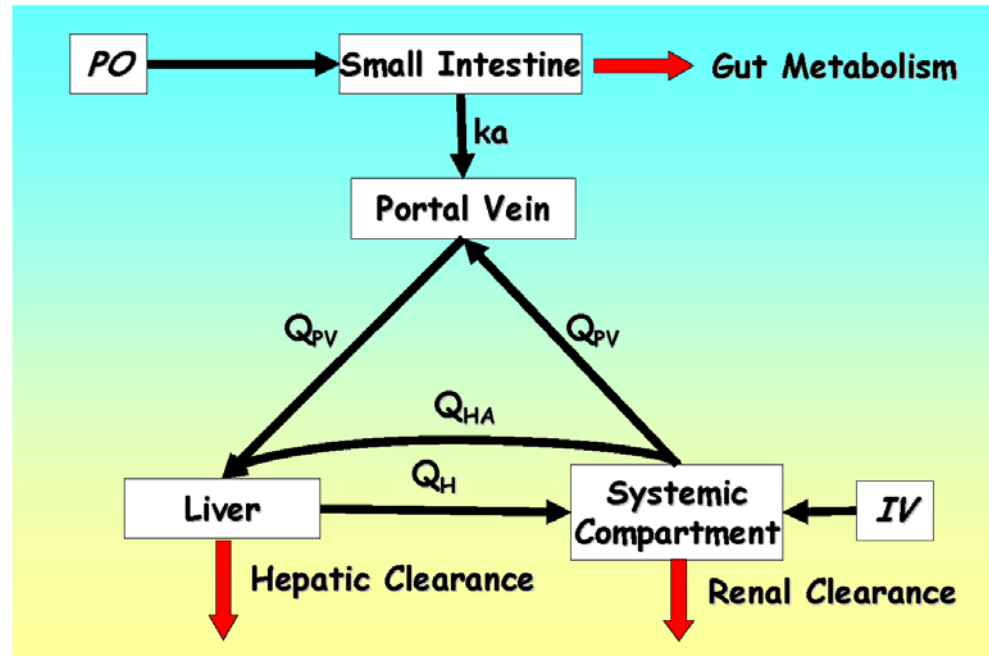
High Throughput Toxicokinetics (HTTK)

Jamei *et al.* (2009)

sim4CYP
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Minimal Model: Lumped Single Distribution Volume

- *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



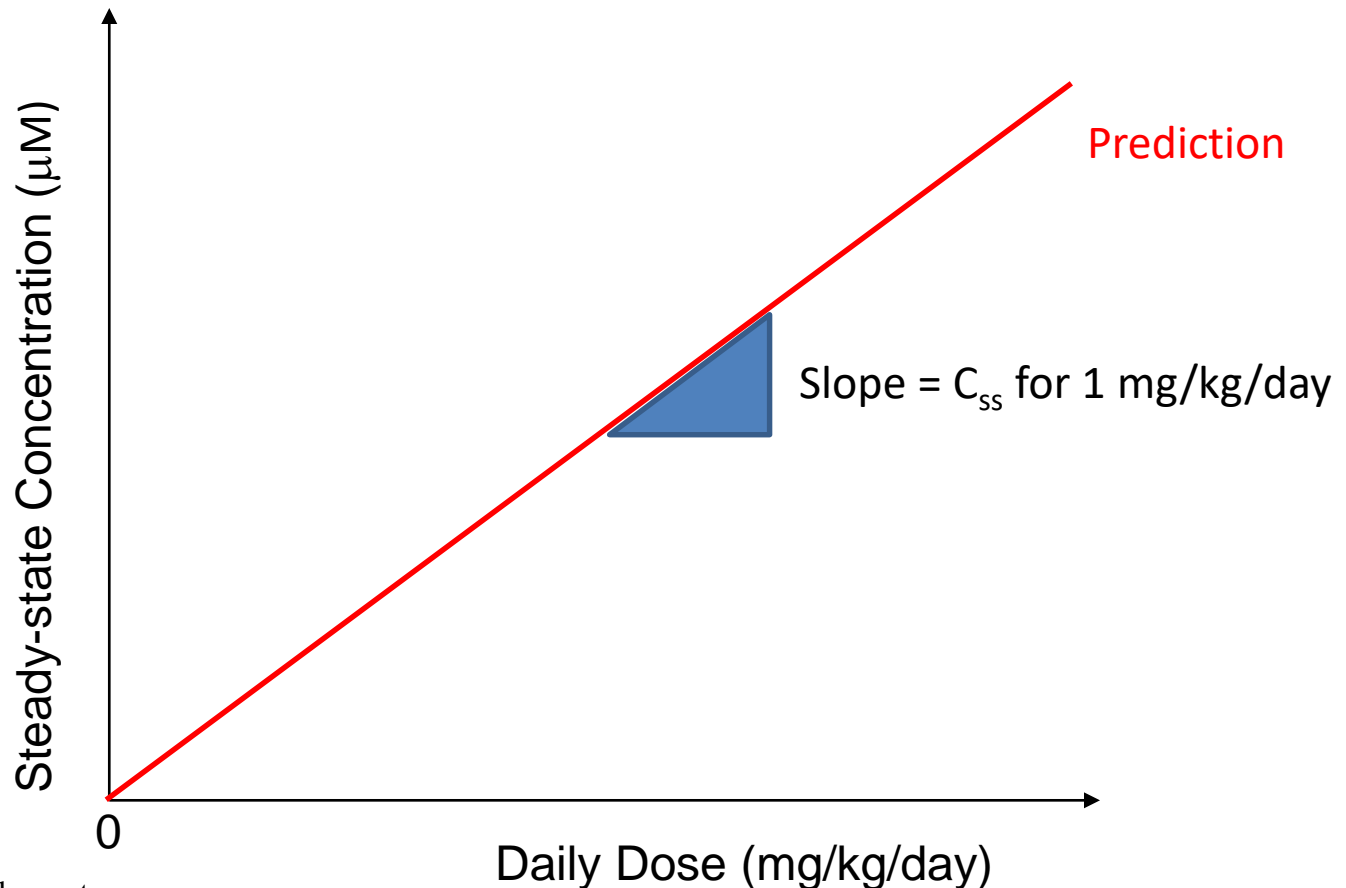
$$C_{ss} = \frac{\text{oral dose rate}}{(GFR * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}} \right)}$$

