The High Throughput Toxicokinetic (HTTK) R Package

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Computational Toxicology Community of Practice Webinar

June 27, 2019

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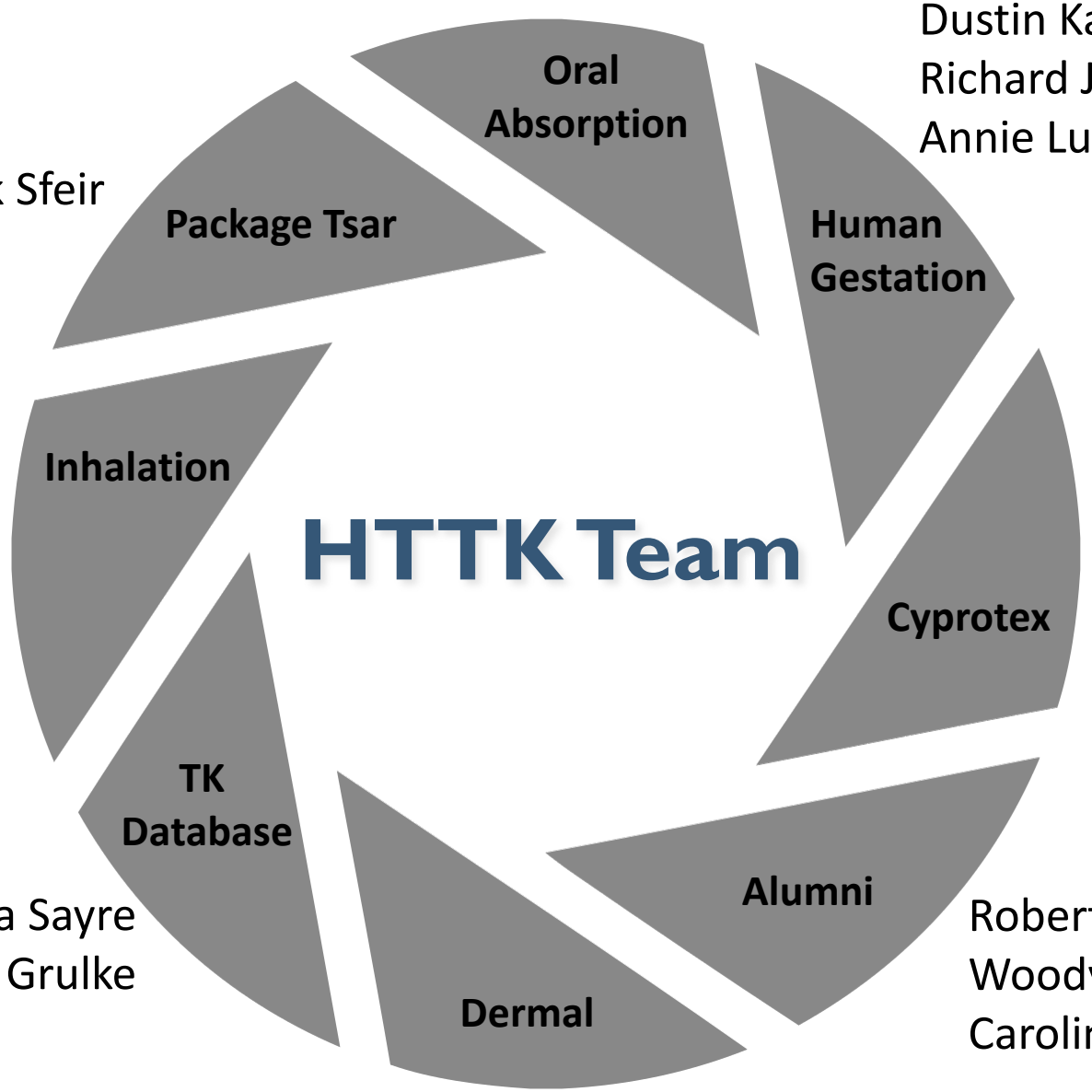
EPA United States Environmental Protection Agency



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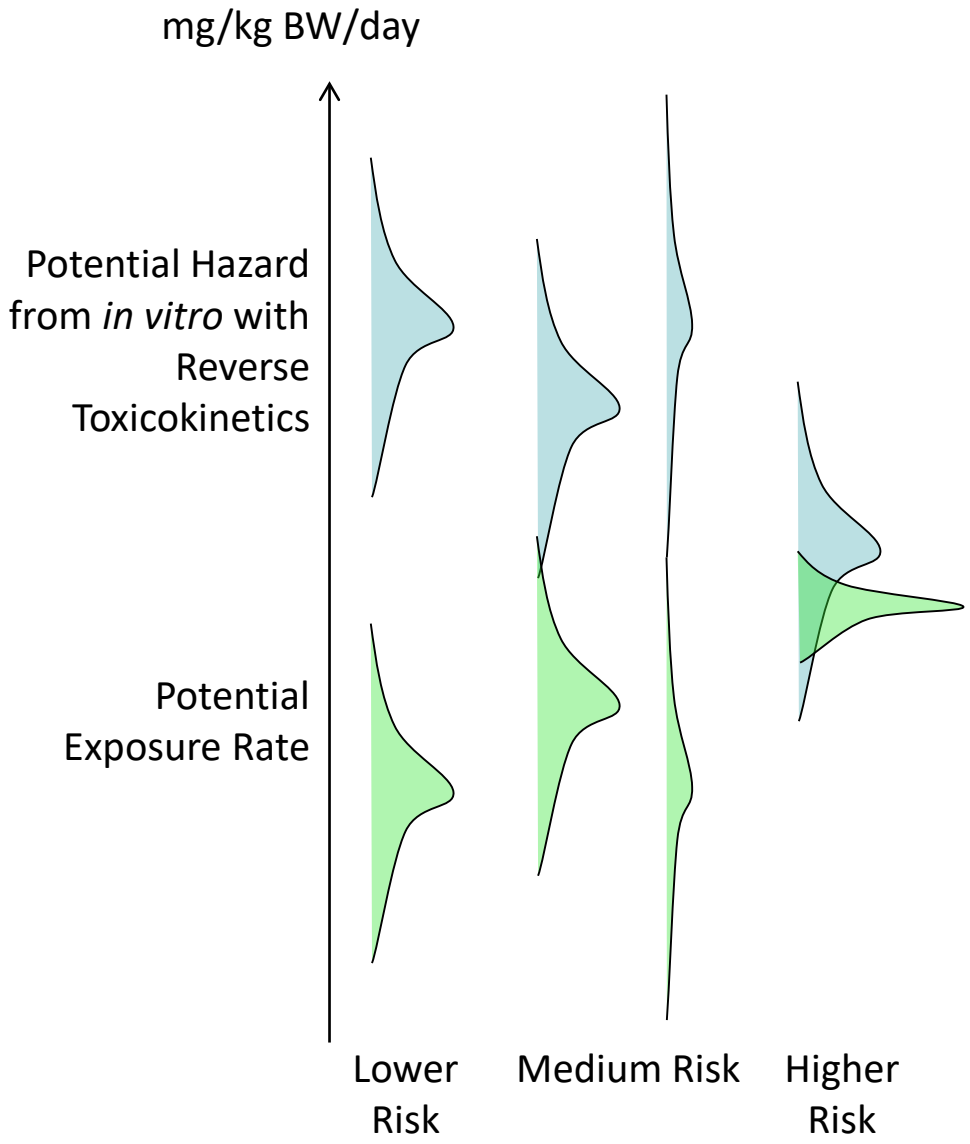
Chemical Risk = Hazard x Exposure

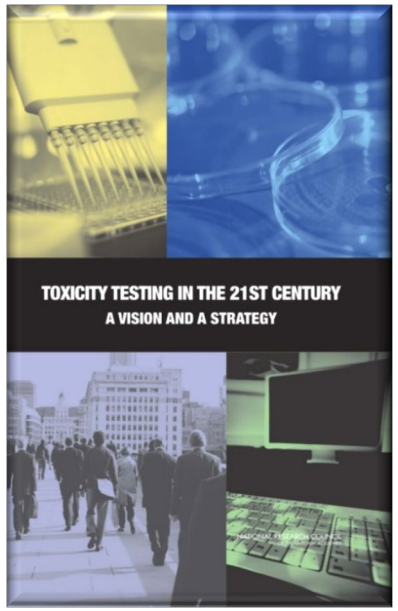
- The U.S. National Research Council (1983) identified chemical risk as a function of both inherent hazard and exposure

- To address the thousands of chemicals in commerce and the environment, we need new approach methodologies (NAMs) that can inform prioritization of chemicals most worthy of additional study

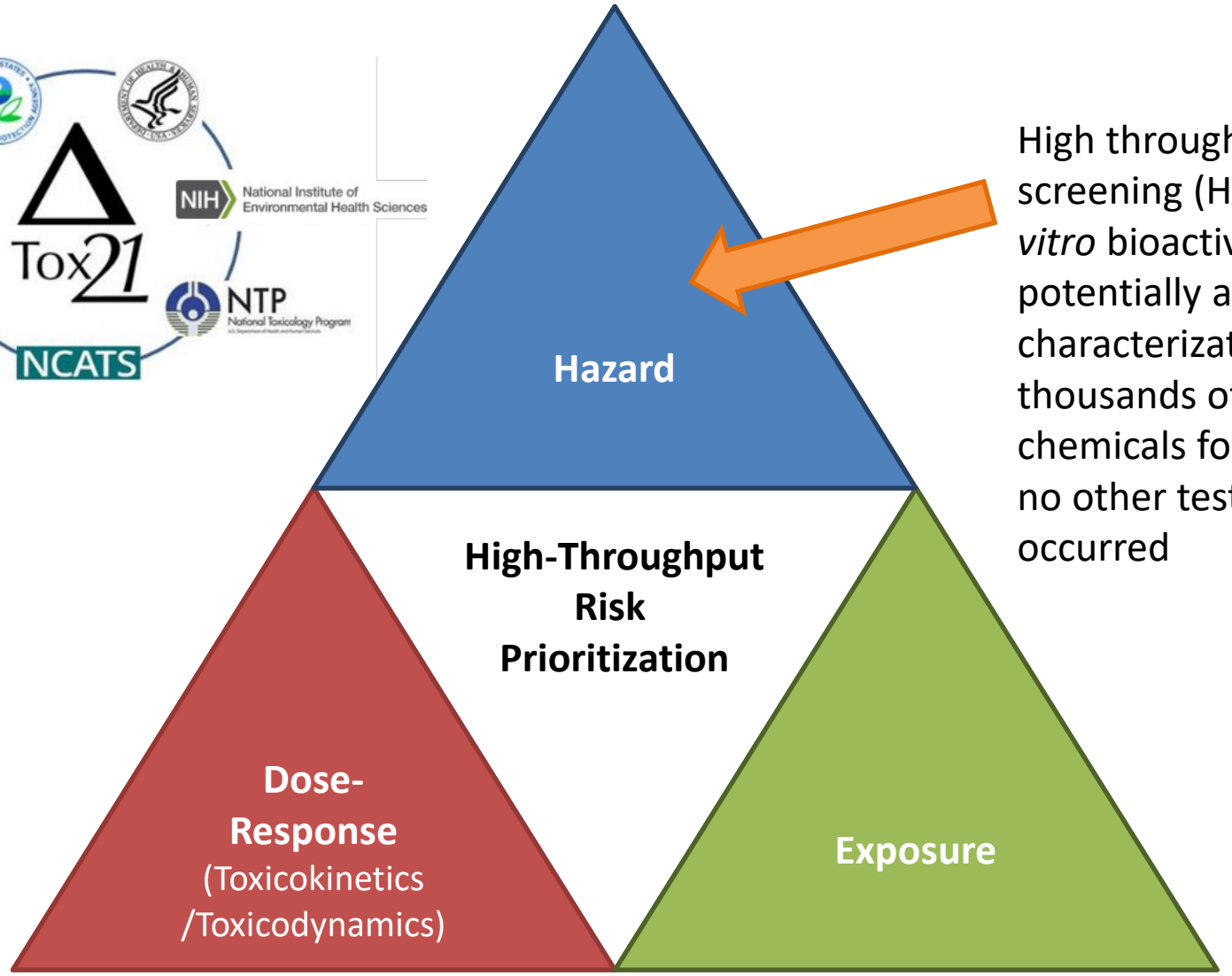
- High throughput risk prioritization needs:
  1. High throughput hazard characterization (Dix et al., 2007, Collins et al., 2008)
  2. High throughput exposure forecasts (Wambaugh et al., 2013, 2014)
  3. High throughput toxicokinetics (i.e., dose-response relationship) linking hazard and exposure (Wetmore et al., 2012, 2015)



High-Throughput Risk Prioritization



NRC (2007)



High throughput screening (HTS) for *in vitro* bioactivity potentially allows characterization of thousands of chemicals for which no other testing has occurred

In Vitro - In Vivo Extrapolation (IVIVE)

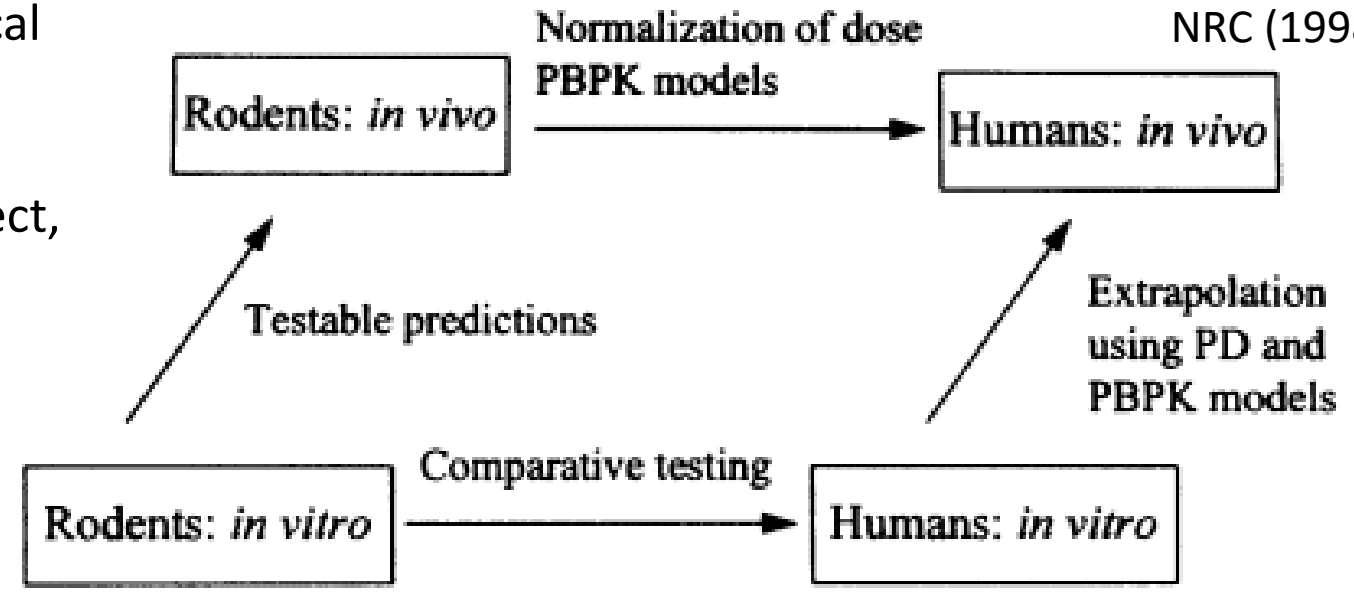
Utilization of *in vitro* experimental data to predict phenomena *in vivo*

- IVIVE-PK/TK (**Pharmacokinetics/Toxicokinetics**):
  - Fate of molecules/chemicals in body
  - Considers absorption, distribution, metabolism, excretion (ADME)
  - Uses empirical PK and physiologically-based (PBPK) modeling

- IVIVE-PD/TD (**Pharmacodynamics/Toxicodynamics**):
  - Effect of molecules/chemicals at biological target *in vivo*
  - Assay design/selection important
  - Perturbation as adverse/therapeutic effect, reversible/ irreversible effeccts

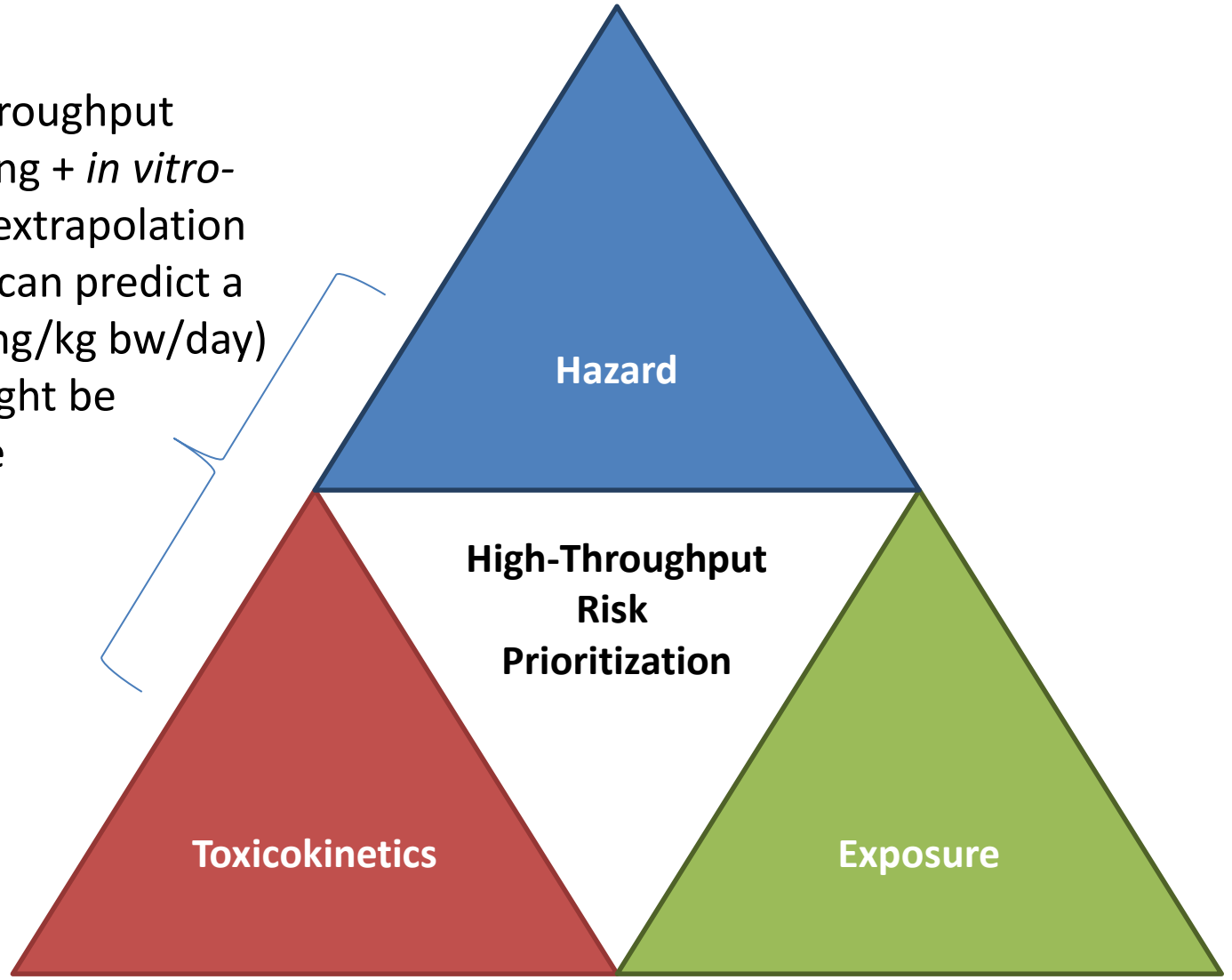
- Both contribute to *in vivo* effect prediction

NRC (1998)