Oral dose in
(mg/kg/day)



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

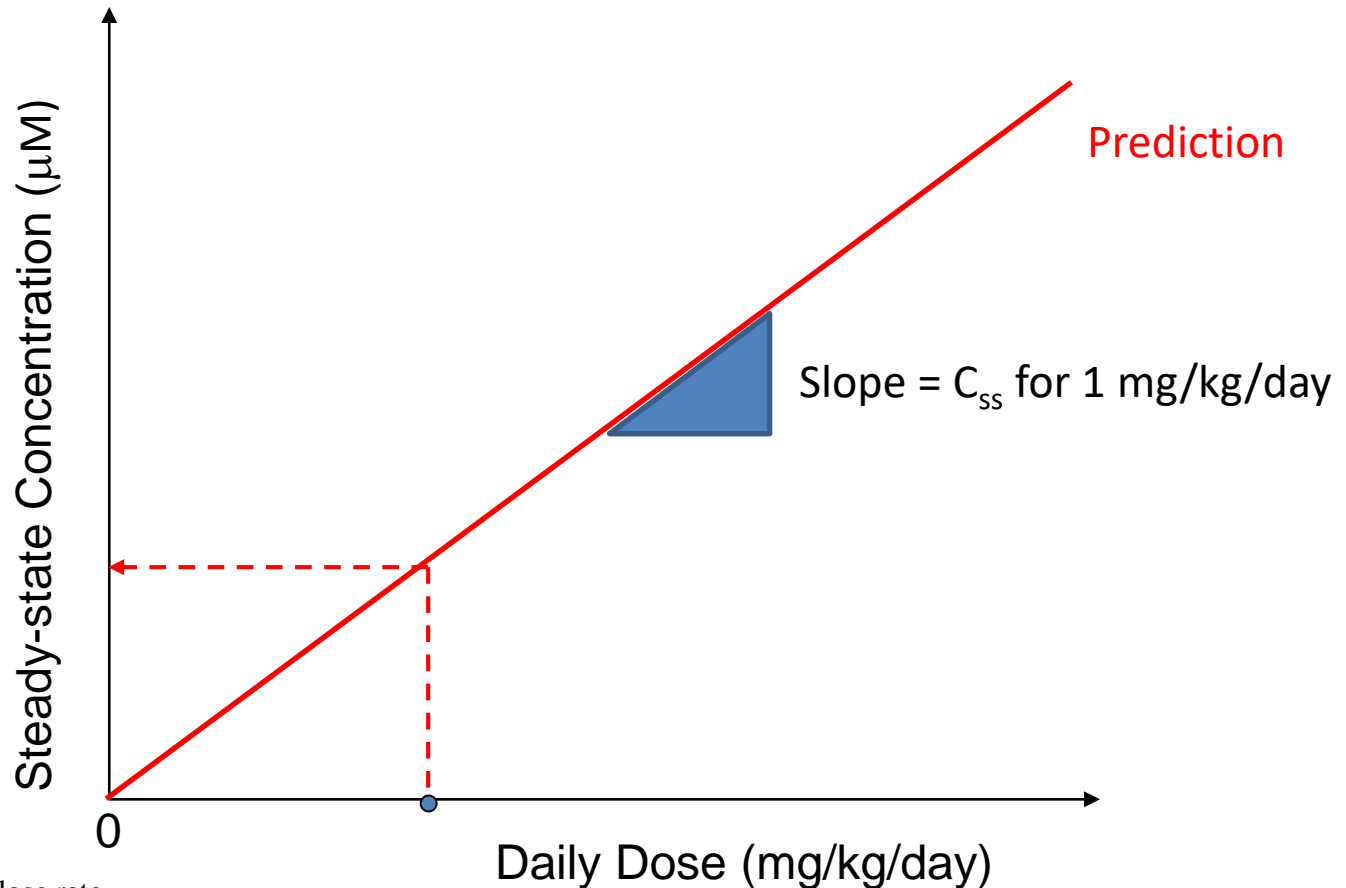
Steady-State is Linear with Dose



$$C_{ss} = \frac{\text{oral dose rate}}{\left(\text{GFR} * F_{ub} \right) + \left(Q_1 * F_{ub} * \frac{Cl_{int}}{Q_1 + F_{ub} * Cl_{int}} \right)}$$

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

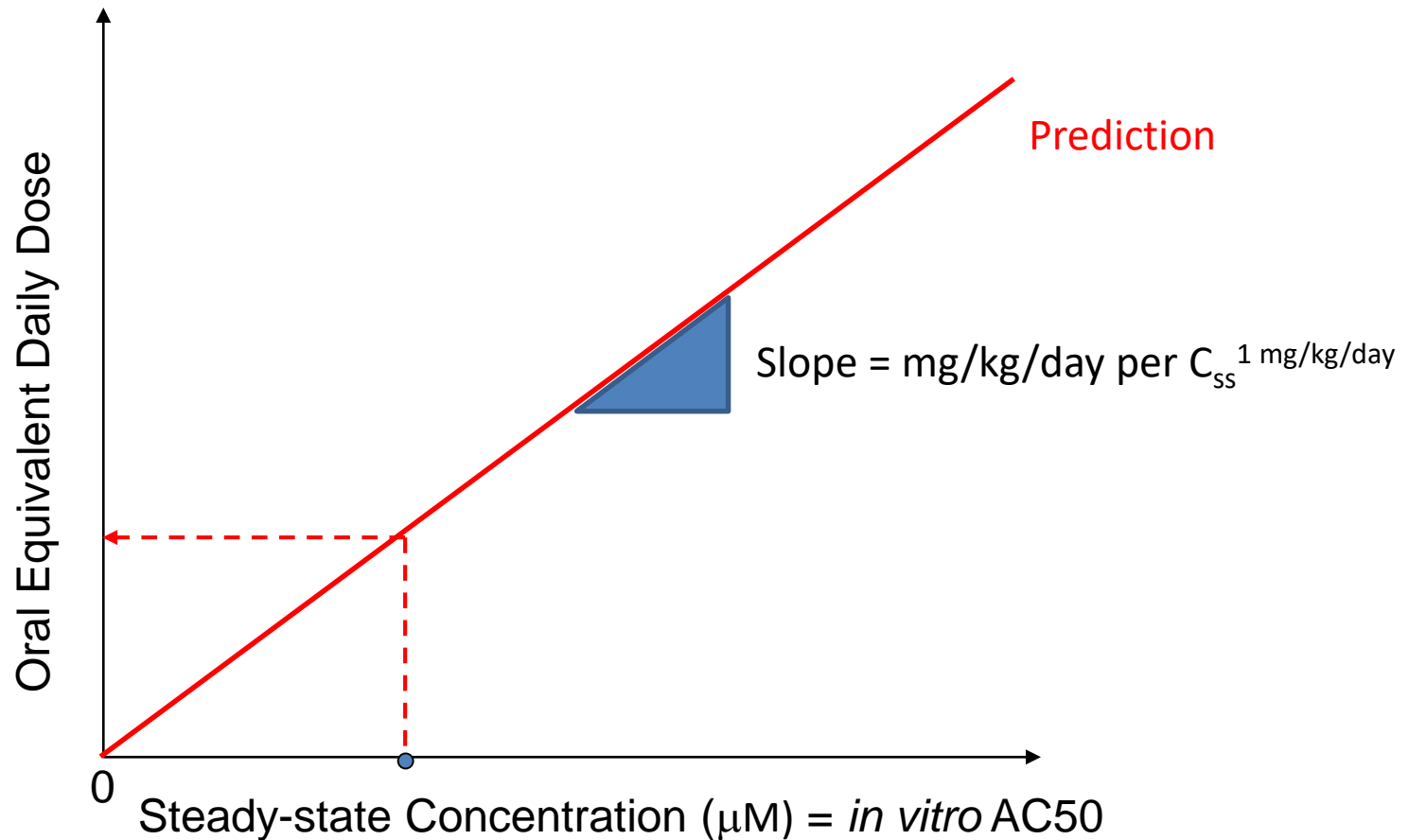
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$$C_{ss} = \frac{\text{oral dose rate}}{\left(\text{GFR} * F_{ub} \right) + \left(Q_1 * F_{ub} * \frac{Cl_{int}}{Q_1 + F_{ub} * Cl_{int}} \right)}$$