New Exposure Data and Models

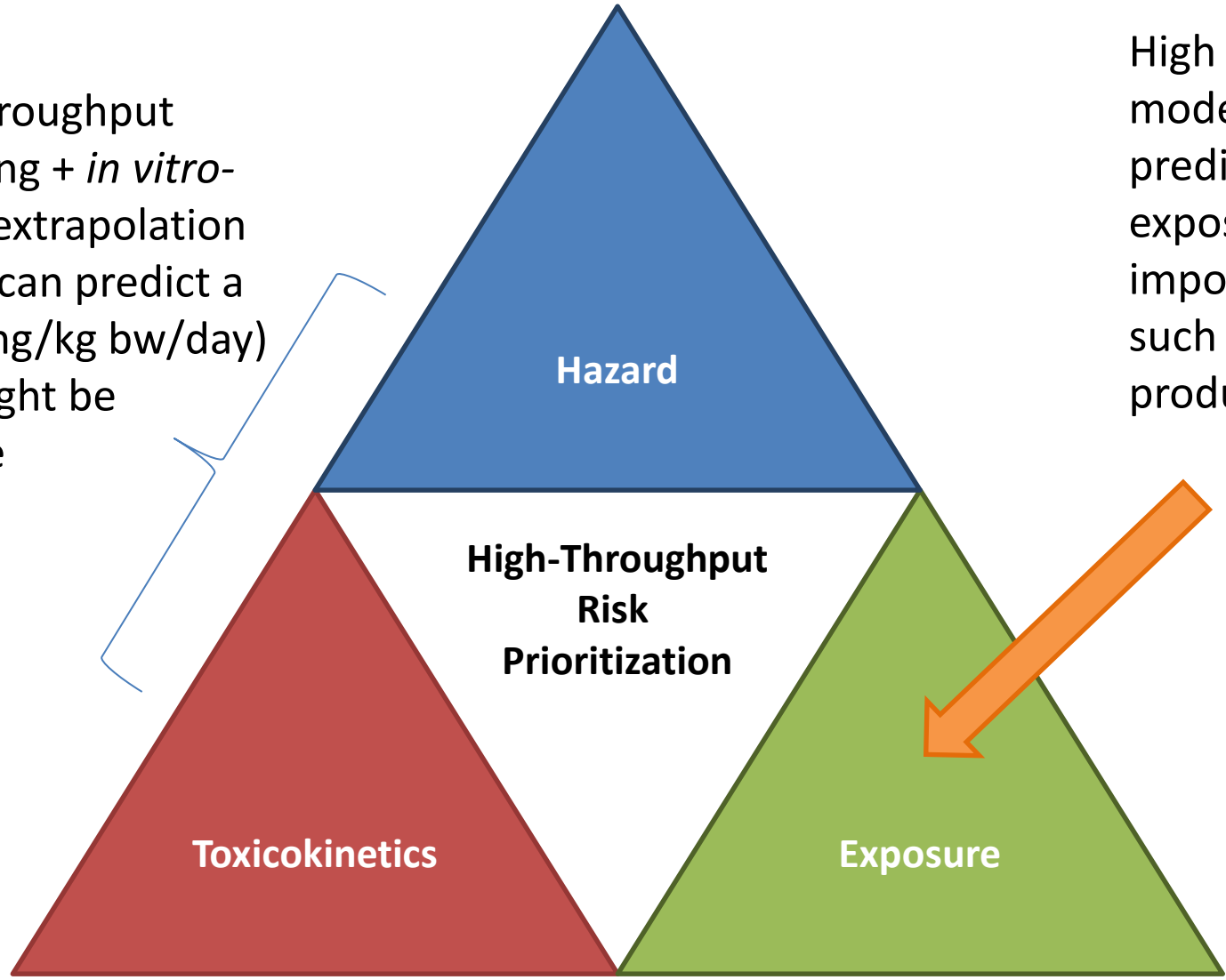
High throughput screening + *in vitro-in vivo* extrapolation (IVIVE) can predict a dose (mg/kg bw/day) that might be adverse



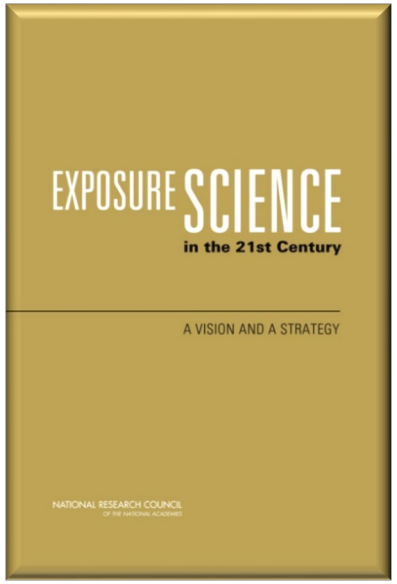
Wetmore et al. (2012, 2015)

New Exposure Data and Models

High throughput screening + *in vitro-in vivo* extrapolation (IVIVE) can predict a dose (mg/kg bw/day) that might be adverse



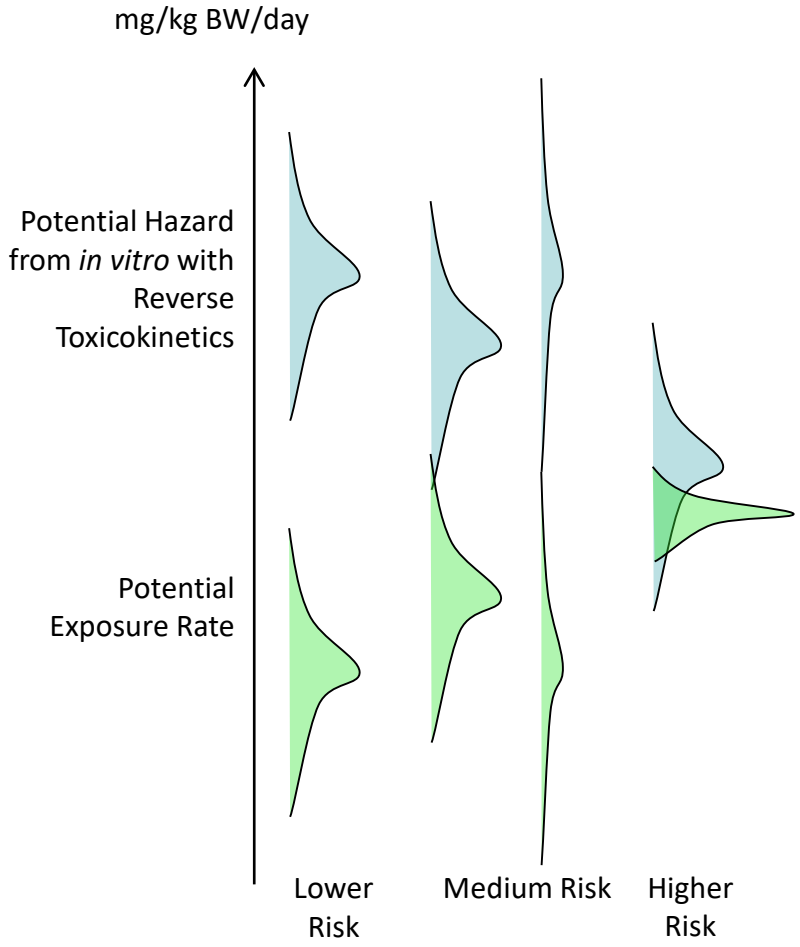
High throughput models exist to make predictions of exposure via specific, important pathways such as residential product use and diet



NRC (2012)

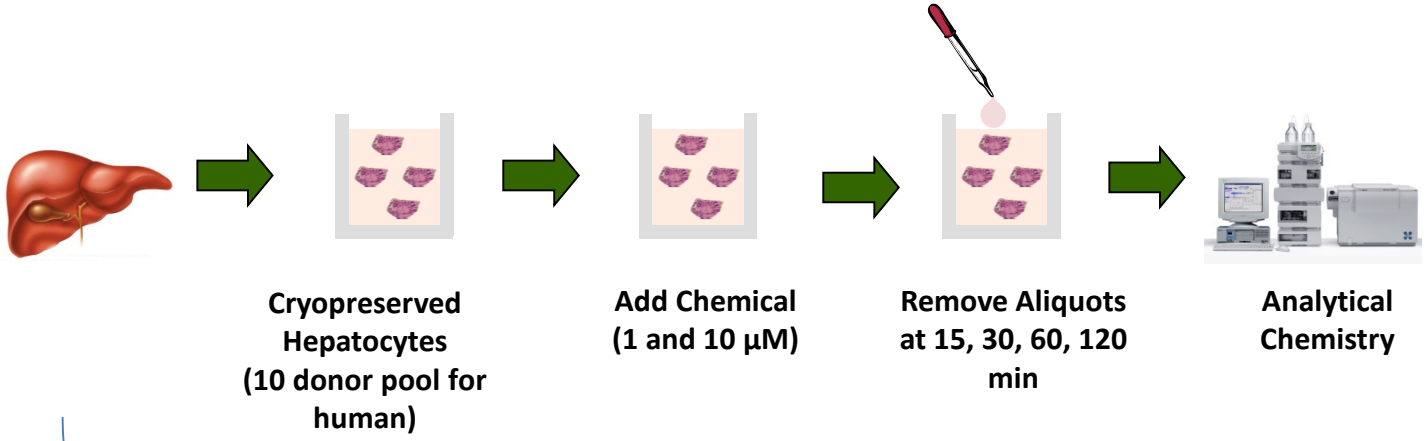
High Throughput Toxicokinetics (HTTK)

- **Most chemicals do not have TK data**
- In order to address greater numbers of chemicals we collect *in vitro*, high throughput toxicokinetic (HTTK) data (Rotroff et al., 2010, Wetmore et al., 2012, 2015)
- 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)
- 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**) (e.g., Wetmore et al., 2015)
- **Secondary goal** is to provide **open source data and models** for evaluation and use by the broader scientific community (Pearce et al, 2017a)

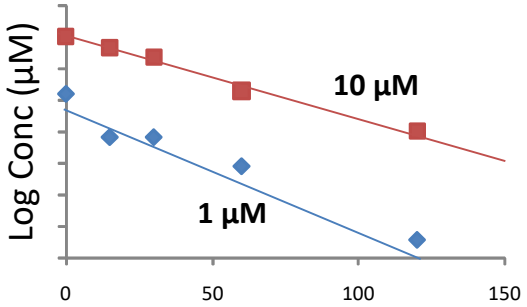


In Vitro Data for HTTK

Cryopreserved hepatocyte suspension Shibata *et al.* (2002)



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



We perform the assay at 1 and 10 µM to check for saturation of metabolizing enzymes.

- **Most chemicals do not have TK data** – we use *in vitro* HTTK methods adapted from pharma to fill gaps
- In drug development, HTTK methods allow IVIVE to estimate therapeutic doses for clinical studies – predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)

In Vitro Data for HTTK

Cryopreserved hepatocyte suspension
Shibata *et al.* (2002)

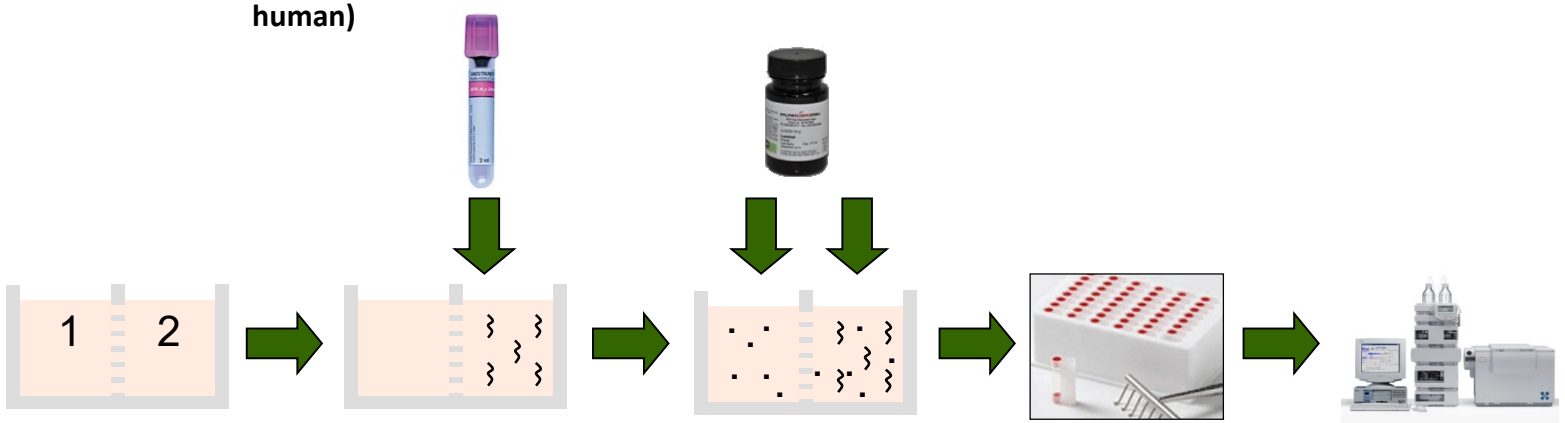
Cryopreserved Hepatocytes (10 donor pool for human)

Add Chemical (1 and 10 µM)

Remove Aliquots at 15, 30, 60, 120 min

Analytical Chemistry

Rapid Equilibrium Dialysis (RED)
Waters *et al.* (2008)



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

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

Add chemical

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

Determine concentration in both wells (analytical chemistry)

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

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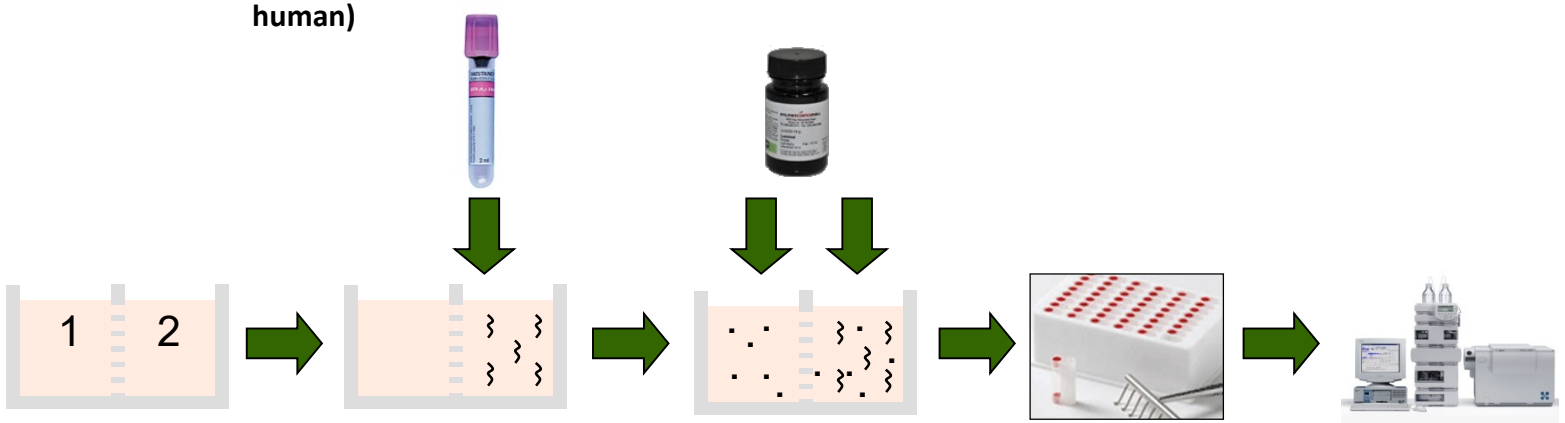
In Vitro Data for HTTK

Cryopreserved hepatocyte suspension Shibata *et al.* (2002)

Rapid Equilibrium Dialysis (RED) Waters *et al.* (2008)



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

- **Most chemicals do not have TK data** – we use *in vitro* HTTK methods adapted from pharma to fill gaps
- Environmental chemicals:
  - Rotroff et al. (2010) **35** chemicals
  - Wetmore et al. (2012) **+204** chemicals
  - Wetmore et al. (2015) **+163** chemicals
  - Wambaugh et al. (submitted) **+389** chemicals

Simple Model for Steady-State Plasma Concentration (C_{ss})

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

Wilkinson and Shand (1975)

Passive Renal Clearance
(GFR: Glomerular filtration rate
f_{up}: fraction unbound in plasma)

Hepatic Metabolism
(Cl_{int}: Scaled hepatic clearance
Q_l: Blood flow to liver)

Assume that Steady-State is Linear with Dose

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

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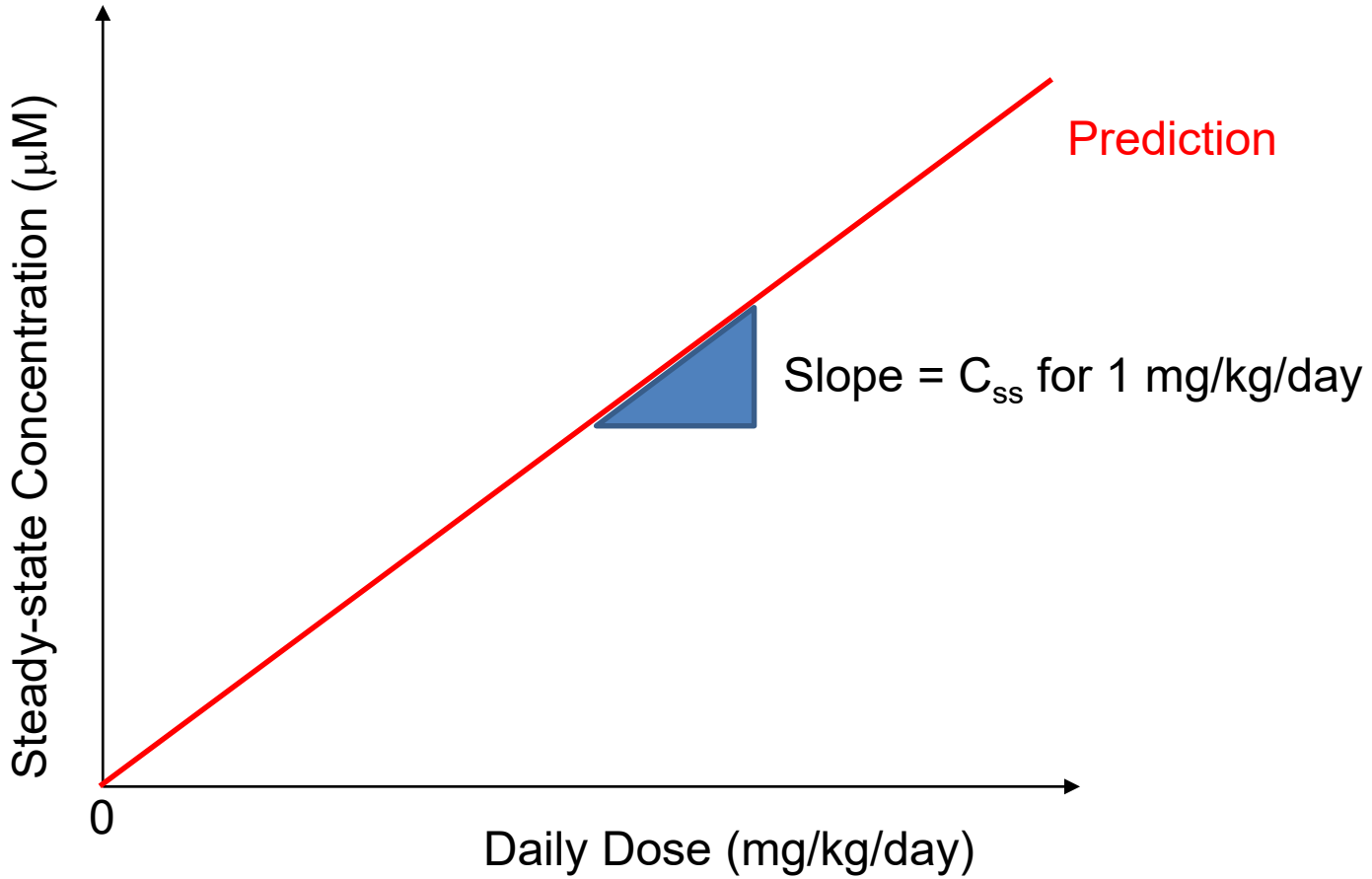
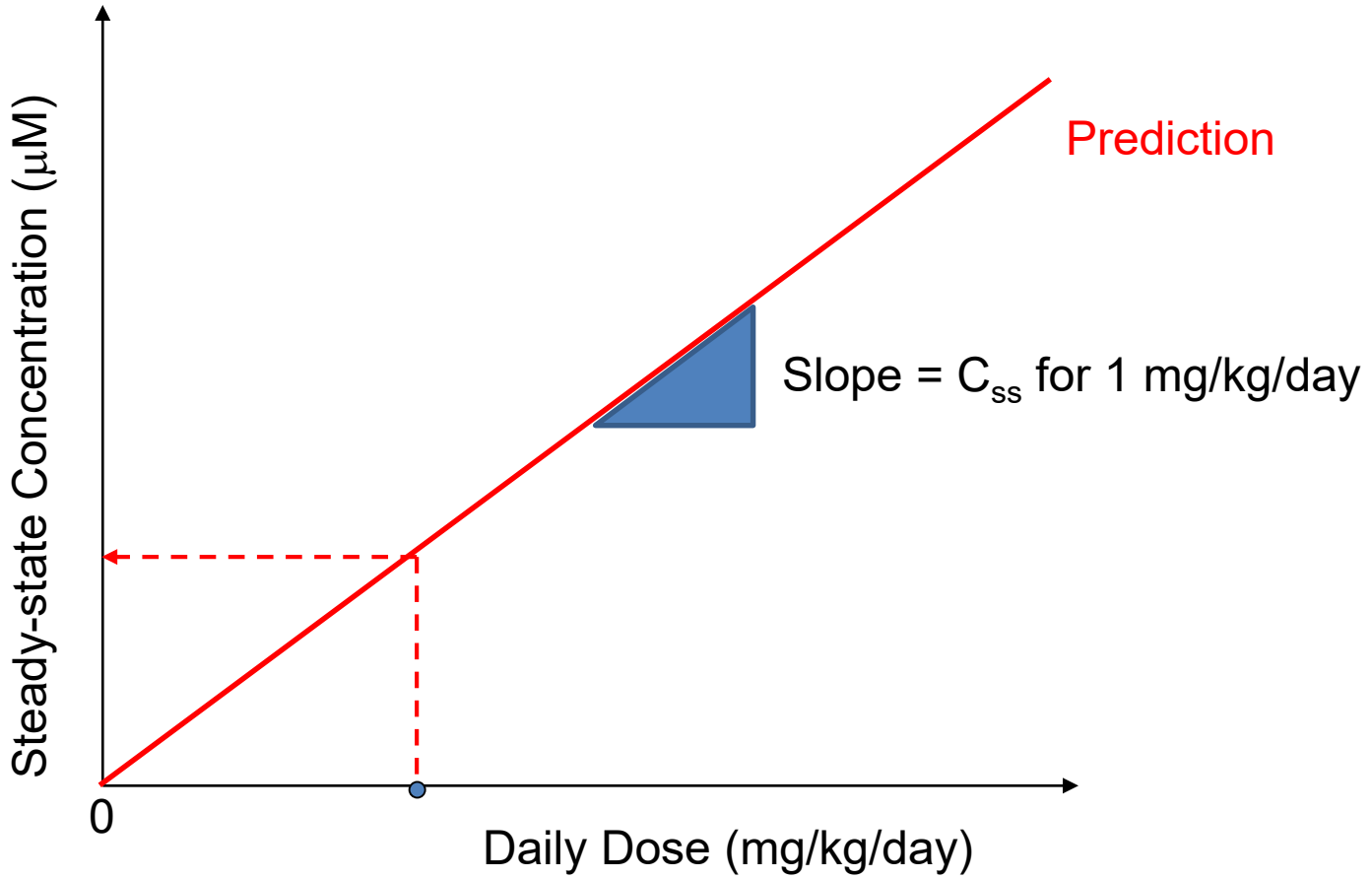


- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al*. (2012)

Assume that Steady-State is Linear with Dose

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

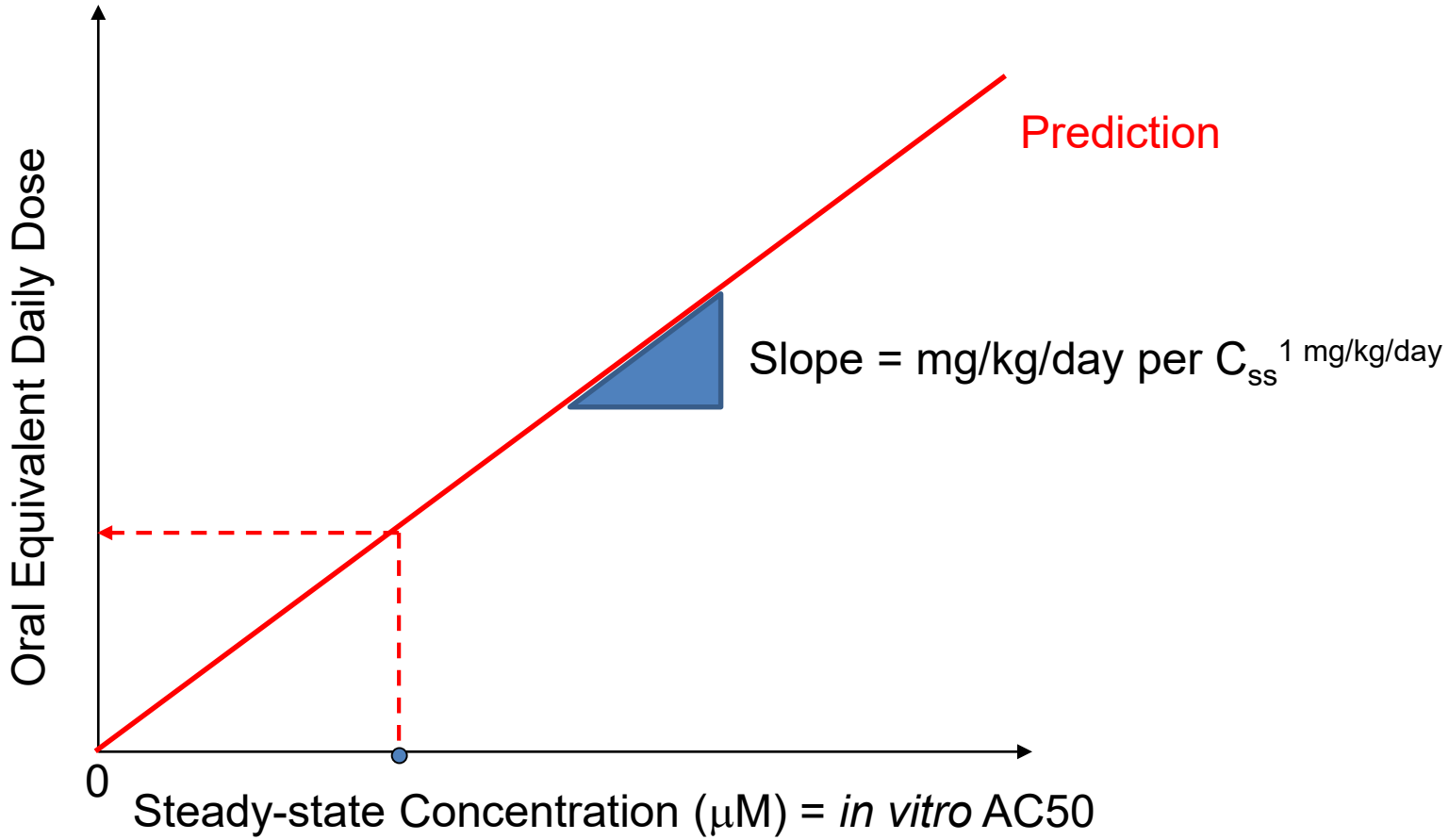


- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al*. (2012)

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

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

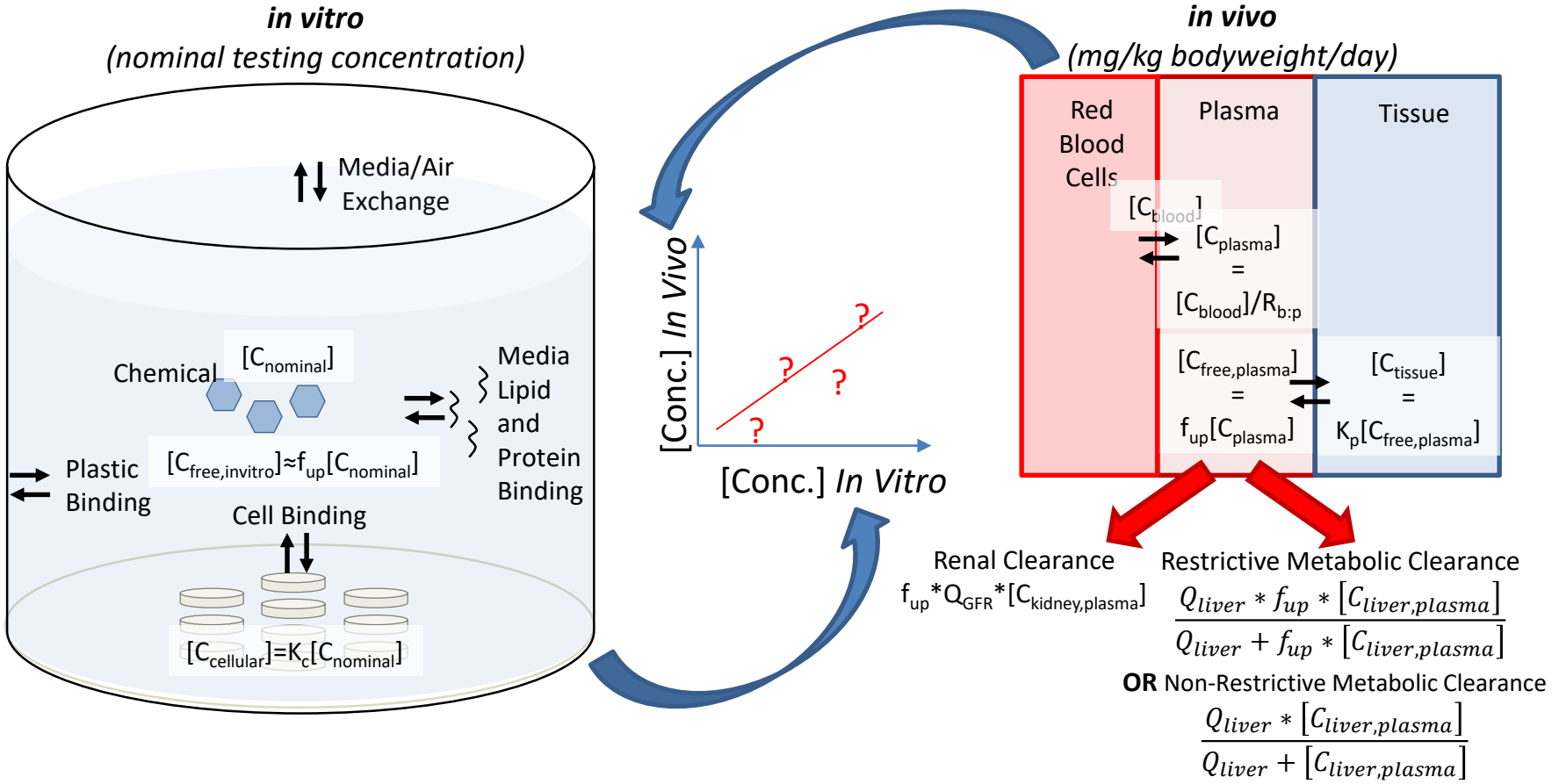


- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al*. (2012)

High-Throughput Toxicokinetics (HTTK) for *In Vitro-In Vivo* Extrapolation (IVIVE)

Using the generic HTTK physiologically based toxicokinetics model to inform IVIVE…



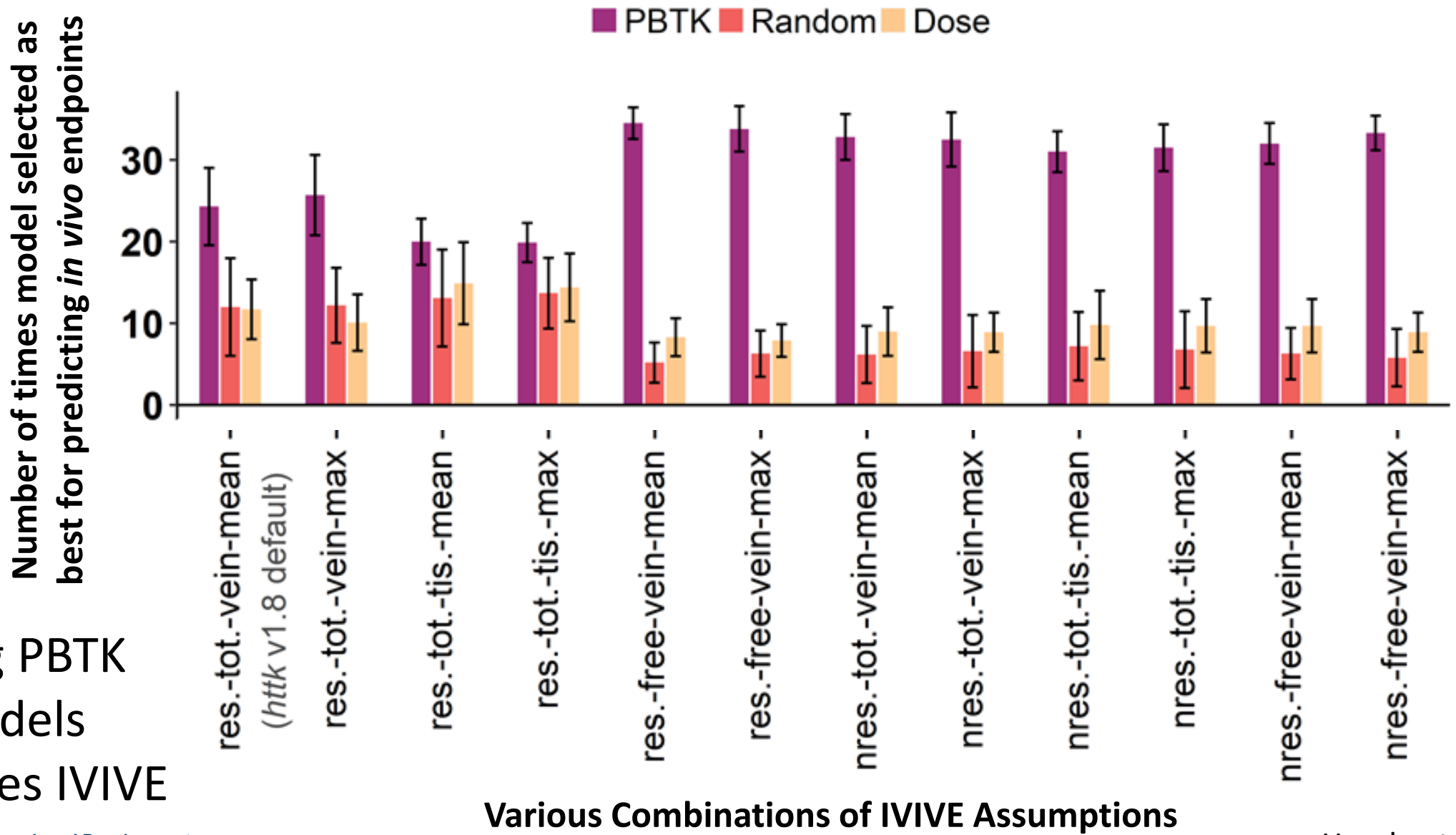
$$\frac{Q_{liver} * f_{up} * [C_{liver,plasma}]}{Q_{liver} + f_{up} * [C_{liver,plasma}]}$$

$$\frac{Q_{liver} * [C_{liver,plasma}]}{Q_{liver} + [C_{liver,plasma}]}$$

Selecting the appropriate *in vitro* and *in vivo* concentrations for extrapolation

Honda et al. (2019)

Optimizing HTTK-based IVIVE

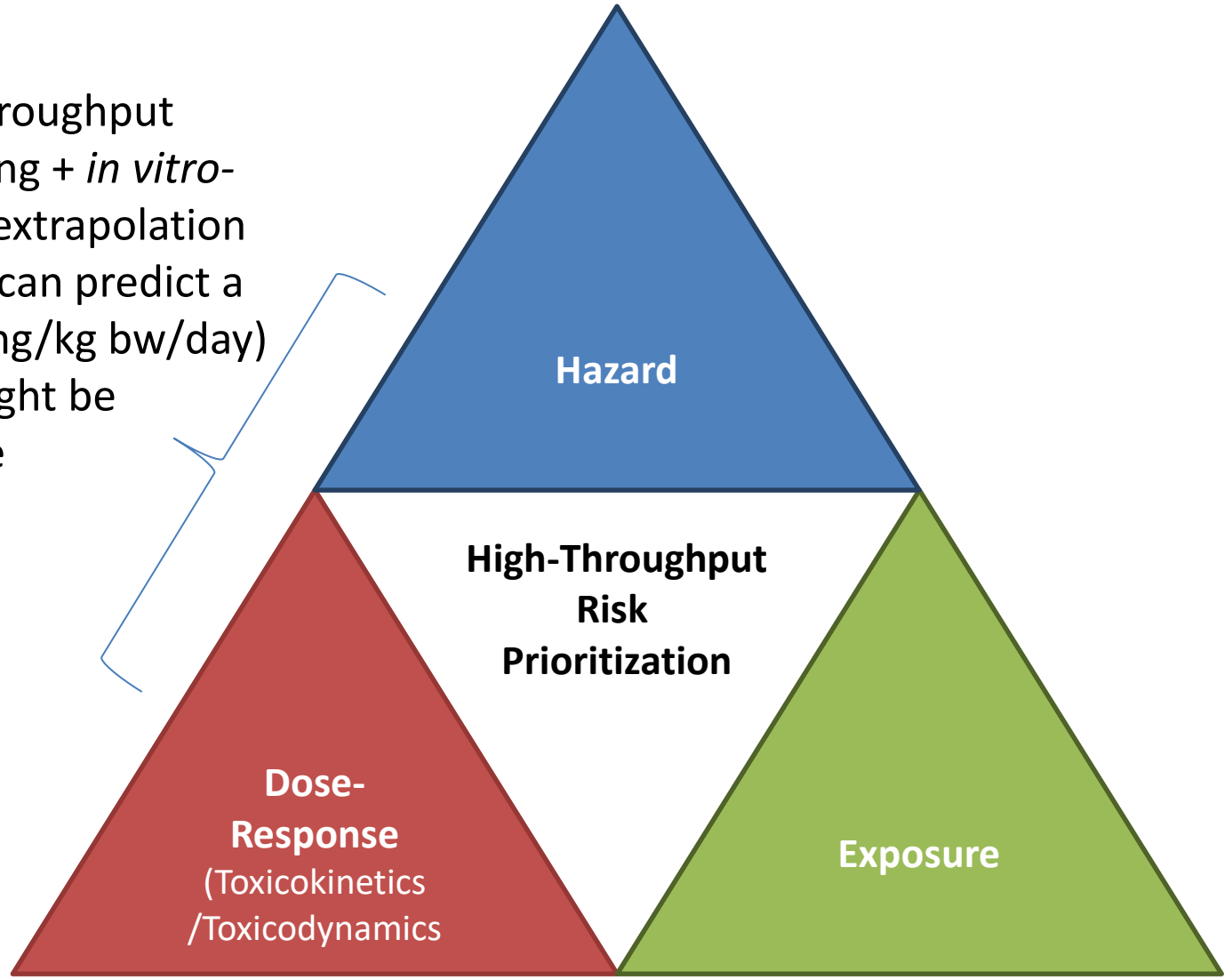


Using PBTK Models Improves IVIVE

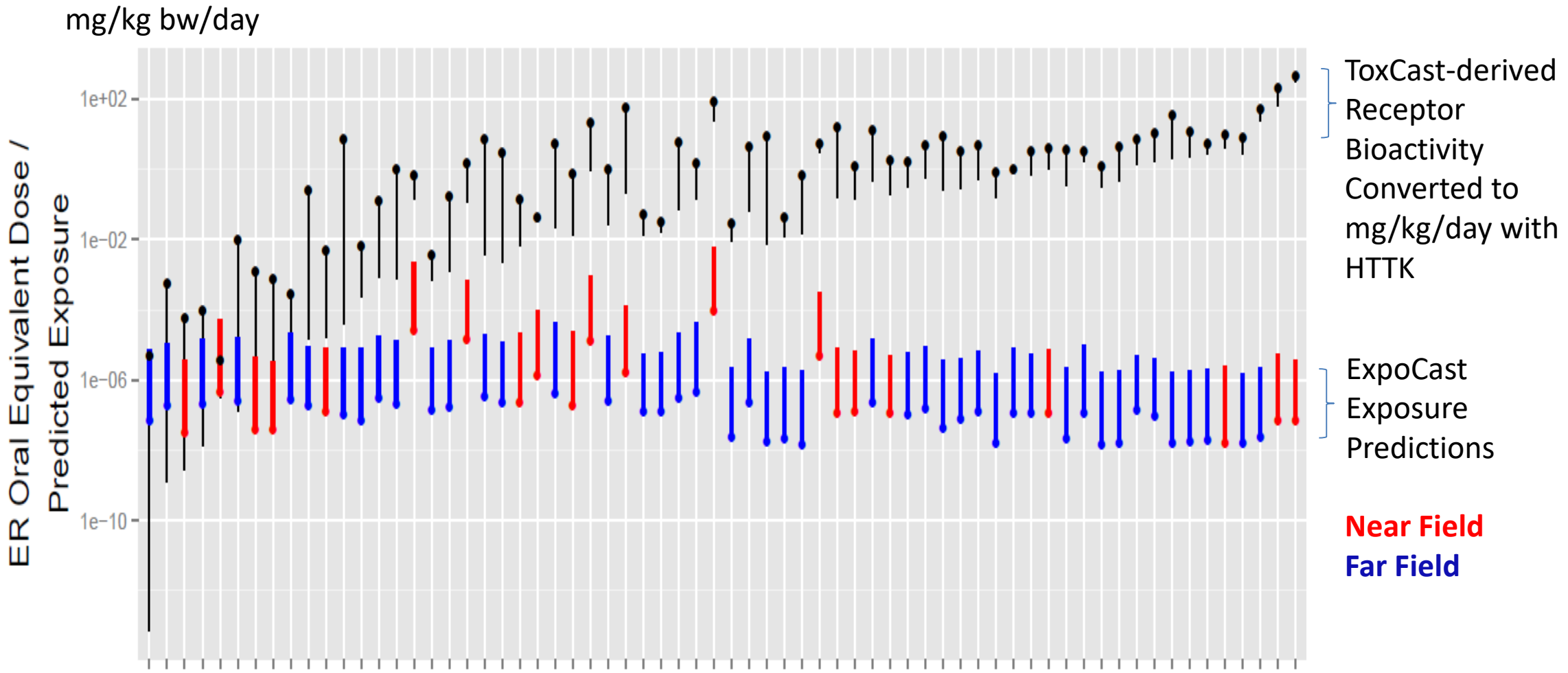
Honda et al. (2019)