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

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

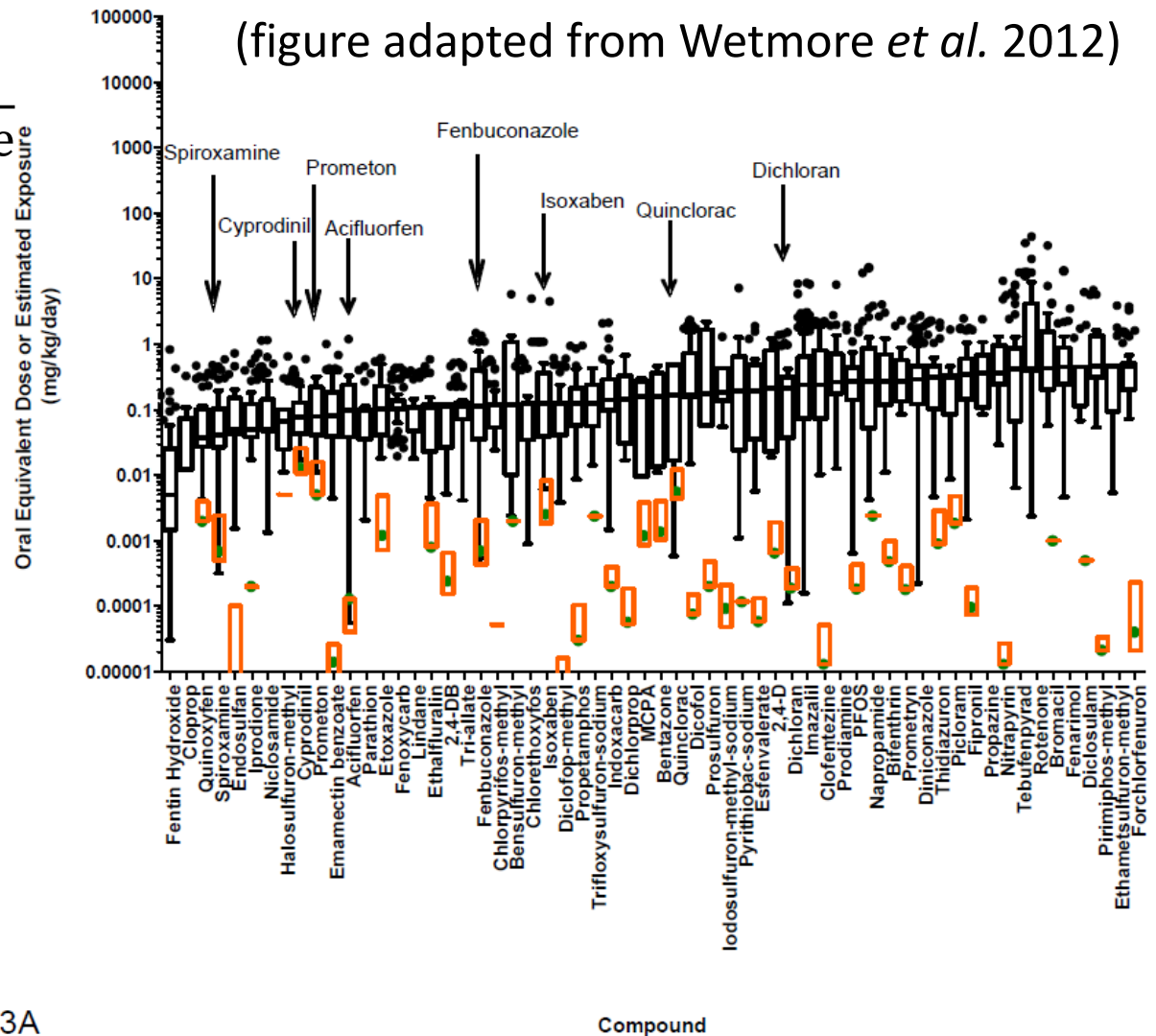
Integrated Bioactivity:Exposure Ratio

(Wetmore et al. 2012, 2014, 2015)

$$IBER = \frac{\text{Bioactive Dose}}{\text{Estimated exposure}}$$

IBER <=1 : Exposure potentially high enough to cause bioactivity

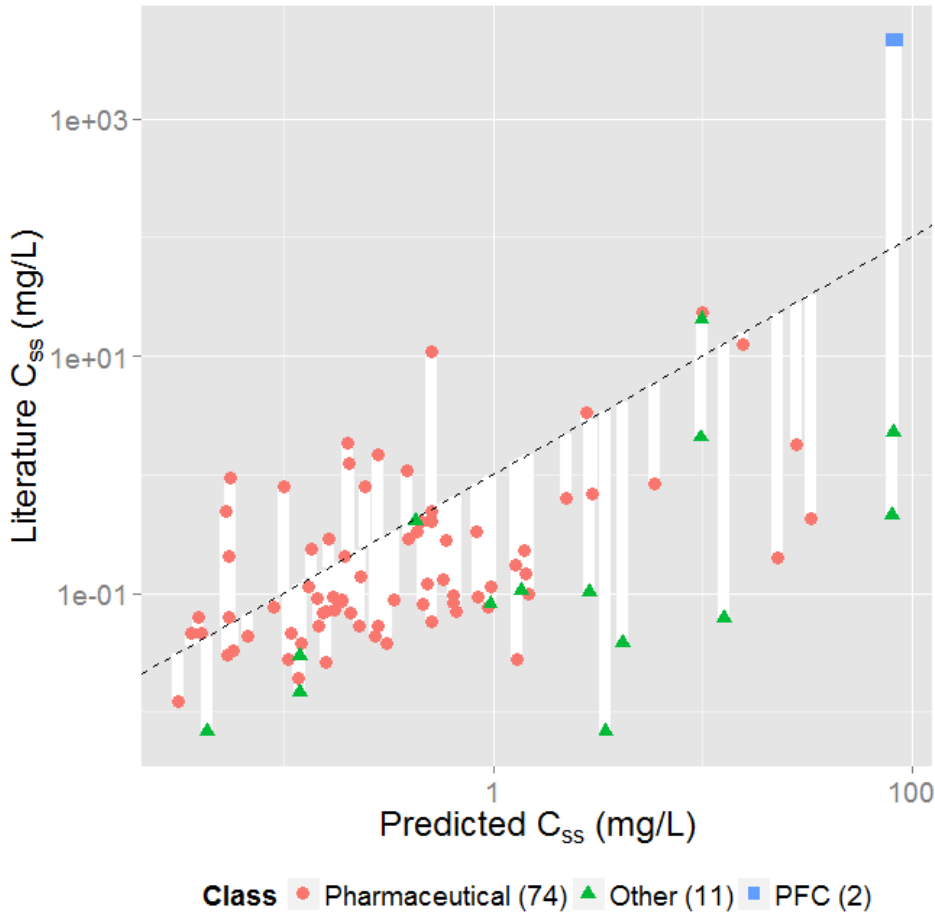
IBER >> 1: Exposure less likely to be high enough to cause bioactivity