New Exposure Data and Models

High throughput screening + *in vitro-in vivo* extrapolation (IVIVE) can predict a dose (mg/kg bw/day) that might be adverse

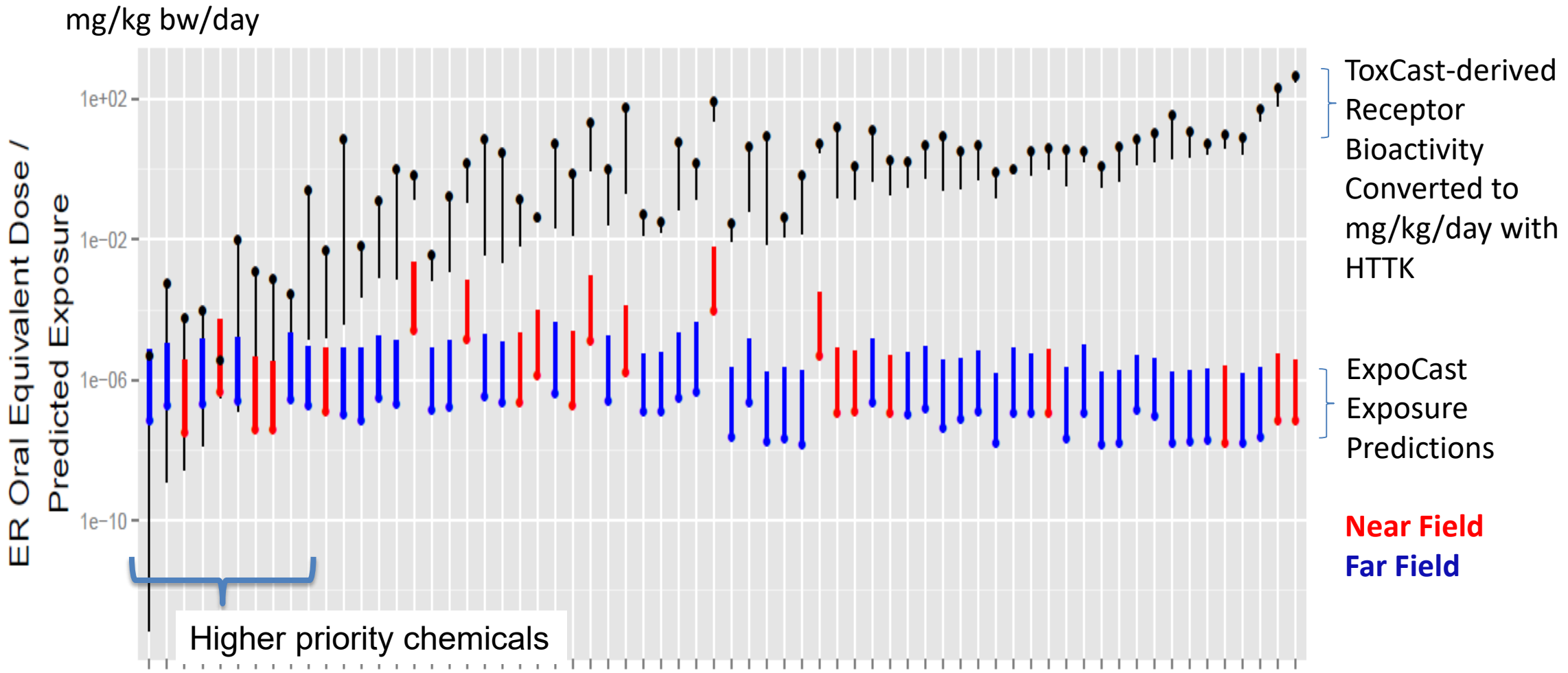


High Throughput Risk Prioritization in Practice



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

High Throughput Risk Prioritization in Practice



December, 2014 Panel:
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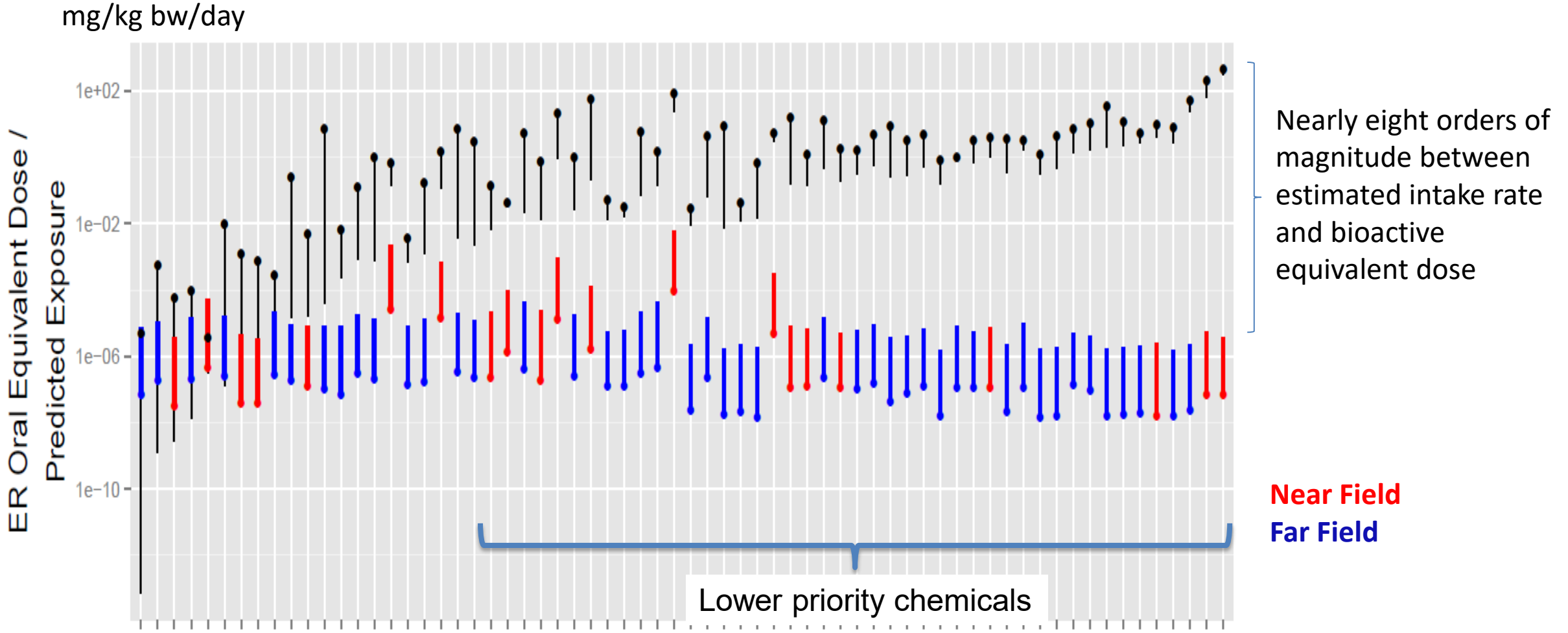
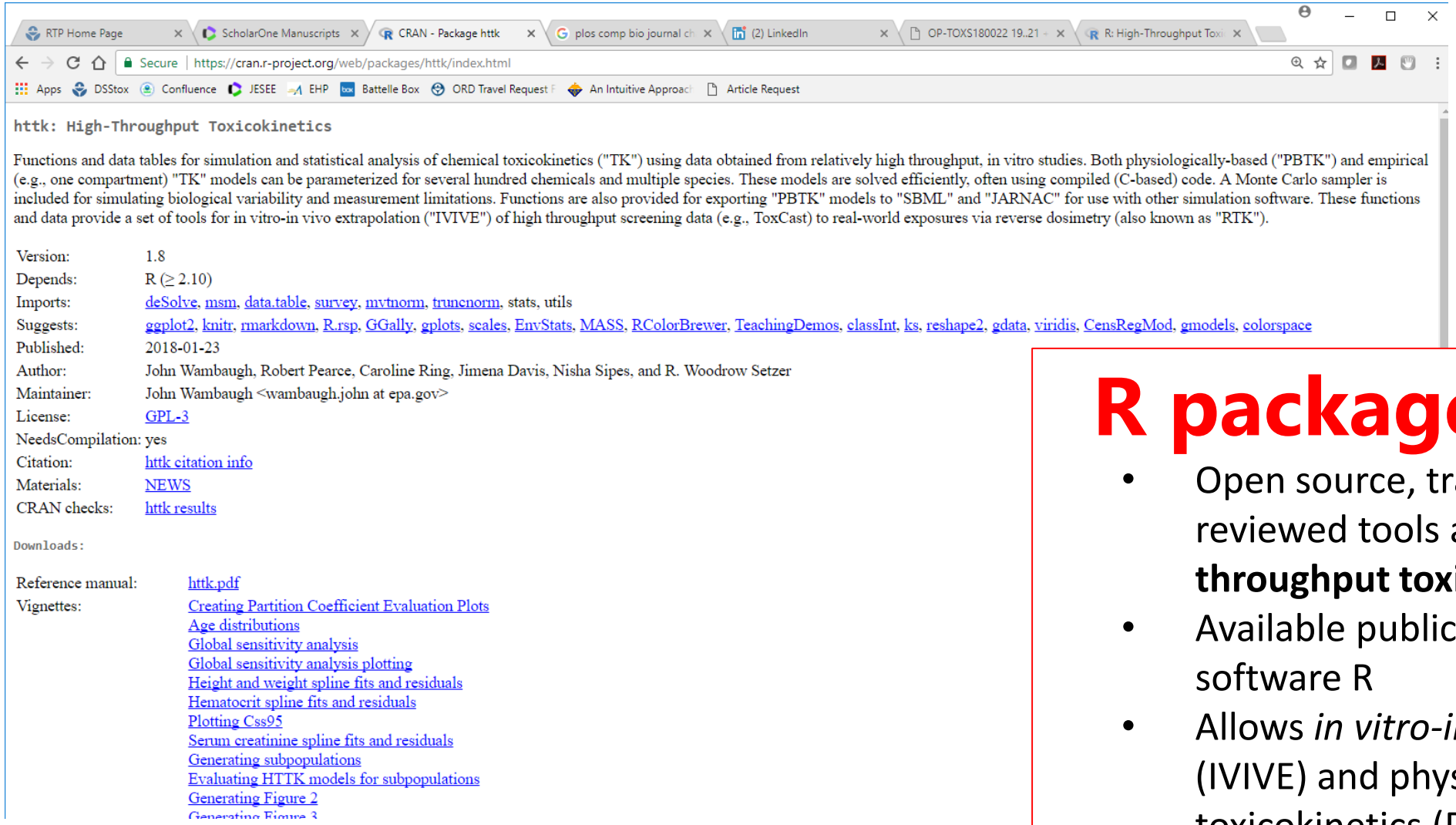
High Throughput Risk Prioritization in Practice



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

Open Source Tools and Data for HTTK

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



RTP Home Page | ScholarOne Manuscripts | CRAN - Package httk | plos comp bio journal ch | (2) LinkedIn | OP-TOXS180022 19..21 | R: High-Throughput Toxi

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

Apps | DSStox | Confluence | JESEE | EHP | Battelle Box | ORD Travel Request F | An Intuitive Approach | Article Request

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:	1.8
Depends:	R (≥ 2.10)
Imports:	deSolve, msm, data.table, survey, mvtnorm, truncnorm, stats, utils
Suggests:	ggplot2, knitr, rmarkdown, R.rsp, GGally, gplots, scales, EnvStats, MASS, RColorBrewer, TeachingDemos, classInt, ks, reshape2, gdata, viridis, CensRegMod, gmodels, colorspace
Published:	2018-01-23
Author:	John Wambaugh, Robert Pearce, Caroline Ring, Jimena Davis, Nisha Sipes, and R. Woodrow Setzer
Maintainer:	John Wambaugh <wambaugh.john at epa.gov>
License:	GPL-3
NeedsCompilation:	yes
Citation:	httk citation info
Materials:	NEWS
CRAN checks:	httk results

Downloads:

Reference manual:	httk.pdf
Vignettes:	Creating Partition Coefficient Evaluation Plots Age distributions Global sensitivity analysis Global sensitivity analysis plotting Height and weight spline fits and residuals Hematocrit spline fits and residuals Plotting Css95 Serum creatinine spline fits and residuals Generating subpopulations Evaluating HTTK models for subpopulations Generating Figure 2 Generating Figure 3

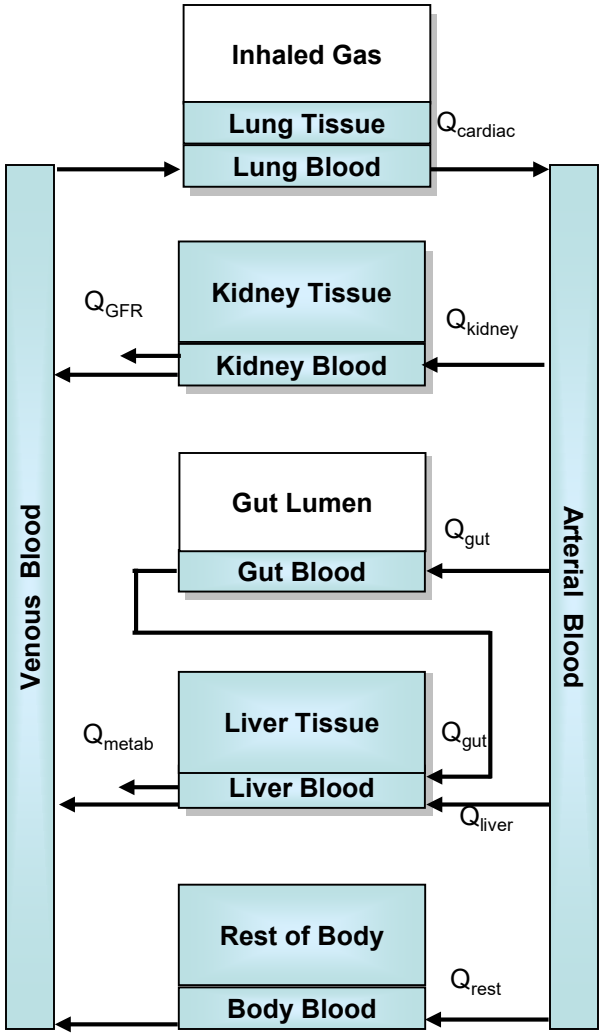
R package "httk"

- Open source, transparent, and peer-reviewed tools and data for **high throughput toxicokinetics (httk)**
- Available publicly for free statistical software R
- Allows *in vitro-in vivo* extrapolation (IVIVE) and physiologically-based toxicokinetics (PBTK)

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 load specific (older) versions of the package
- You can use specific demographics in the population simulator (Ring et al., 2017)
- You can control the built in random number generator to reproduce the same random sequence (function set.seed())

A General Physiologically-based Toxicokinetic (PBTK) Model



- "httk" 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.
- The only ways 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 (which filter into the lumen of the kidney).

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
Reference	Jamei et al. (2009)	Lukacova et al., (2009)	Loizou et al. (2011)	Jongeneelen et al., (2013)	Pearce et al. (2017a)
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

Oral Equivalent Dose Examples

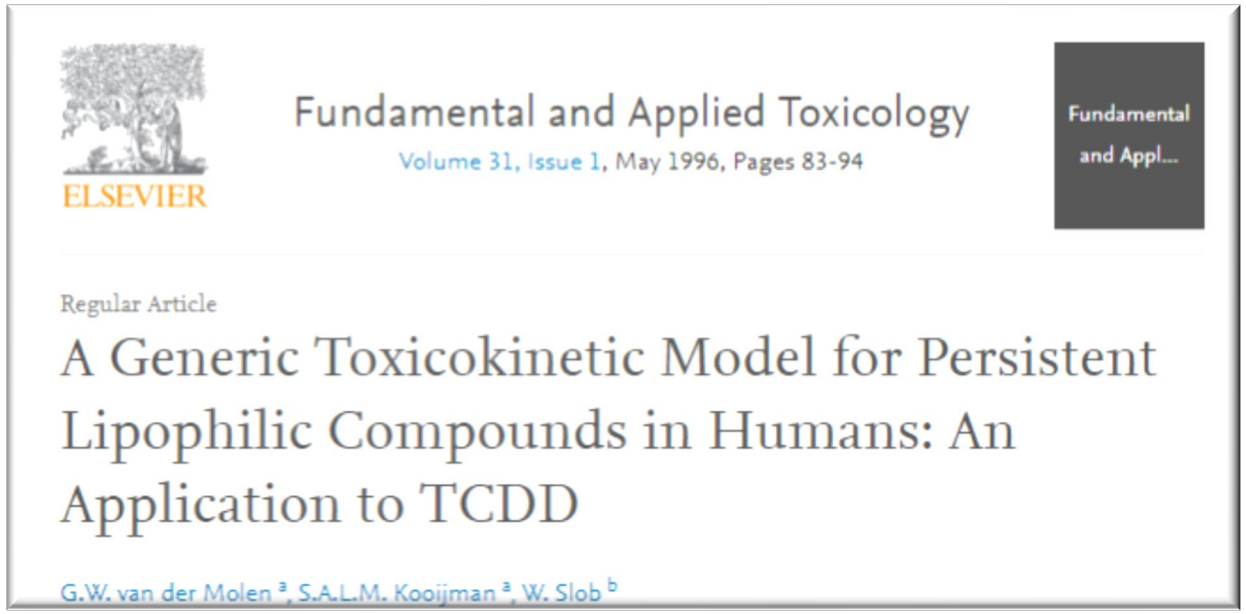
```
#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.95 quantile, for Acetochlor (published value):
get_wetmore_oral_equiv(0.1,chem.cas="34256-82-1")

#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")

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.05, 0.5, and 0.95 quantile, for Acetochlor (published values):
get_wetmore_oral_equiv(0.1,chem.cas="34256-82-1",which.quantile=c(0.05,0.5,0.95))

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.05, 0.5, and 0.95 quantiles, for Acetochlor (calculated value):
calc_mc_oral_equiv(0.1,chem.cas="34256-82-1",which.quantile=c(0.05,0.5,0.95))

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

Interspecies Extrapolation Examples

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

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for rat for Acetochlor (should produce errors since
there is no published value, 0.5 quantile only):
get_wetmore_css(chem.cas="34256-82-1",species="Rat")

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for rat for Acetochlor (calculated value):
calc_mc_css(chem.cas="34256-82-1",species="Rat")

#Steady-state concentration (uM) for 1 mg/kg/day for 0.5 quantile for rat for Acetochlor (published value):
get_wetmore_css(chem.cas="34256-82-1",species="Rat",which.quantile=0.5)

#Steady-state concentration (uM) for 1 mg/kg/day for 0.5 quantile for rat for Acetochlor (calculated value):
calc_mc_css(chem.cas="34256-82-1",species="Rat",which.quantile=0.5)

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for mouse for Acetochlor (should produce error since
there is no published value, human and rat only):
get_wetmore_css(chem.cas="34256-82-1",species="Mouse")

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for  mouse for Acetochlor (calculated value):
calc_mc_css(chem.cas="34256-82-1",species ="Mouse")
calc_mc_css(chem.cas="34256-82-1",species ="Mouse",default.to.human=T)
```

Generic PBTK Models

There is nothing new about the idea of generic PBTK models…



ELSEVIER

Fundamental and Applied Toxicology

Volume 31, Issue 1, May 1996, Pages 83-94

Fundamental and Appl...

Regular Article

A Generic Toxicokinetic Model for Persistent Lipophilic Compounds in Humans: An Application to TCDD

G.W. van der Molen [a], S.A.L.M. Kooijman [a], W. Slob [b]

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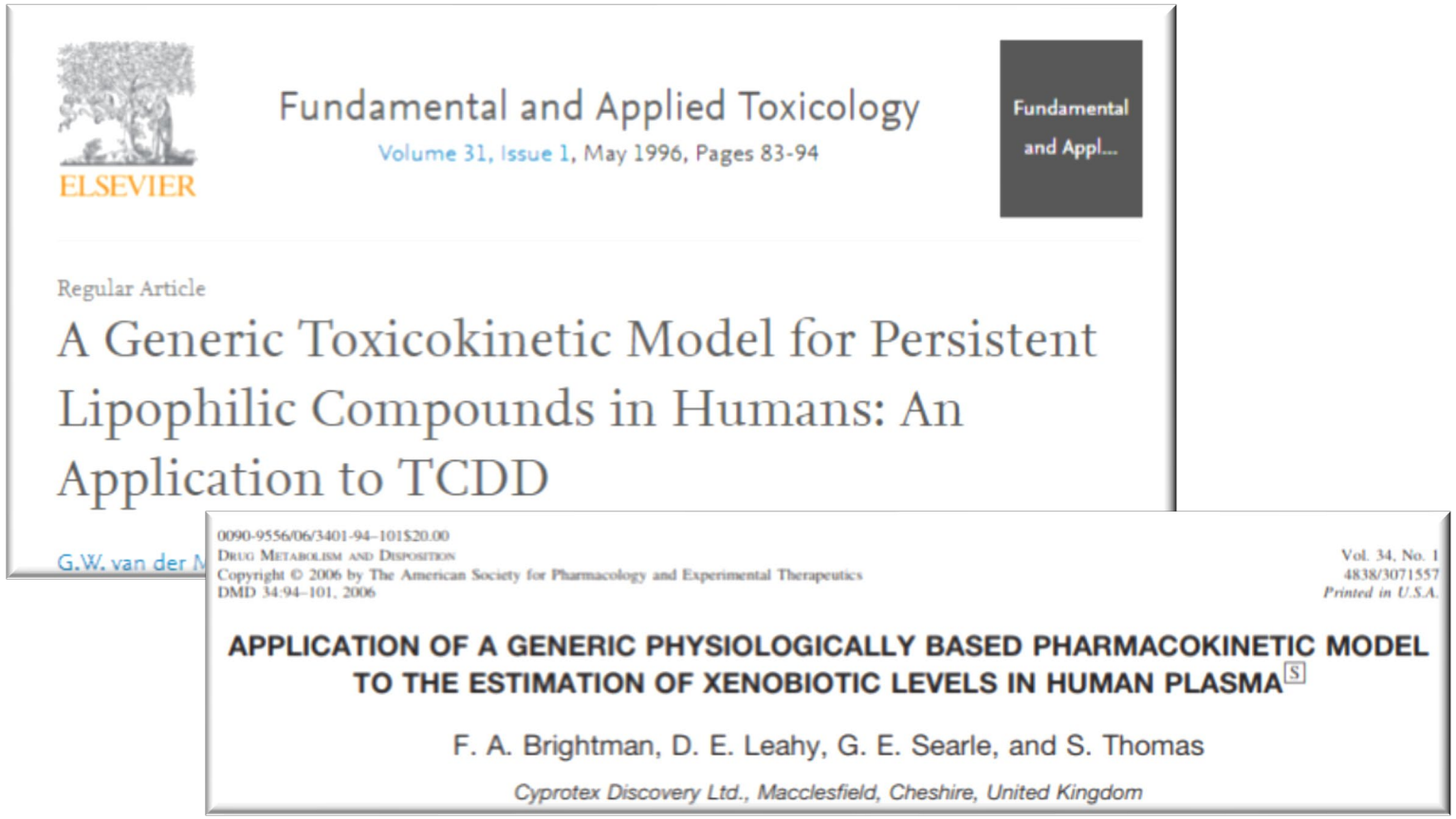

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DMD 34:94–101, 2006

Vol. 34, No. 1
4838/3071557
Printed in U.S.A.

APPLICATION OF A GENERIC PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL TO THE ESTIMATION OF XENOBIOTIC LEVELS IN HUMAN PLASMA[S]

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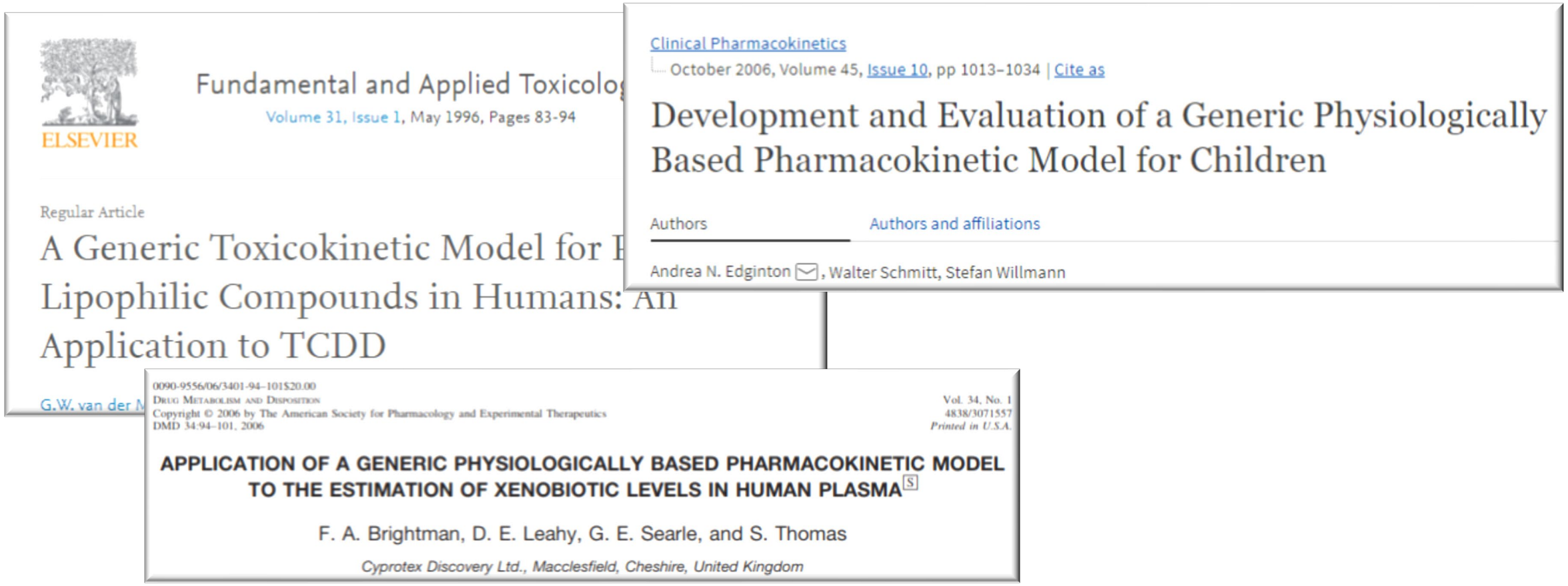

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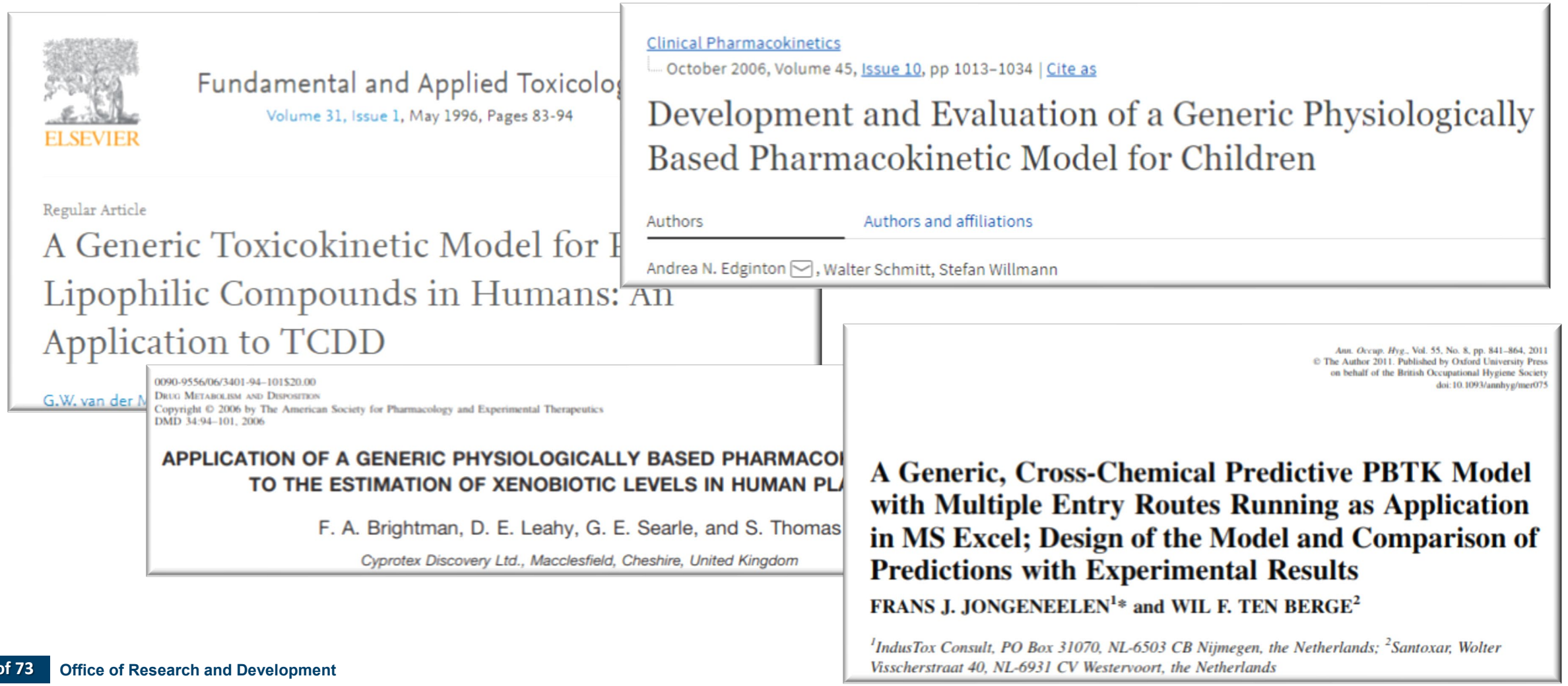

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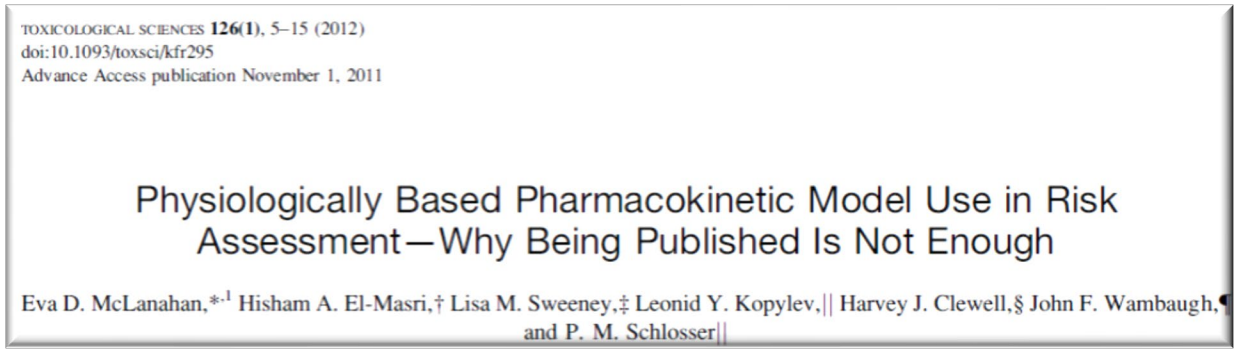
Ann. Occup. Hyg., Vol. 55, No. 8, pp. 841–864, 2011
© The Author 2011. Published by Oxford University Press on behalf of the British Occupational Hygiene Society
doi:10.1093/annhyg/mer075

A Generic, Cross-Chemical Predictive PBTK Model with Multiple Entry Routes Running as Application in MS Excel; Design of the Model and Comparison of Predictions with Experimental Results

FRANS J. JONGENEELEN[1]* and WIL F. TEN BERGE[2]

[1]IndusTox Consult, PO Box 31070, NL-6503 CB Nijmegen, the Netherlands; [2]Santoxar, Wolter Visscherstraat 40, NL-6931 CV Westervoort, the Netherlands

Open Source, Verifiable, Reproducible

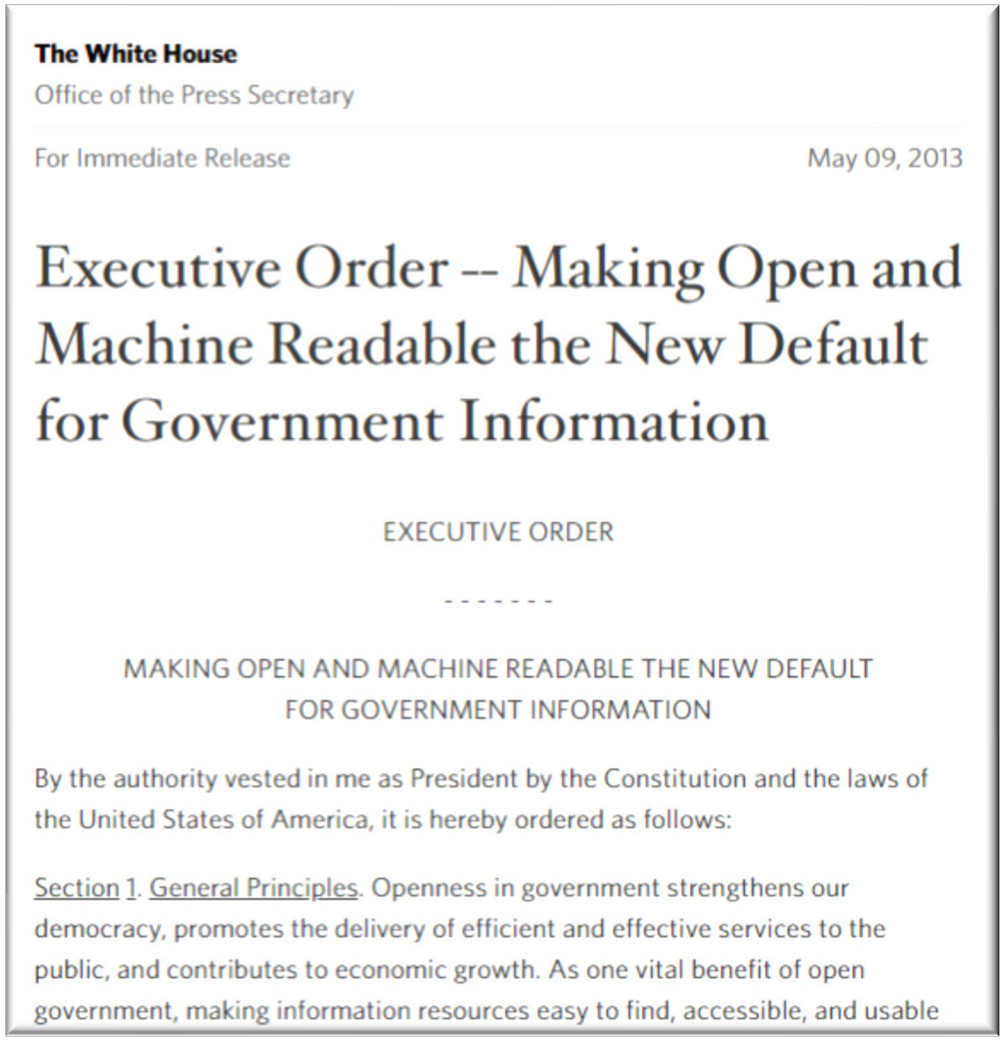


TOXICOLOGICAL SCIENCES 126(1), 5–15 (2012)
doi:10.1093/toxsci/kfr295
Advance Access publication November 1, 2011

Physiologically Based Pharmacokinetic Model Use in Risk Assessment—Why Being Published Is Not Enough

Eva D. McLanahan,*[,1] Hisham A. El-Masri,† Lisa M. Sweeney,‡ Leonid Y. Kopylev,|| Harvey J. Clewell,§ John F. Wambaugh,¶ and P. M. Schlosser||

"Although publication of a PBPK model in a peer-reviewed journal is a mark of good science, subsequent evaluation of published models and the supporting computer code is necessary for their consideration for use in [Human Health Risk Assessments]"



The White House

Office of the Press Secretary

For Immediate Release May 09, 2013

Executive Order -- Making Open and Machine Readable the New Default for Government Information

EXECUTIVE ORDER

- - - - - - -

MAKING OPEN AND MACHINE READABLE THE NEW DEFAULT FOR GOVERNMENT INFORMATION

By the authority vested in me as President by the Constitution and the laws of the United States of America, it is hereby ordered as follows:

Section 1. General Principles. Openness in government strengthens our democracy, promotes the delivery of efficient and effective services to the public, and contributes to economic growth. As one vital benefit of open government, making information resources easy to find, accessible, and usable

"…the default state of new and modernized Government information resources shall be open and machine readable."

Doing Statistical Analysis with HTTK

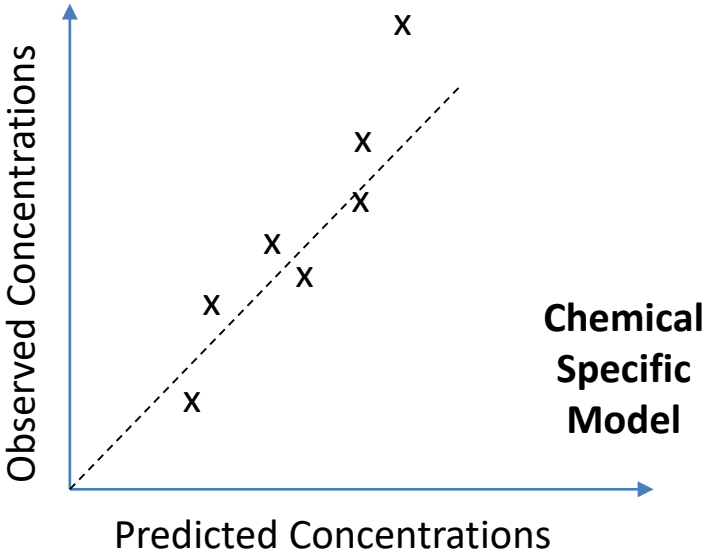
- If we are to use HTTK, we need confidence in predictive ability

- 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**
  - ORD has both compiled existing (literature) TK data (Wambaugh *et al*., 2015) and conducted new experiments in rats on chemicals with HTTK *in vitro* data (Wambaugh *et al*., 2018)
  - Any approximations, omissions, or mistakes should work to increase the estimated uncertainty when evaluated systematically across chemicals

Building Confidence in TK Models

- In order to evaluate 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



Cohen Hubal et al. (2018)

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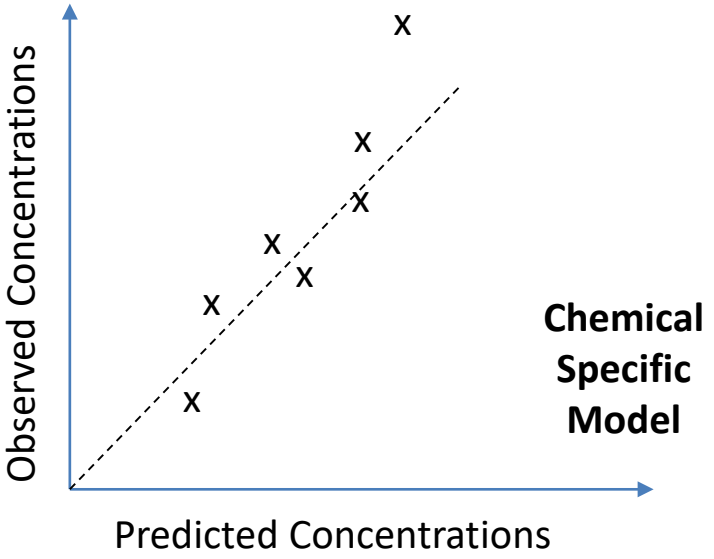
- However, we do not typically have TK data