In vivo Predictive Ability and Domain of Applicability

- In drug development, HTK 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

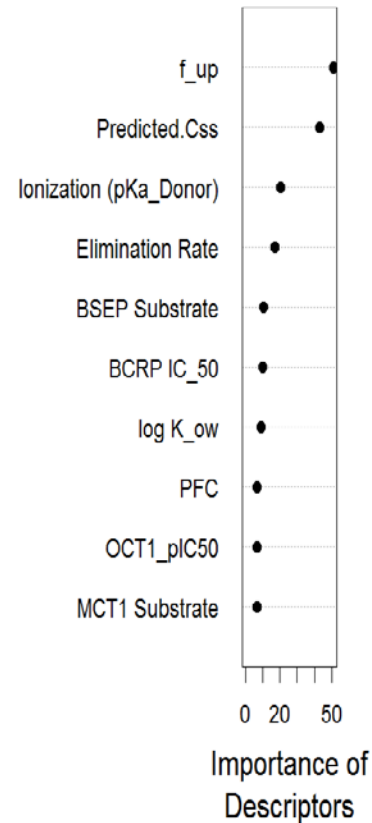
Using *in vivo* Data to Evaluate HTTK



- 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 ($R^2 \sim 0.34$)

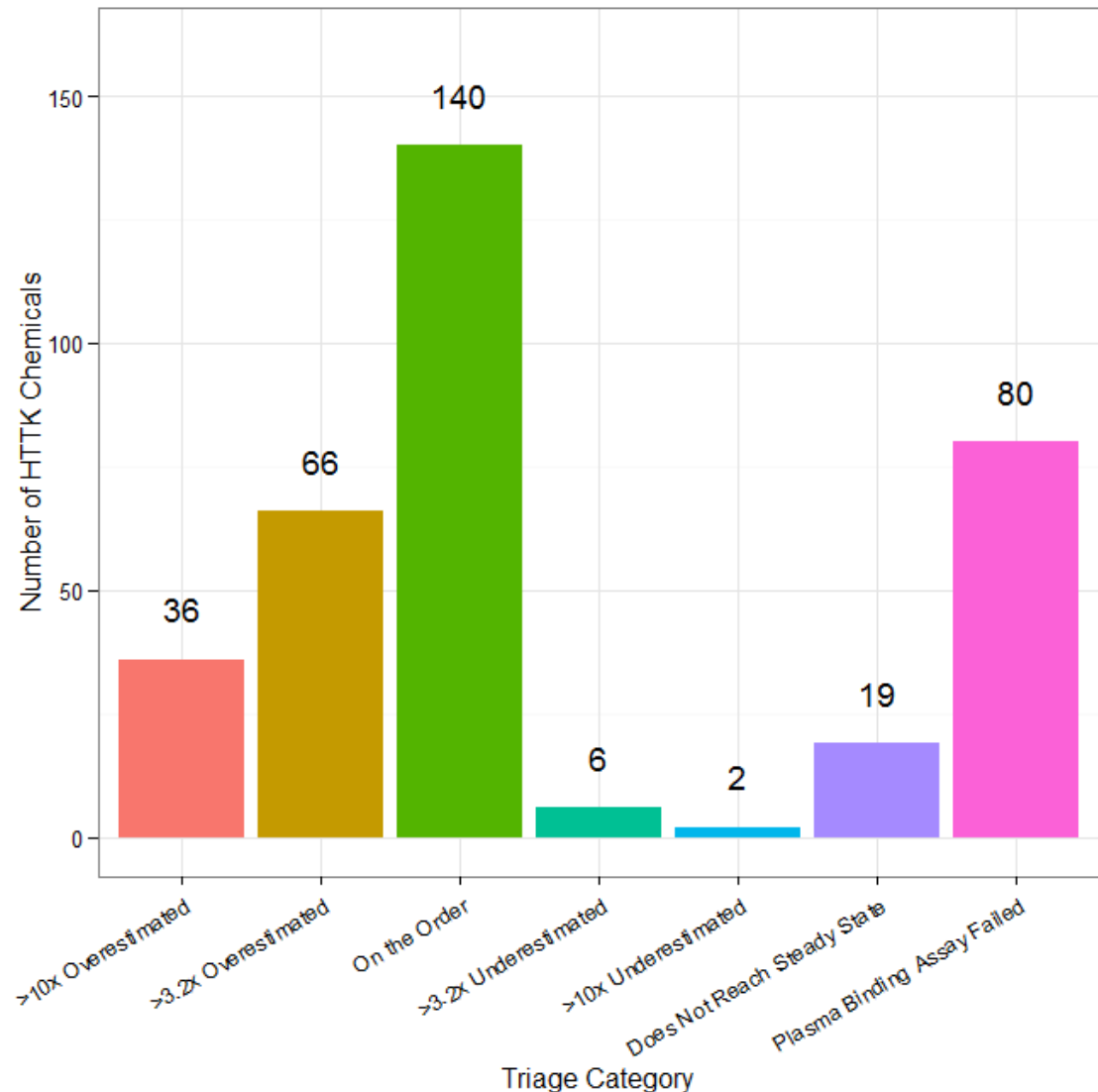
- The dashed line indicates the identity (perfect predictor) line:
 - Over-predict for 65
 - Under-predict for 22