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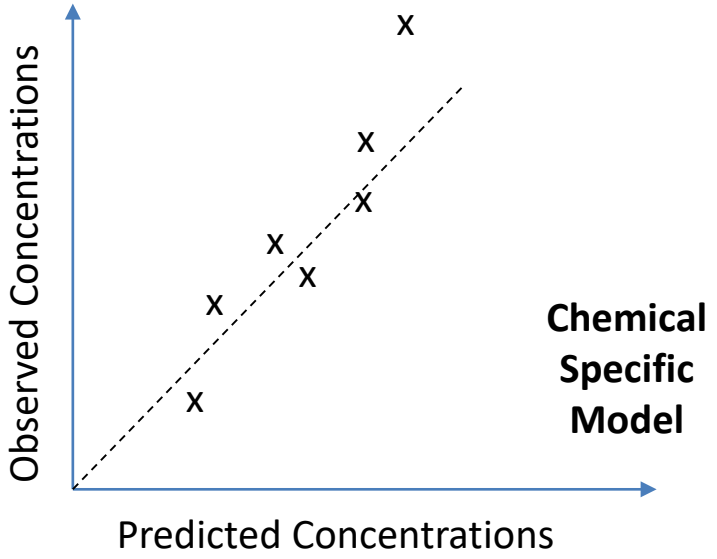
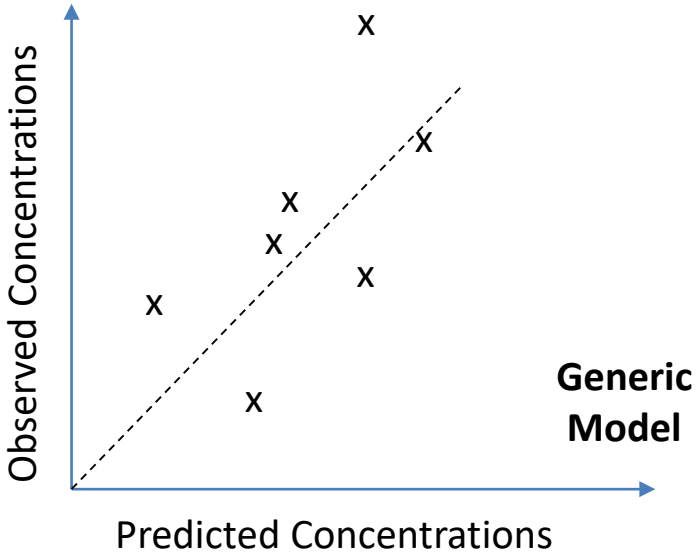
- We can parameterize a **generic TK model**, and evaluate that model for as many chemicals as we do have data
  - We do expect larger uncertainty, but also greater confidence in model implementation
  - Estimate bias and uncertainty, and try to correlate with chemical-specific properties





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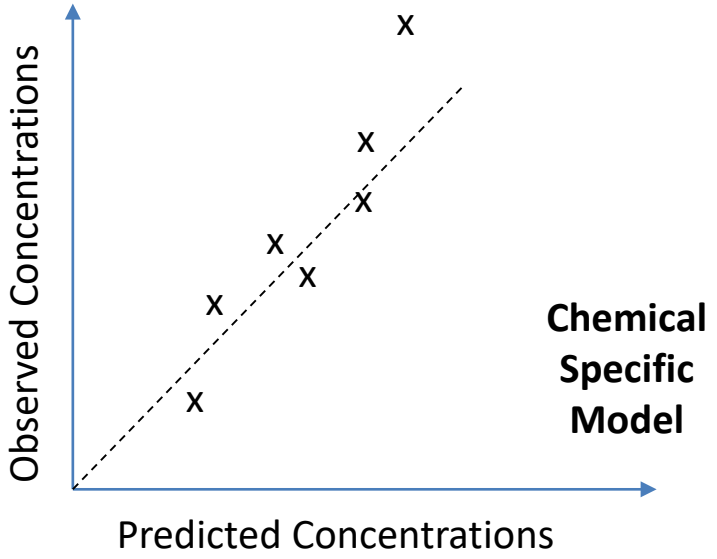

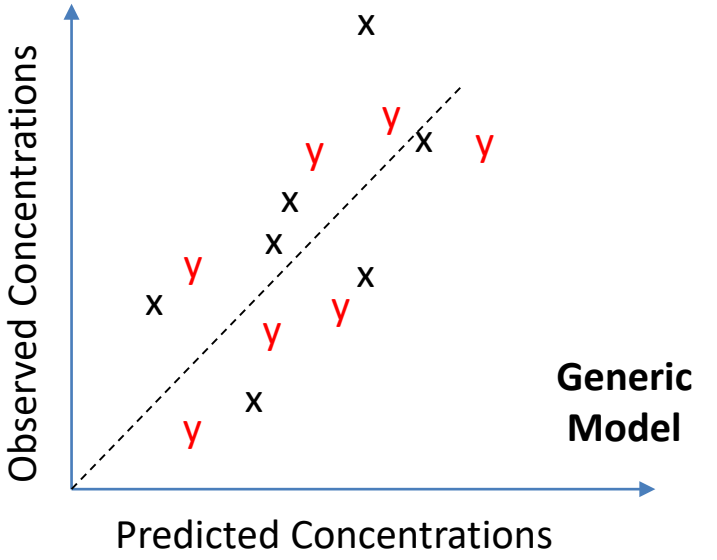

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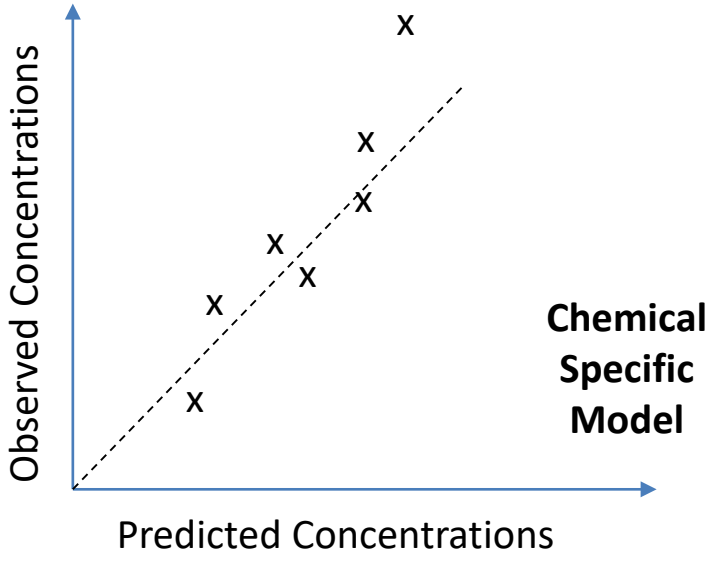
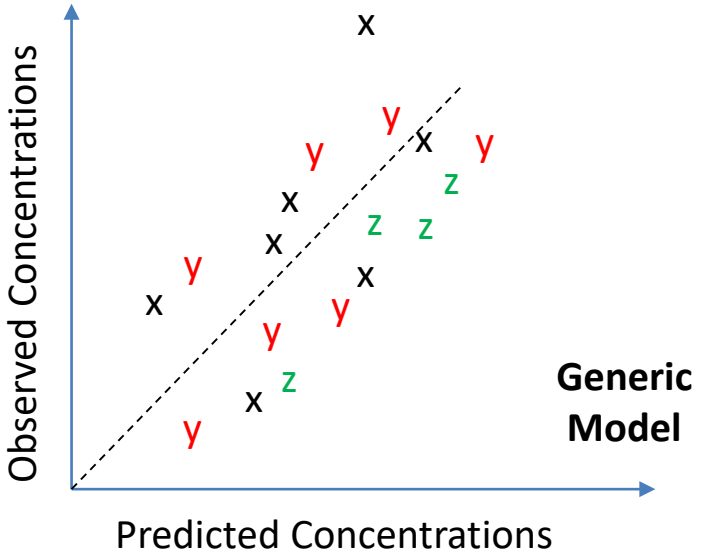




Cohen Hubal et al. (2018)

In Vivo TK Database

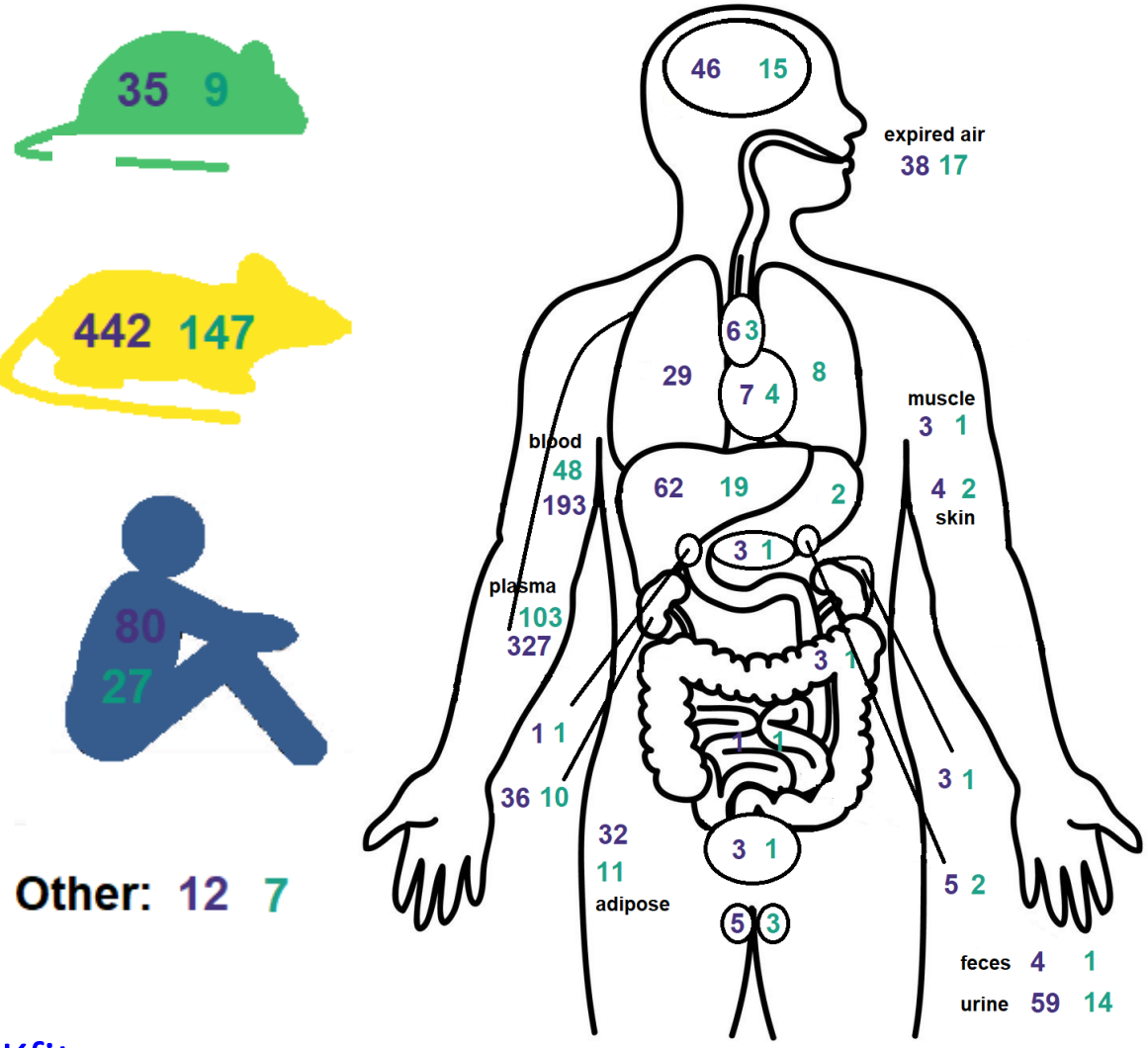
- EPA is developing a **public database** of **concentration vs. time data** for building, calibrating, and evaluating TK models

- Curation and development ongoing, but to date includes:
  - 198 analytes (EPA, National Toxicology Program, literature)
  - Routes: Intravenous, dermal, oral, sub-cutaneous, and inhalation exposure

- Database will be made available through web interface and through the “httk” R package

- Standardized, open source curve fitting software invivoPKfit used to calibrate models to all data:

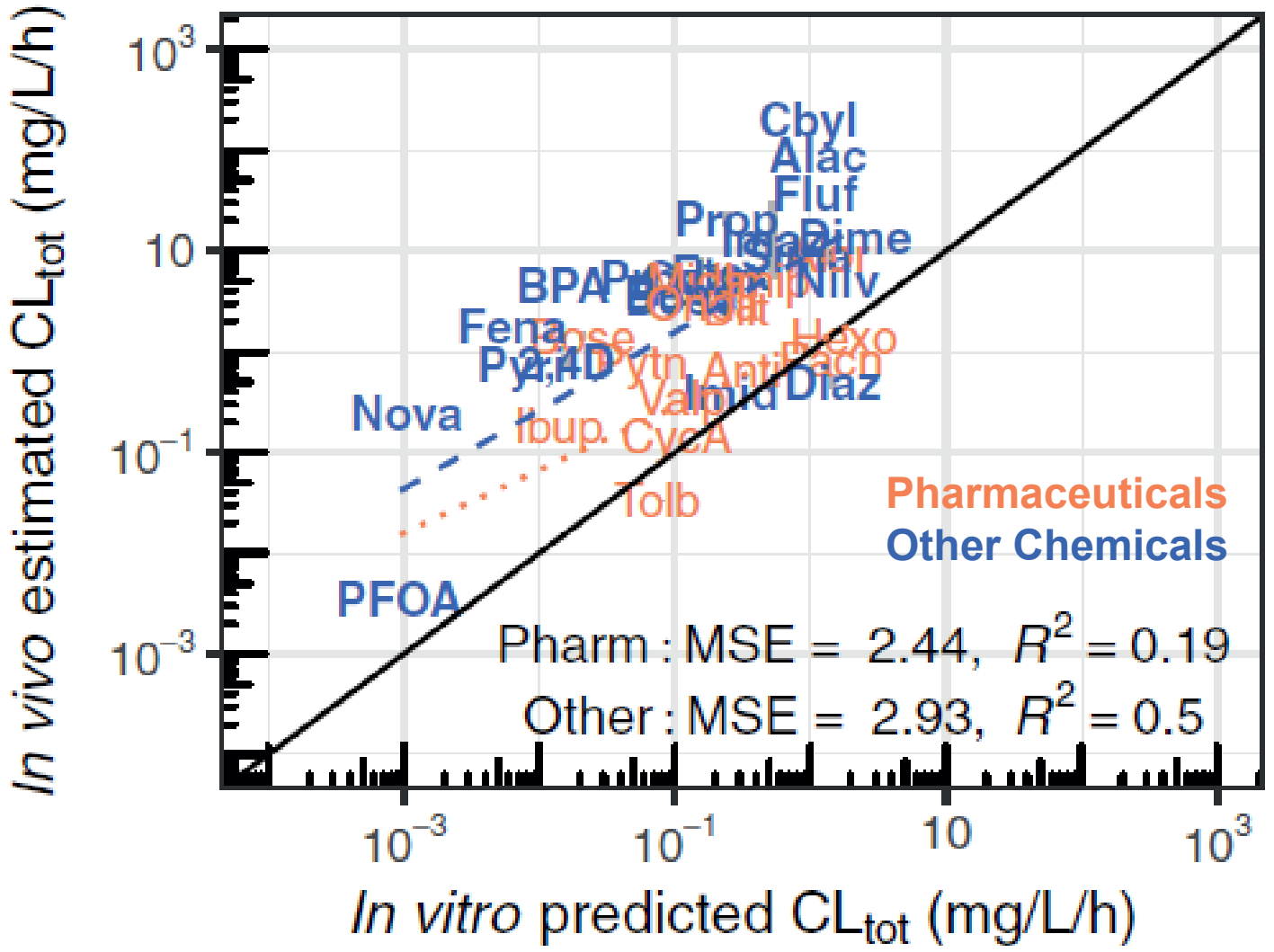
  https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit



Sayre et al. (in preparation)

Observed Total Clearance

- We estimate clearance from two processes – hepatic metabolism (liver) and passive glomerular filtration (kidney)
- This appears to work better for pharmaceuticals than other chemicals:
  - ToxCast chemicals are overestimated
- Non-pharmaceuticals may be subject to extrahepatic metabolism and/or active transport



Wambaugh et al. (2018)

Variability

Different crayons have different colors…

Variability

Different crayons have different colors, and none of them are the "average" color

Population simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters built into R “httk” package (Pearce et al., 2017):

Sample NHANES biometrics for actual individuals:

- Sex
- Race/ethnicity
- Age
- Height
- Weight
- Serum creatinine

Slide from Caroline Ring (ToxStrategies)

Ring *et al.* (2017)

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Regression equations from literature (McNally *et al.*, 2014) (+ residual marginal variability)

(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.)

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Predict physiological quantities

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

Slide from Caroline Ring (ToxStrategies)

Ring *et al.* (2017)

Uncertainty

Until I open the box, I don't know what colors I have...

...especially if my six-year-old has been around.

Analytical Chemistry is an HTTK Bottleneck

- For HTTK we always need to develop a chemical-specific method for quantitating amount of chemical *in vitro*
  - This is very different from HTS where the same readout (e.g., bioluminescence) can be used for most chemicals

- In Wetmore et al. (2012), the rapid equilibrium dialysis (RED) assay (Waters et al. 2008) failed for fraction unbound in plasma (f_{up}) 38% of the chemicals.

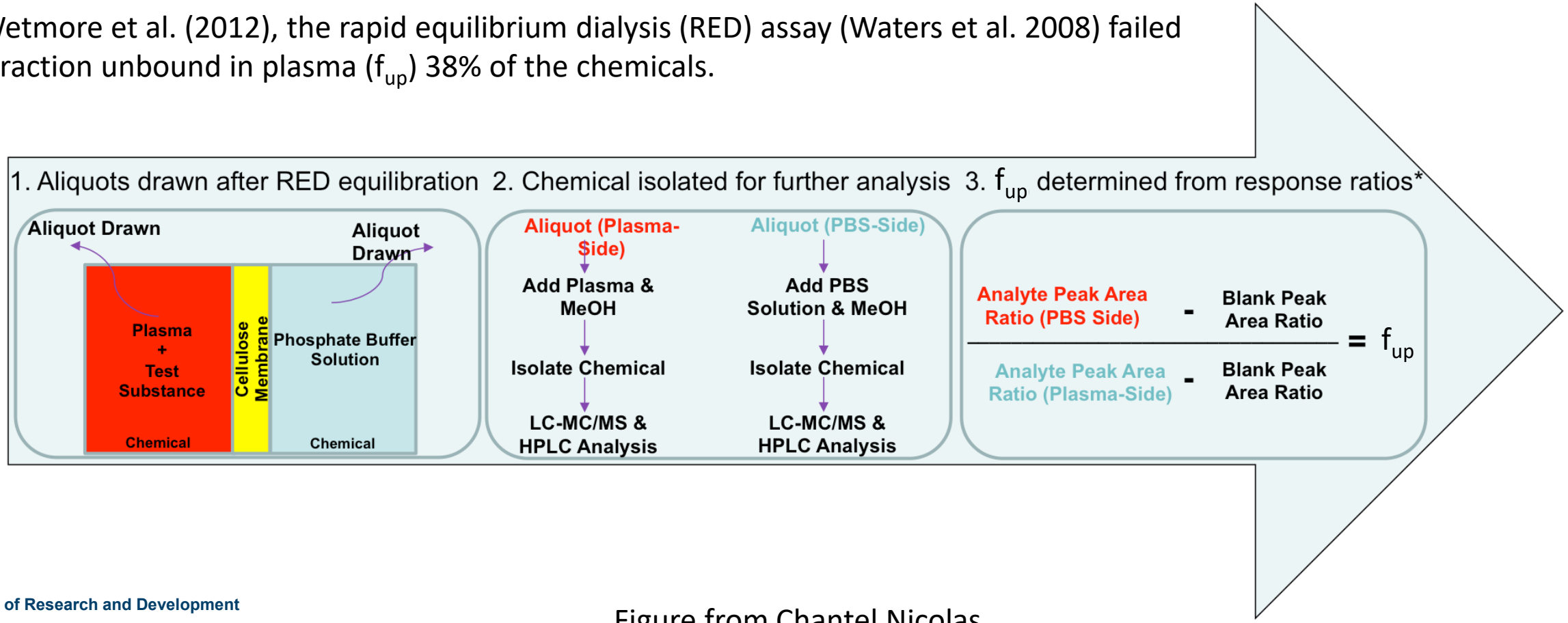
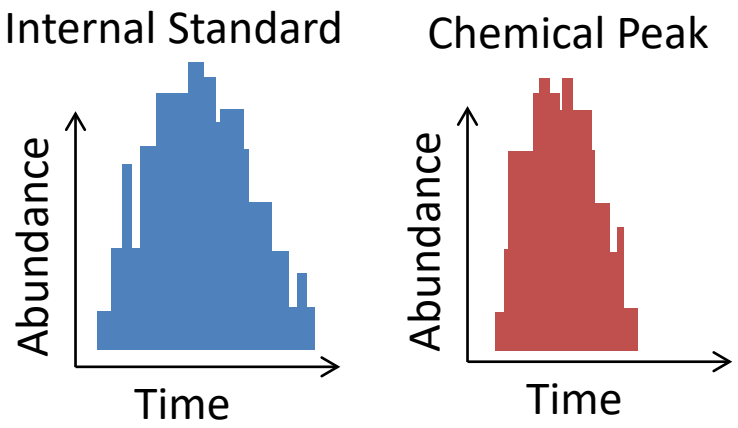


Figure from Chantel Nicolas

New HTTK Measurements and Uncertainty Analysis

The HTTK *in vitro* assays need to measure differences in chemical concentration



- Area of the internal standard (ITSD) at a known, fixed concentration fluctuates with time
- Find a peak that corresponds to chemical of interest, and then follow the ratio R of the chemical peak to the ITSD

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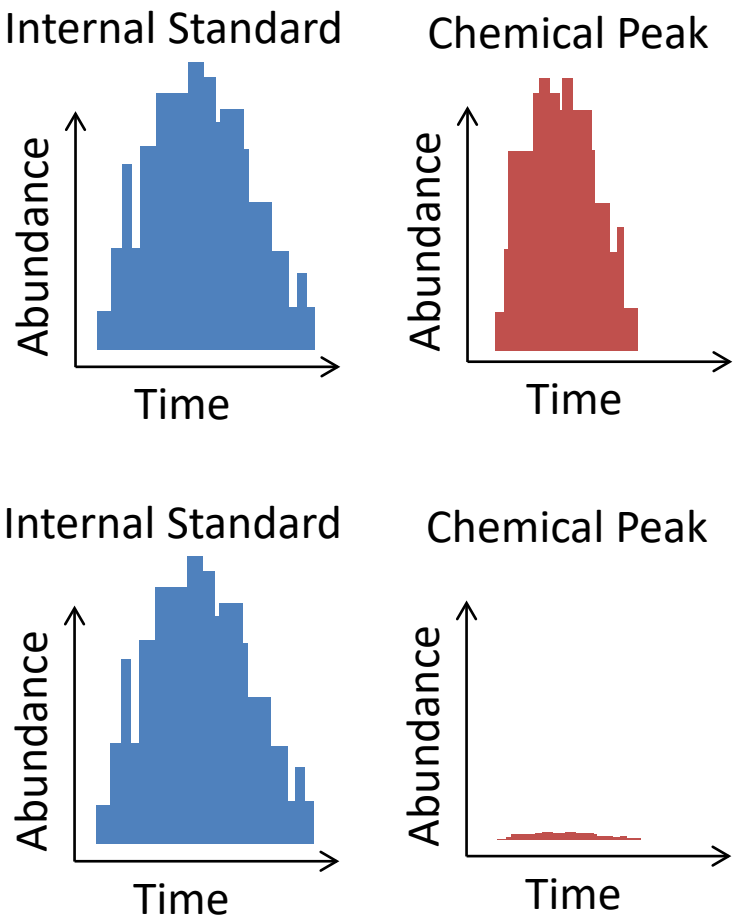
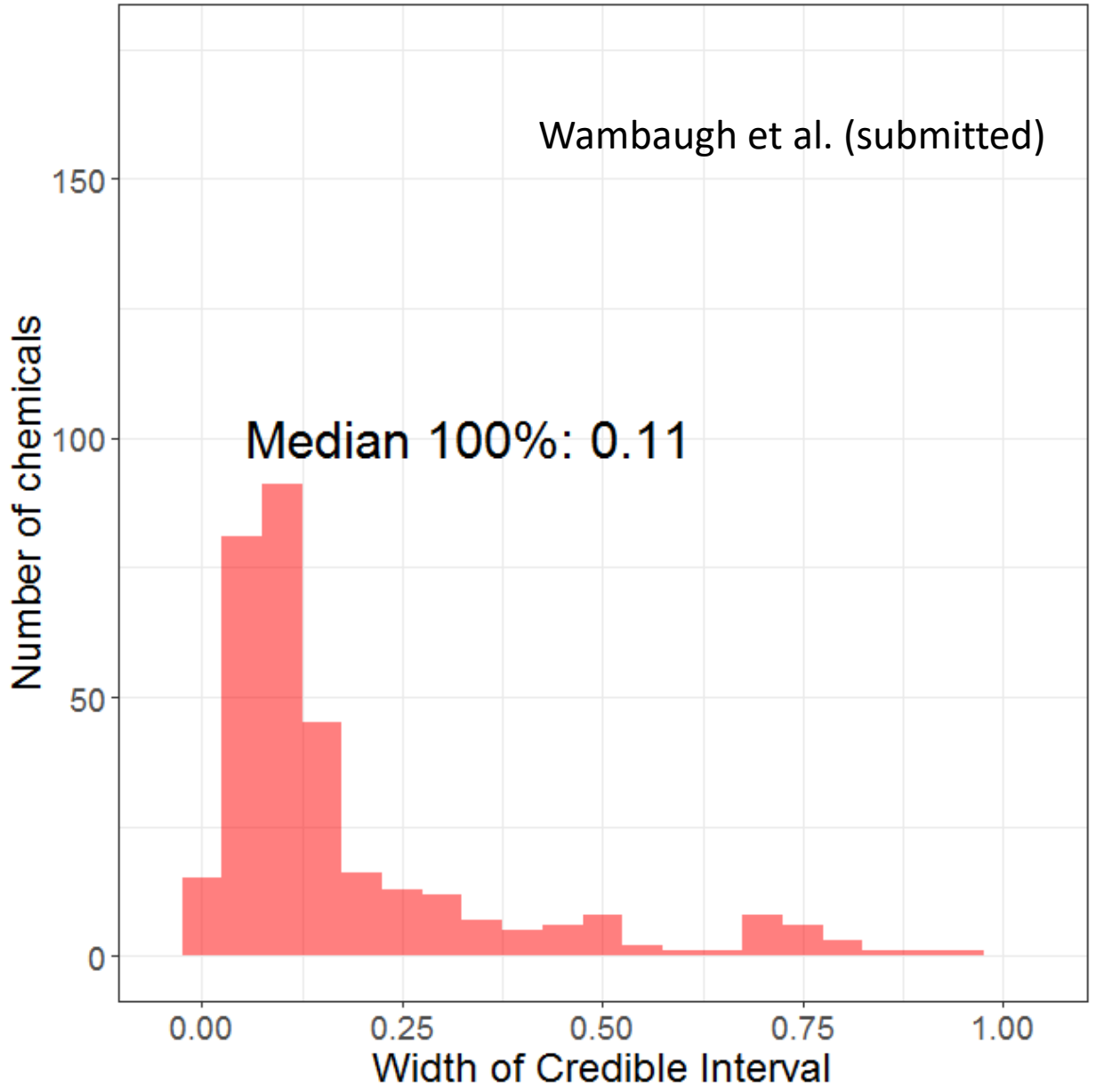


- Area of the internal standard (ITSD) at a known, fixed concentration fluctuates with time
- Find a peak that corresponds to chemical of interest, and then follow the ratio R of the chemical peak to the ITSD
- For new measurements HTTK (>200 compounds to data) performed by Cyprotex, we have modified RED protocol to use a titration of plasma protein (10%, 30%, 100%) of physiological concentration
    - Keeps chemical concentration in the same range
- Analyzed data in Bayesian framework that included a model for analytical chemistry
    - Bayesian approach gives a credible interval (range of values that would be consistent with the data) – quantitative uncertainty

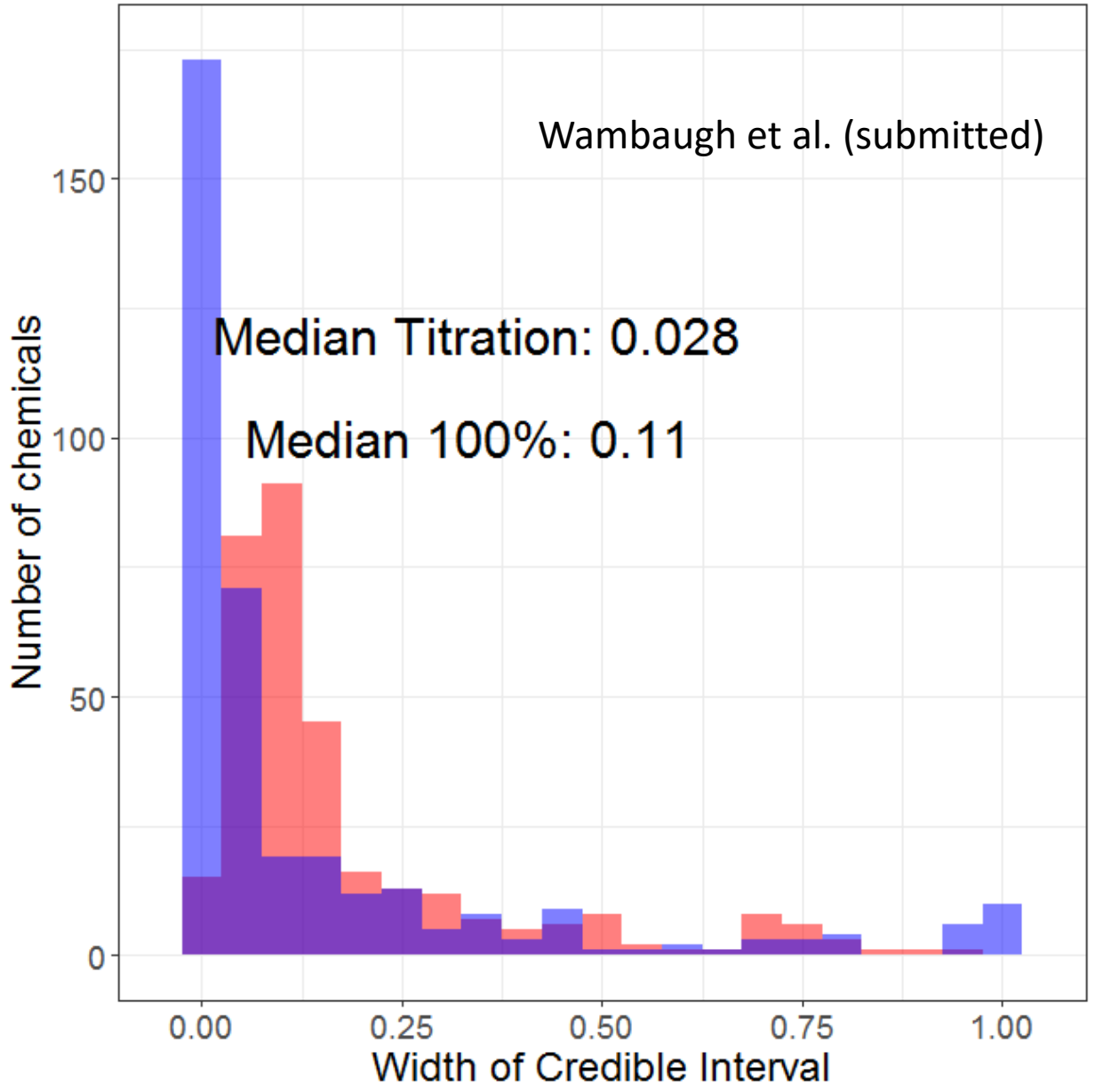
New Plasma Binding Protocol Reduces Uncertainty

- New protocol performs assay at 100%, 30%, and 10% of physiologic protein concentration
- Median uncertainty for 100% physiological concentration only: +-5.5%



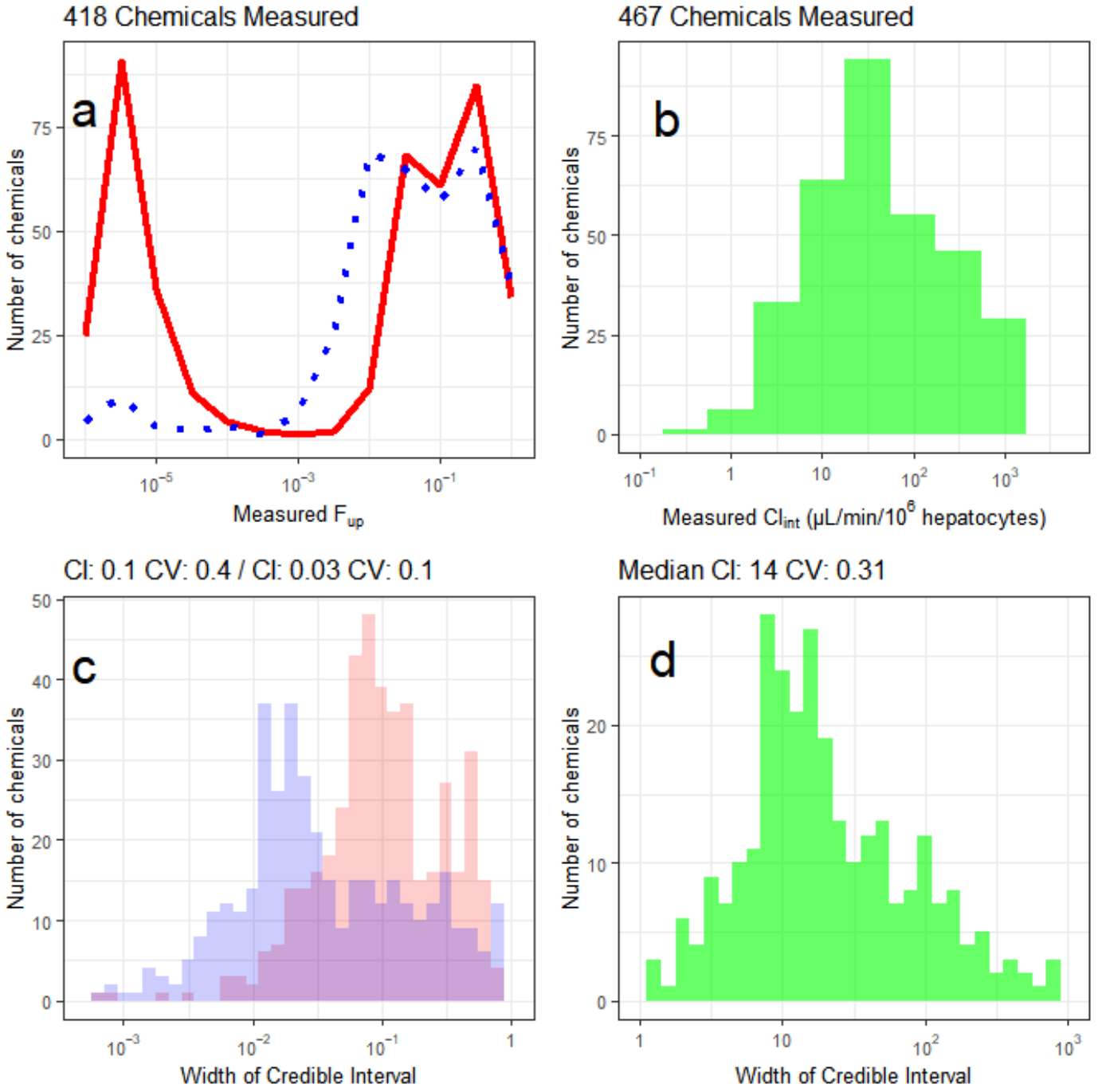
New Plasma Binding Protocol Reduces Uncertainty

- New protocol performs assay at 100%, 30%, and 10% of physiologic protein concentration
- Median uncertainty for 100% physiological concentration only: +-5.5%
- Median uncertainty for three-point assay: +-1.4%

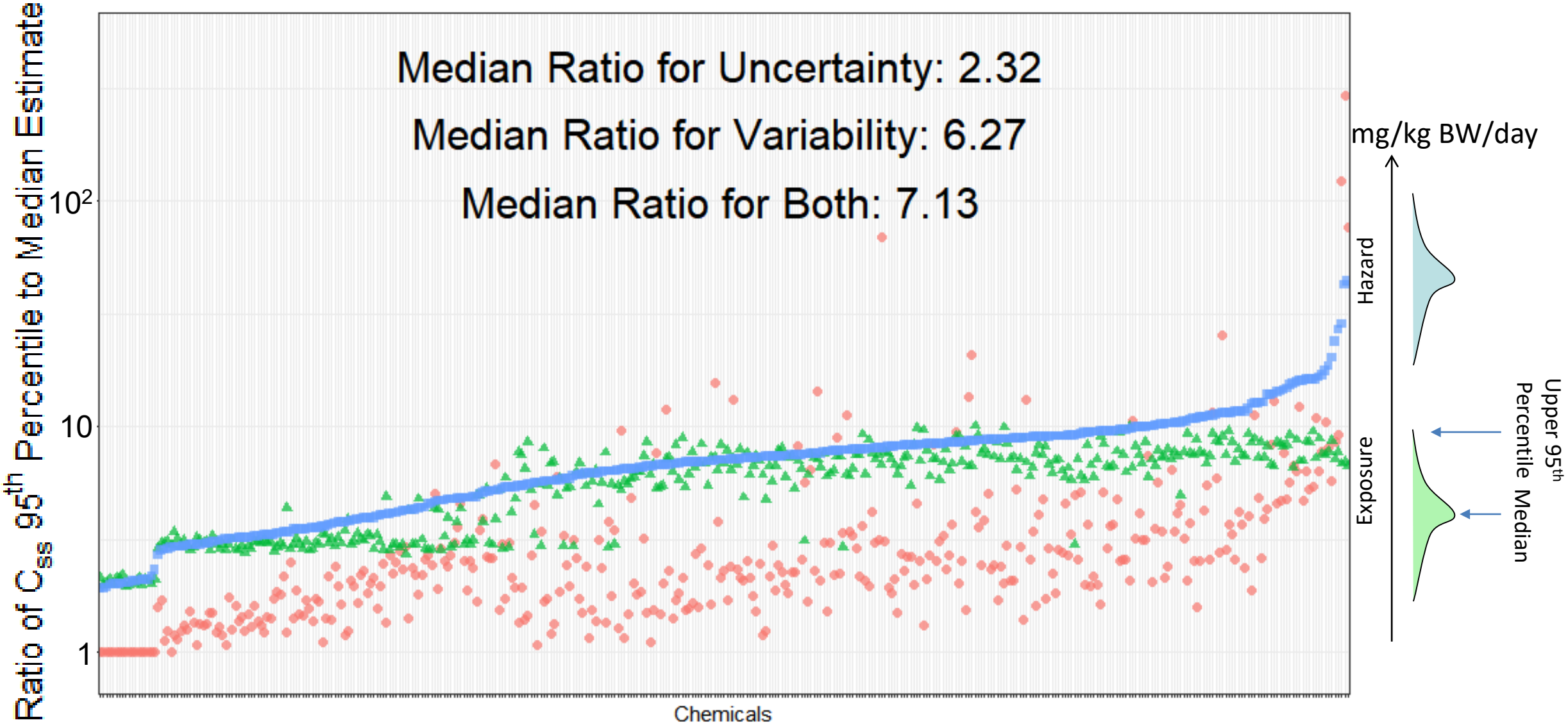


New Data!

New experimental measurements of f_{up} and Cl_{int} are reported for 418 and 467 chemicals, respectively. These data raise the HTTK chemical coverage of the ToxCast Phase I and II libraries to 57%.