- The white lines indicate the discrepancy between measured and predicted values (the residual)

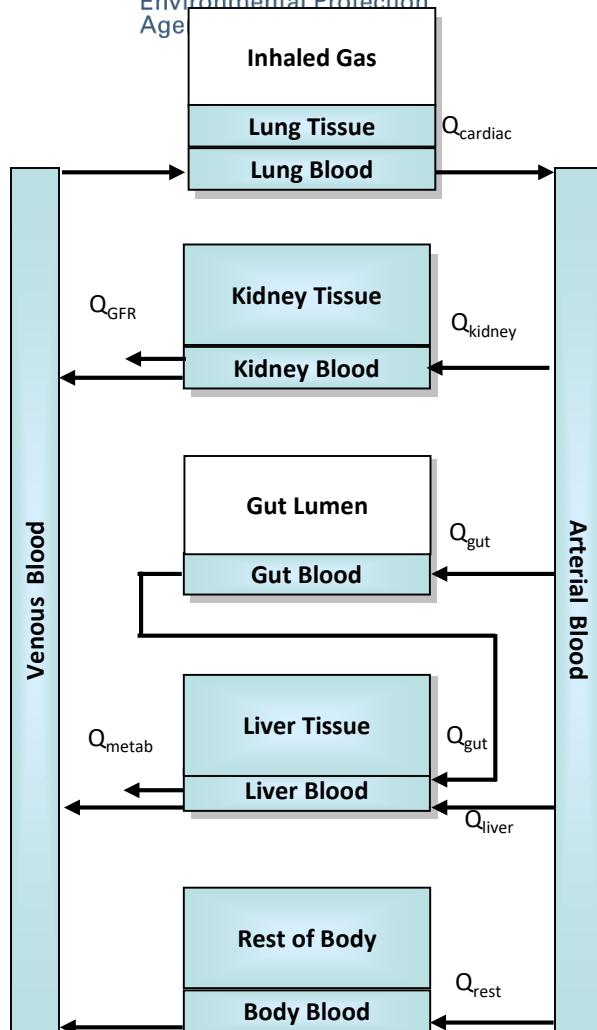


- Through comparison to *in vivo* data, a cross-validated (random forest) predictor of success or failure of HTTK has been constructed
- Add categories for chemicals that do not reach steady-state or for which plasma binding assay fails
- All chemicals can be placed into one of seven confidence categories

Toxicokinetic Triage

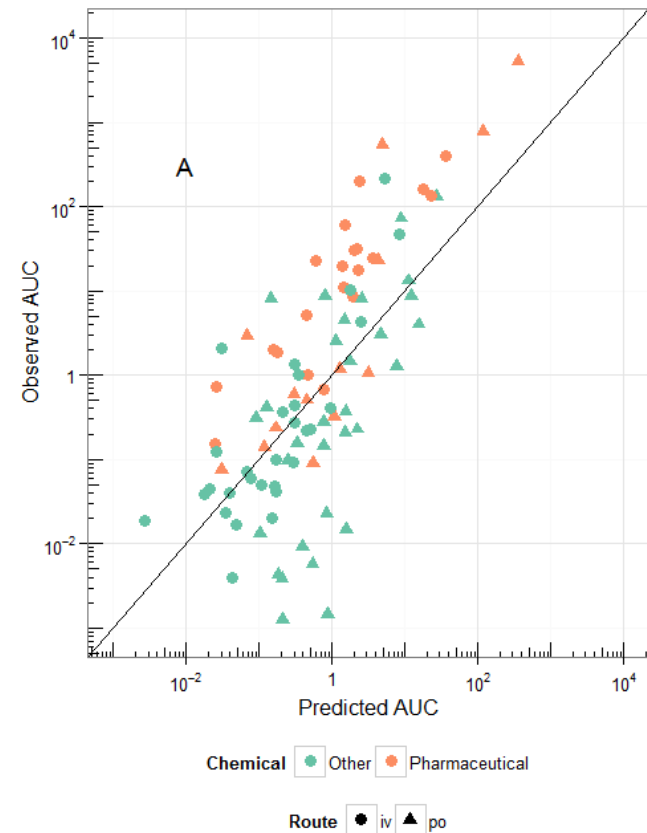


A General Physiologically-based Toxicokinetic (PBTK) Model



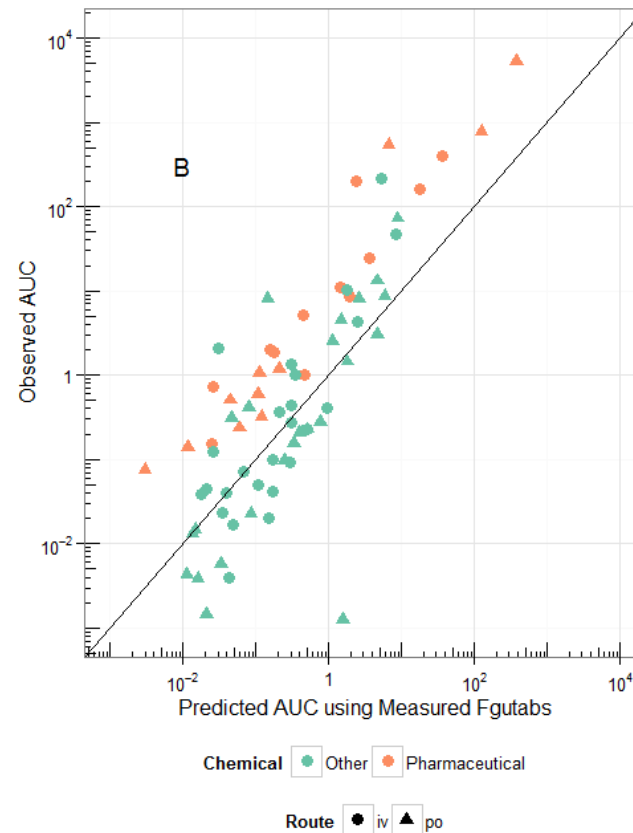
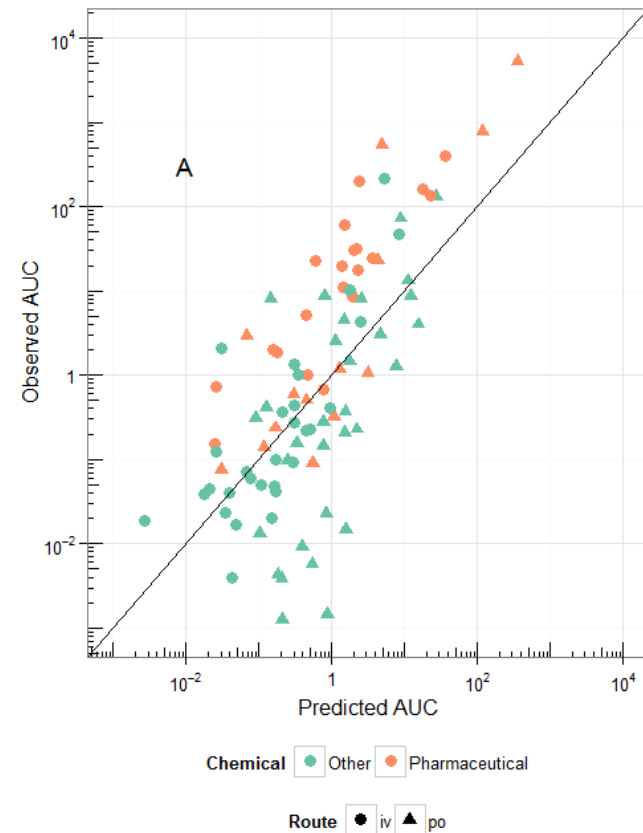
- Some tissues (e.g. arterial blood) are simple compartments, while others (e.g. kidney) are compound compartments consisting of separate blood and tissue sections with constant partitioning (i.e., tissue specific partition coefficients)
- Exposures are absorbed from reservoirs (gut lumen)
- Some specific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

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



- PBTK predictions for the AUC (time integrated plasma concentration or Area Under the Curve)
- Oral and *iv* studies for 26 ToxCast compounds
 - Collaboration with NHEERL (Mike Hughes and Jane Ellen Simmons)
 - Additional work by Research Triangle Institute (Tim Fennell)
- Can estimate
 - Fraction absorbed
 - Absorption Rate
 - Elimination Rate
 - Volume of Distribution

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

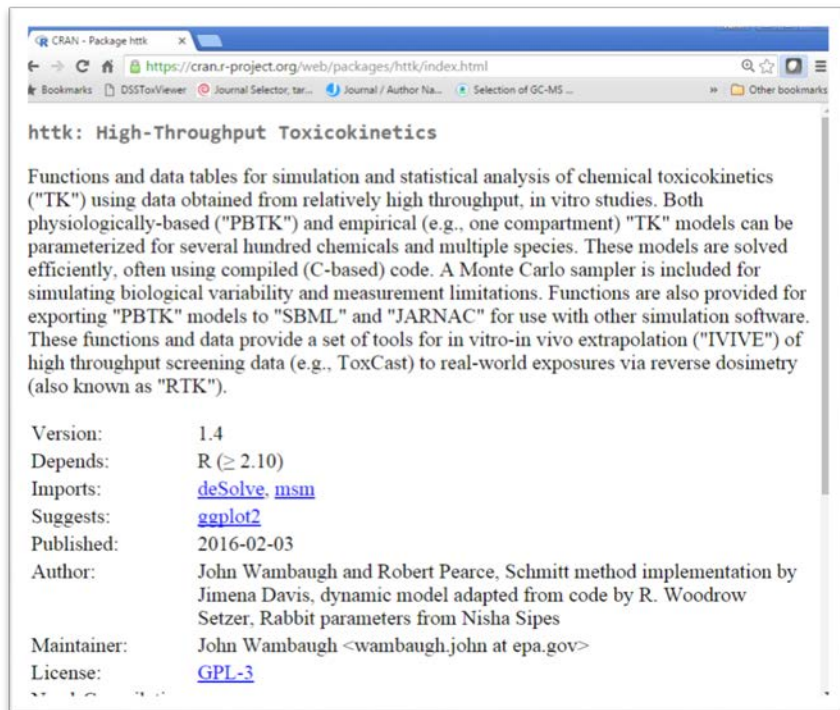


- PBTK predictions for the AUC (time integrated plasma concentration or Area Under the Curve)
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Cyprotex is now measuring bioavailability (CACO2) for all HHTK chemicals

httk: An Up-to-Date Tool

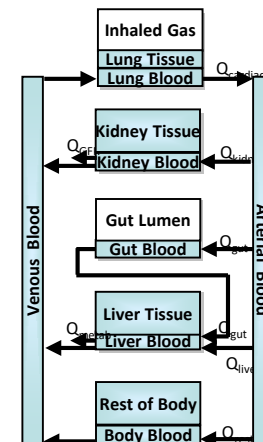
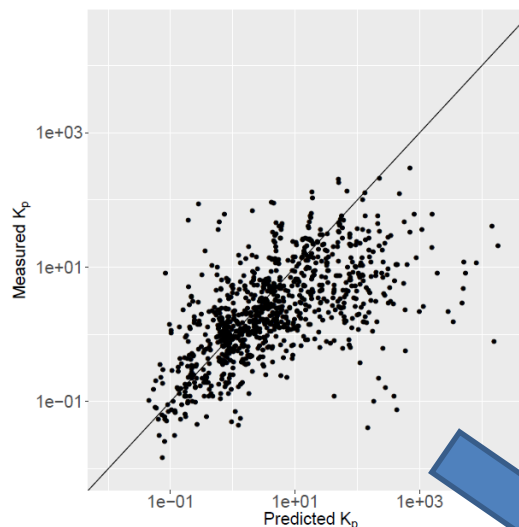
Old versions are archived



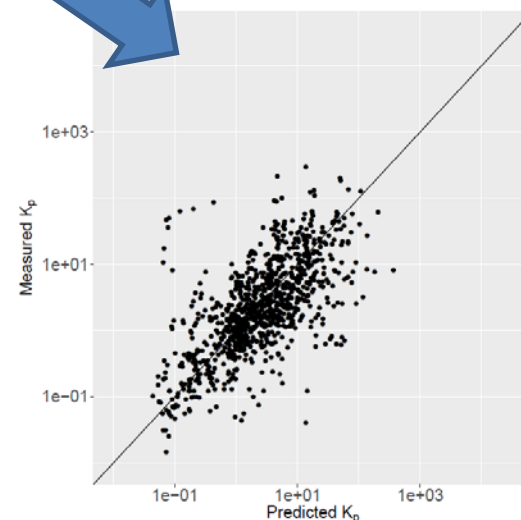
httk: High-Throughput Toxicokinetics

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: 1.4
 Depends: R (≥ 2.10)
 Imports: [deSolve](#), [msm](#)
 Suggests: [ggplot2](#)
 Published: 2016-02-03
 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](#)



Ongoing refinements:
 High log P, ionization
 (Pearce et al., in preparation)




- "httk" R Package for reverse dosimetry and PBTK
- 543 Chemicals to date
- 100's of additional chemicals being studied
- Pearce *et al.* package documentation manuscript accepted at Journal of Statistical Software

Application to High Throughput Risk Prioritization

More Plausible Biologically Active Exposures

mg/kg BW/day



} 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”

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



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

NCCT

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Parichehr
Saranjampour*
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Woody Setzer
Rusty Thomas
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Harvey Clewell

Chantel Nicolas

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