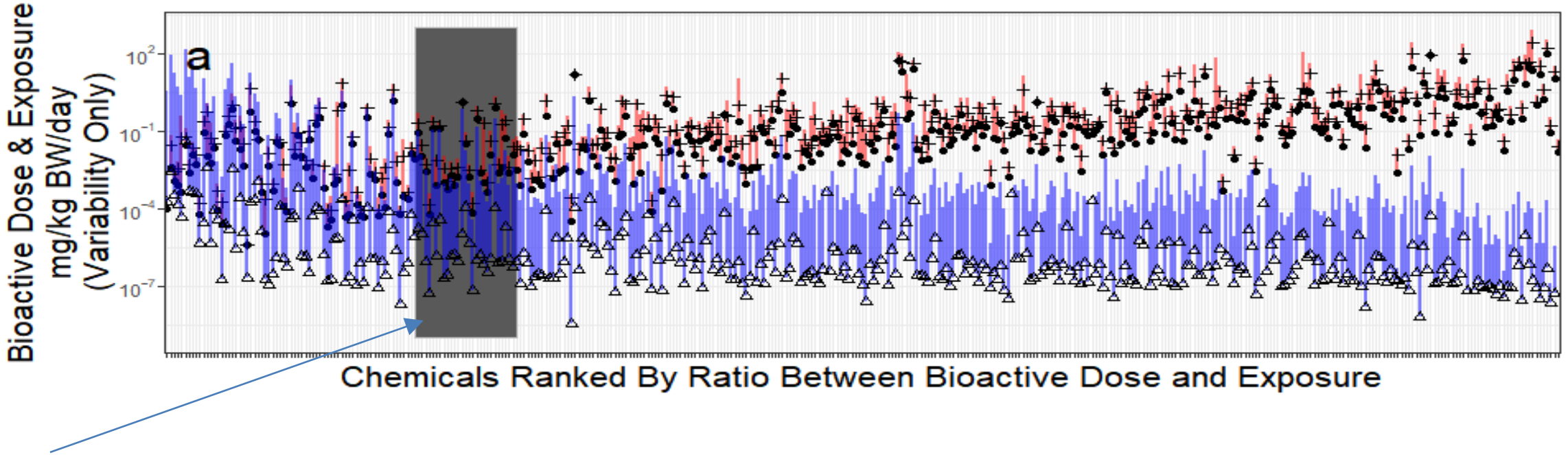


Quantifying the Impact of Uncertainty



Wambaugh et al. (submitted)

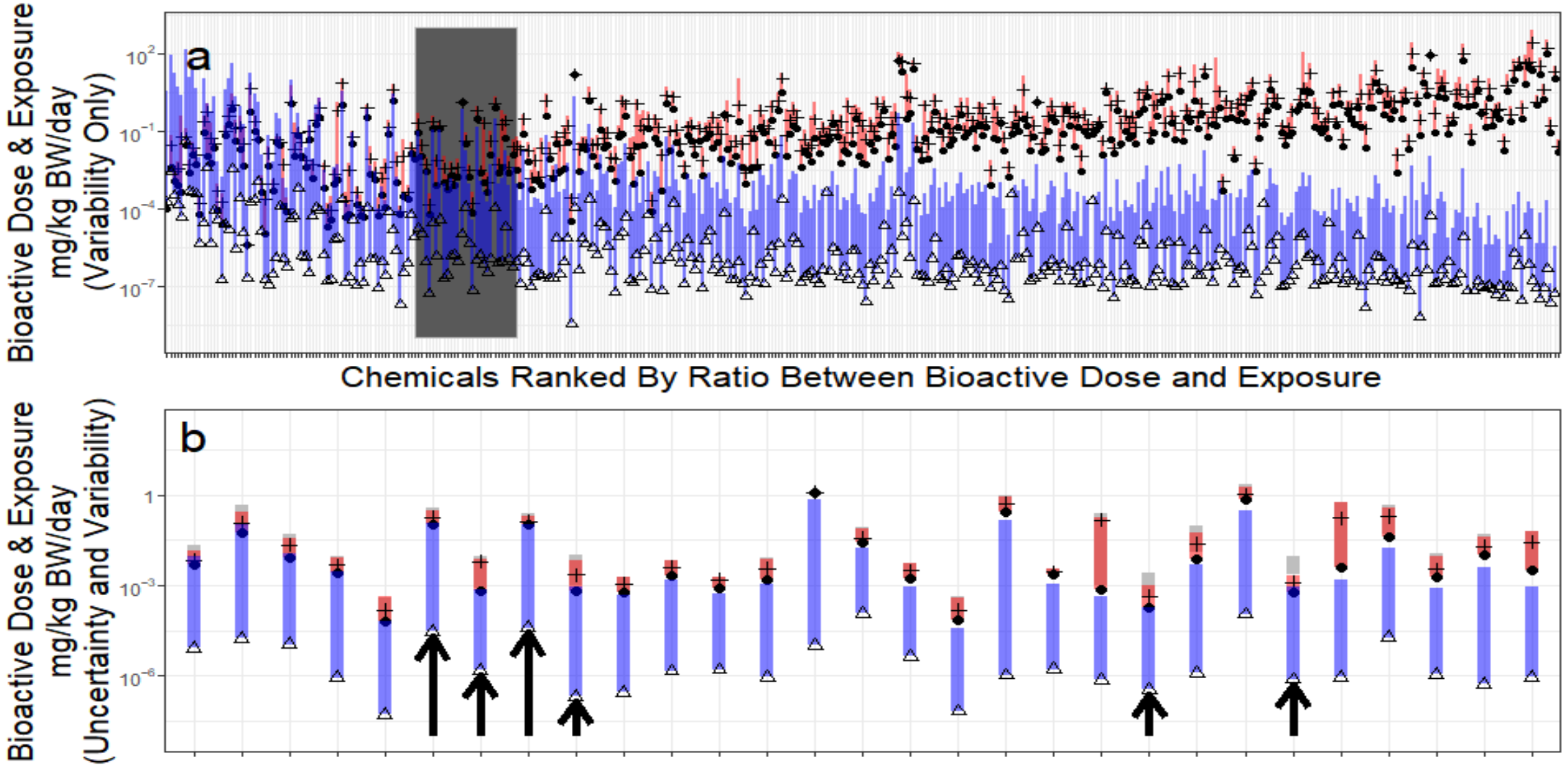
New IVIVE For 393 ToxCast Chemcials



Including chemical-specific uncertainty only caused changes in whether or not exposure and bioactivity overlapped in a small region

Wambaugh et al. (submitted)

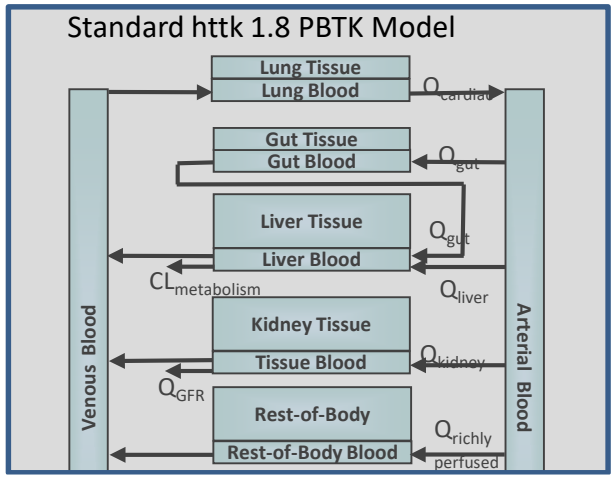
The Impact of Measurement Uncertainty



Only six more chemicals overlap

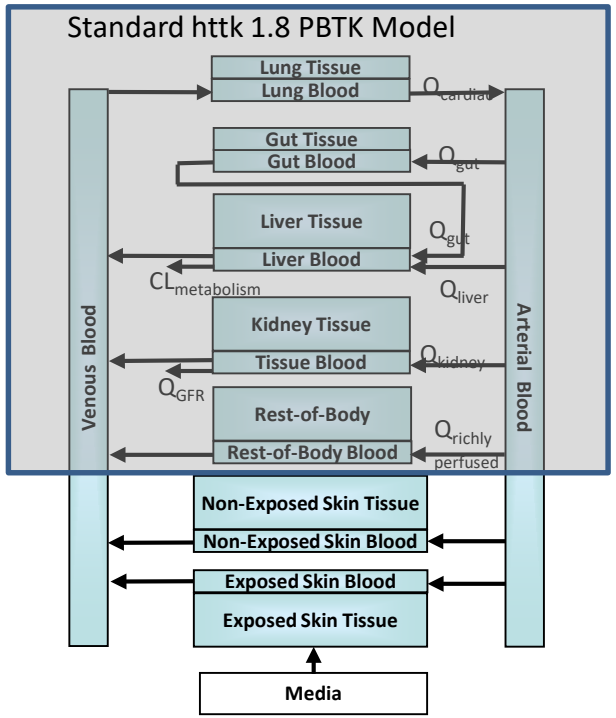
Wambaugh et al. (submitted)

New HT-PBTK Models



- We are working to augment the basic HT-PBPTK model with new PBTK models
  - For example, inhalation PBTK will allow for calculation of “inhalation equivalent doses” instead of oral equivalents
- Each model will be released publicly upon peer-reviewed publication
- Pre-publication models can be shared under a MTA
- We assume there will be coding errors and over-simplifications, so each publication involves curation of evaluation data from the scientific literature and through statistical analysis

New HT-PBTK Models



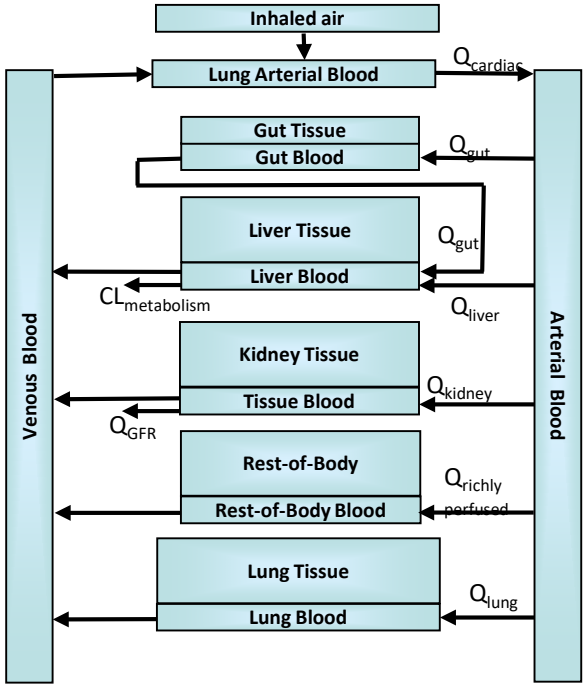
Dermal Exposure Route

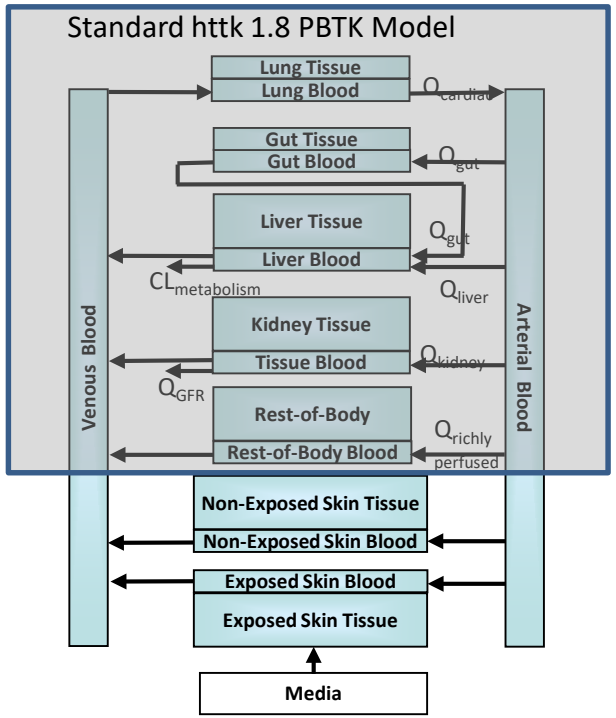
EPA, Unilever, INERIS

New HT-PBTK Models

Gas Inhalation Exposure Route

EPA, USAF



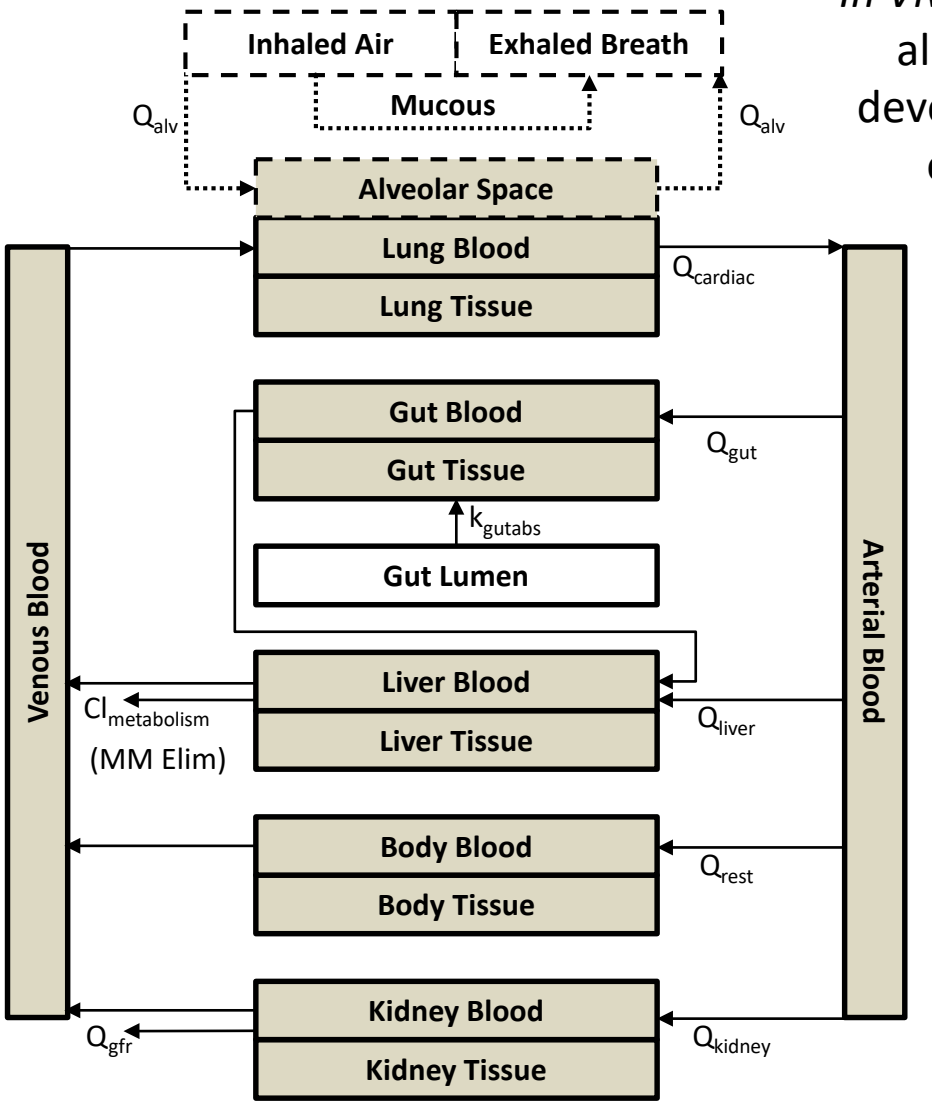


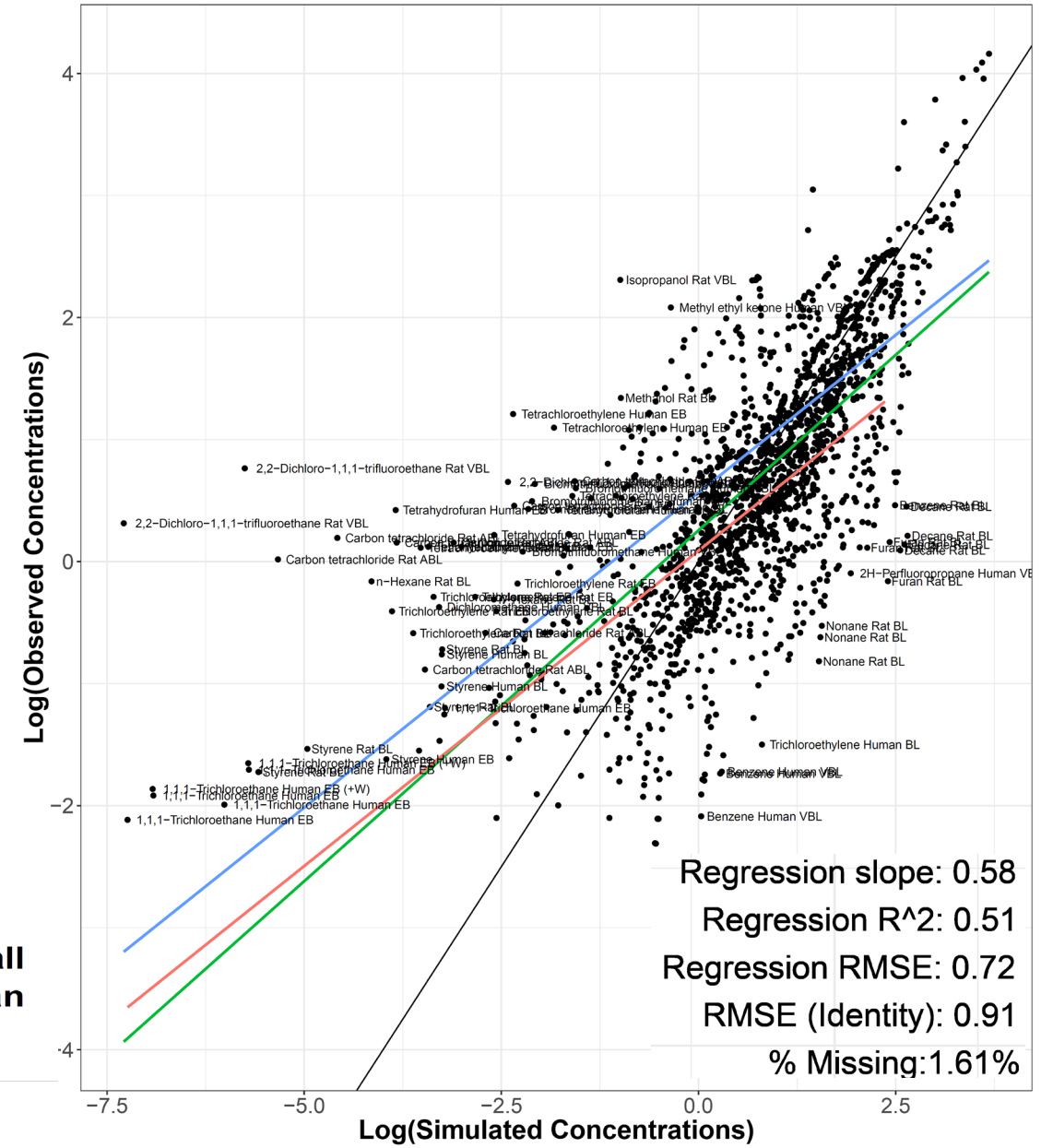
Dermal Exposure Route

EPA, Unilever, INERIS

Generic Gas Inhalation Model

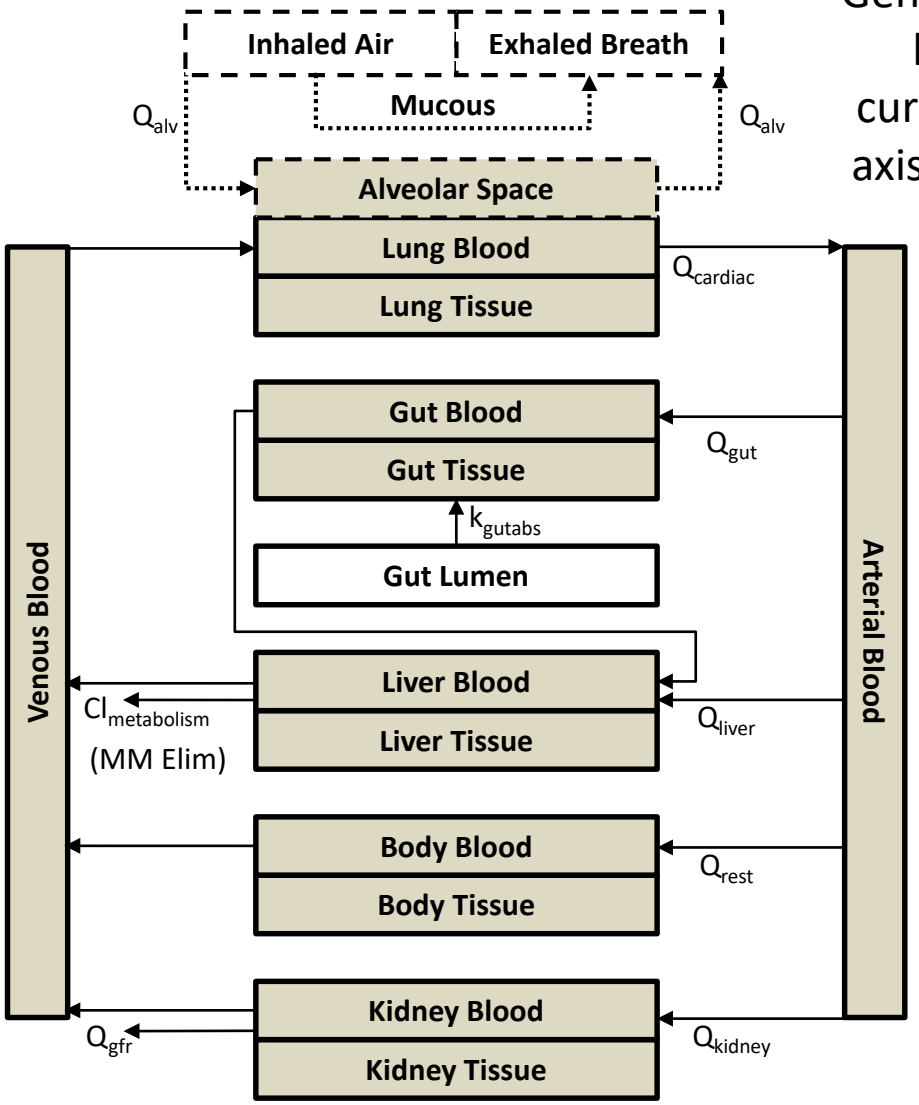
In Vivo TK database allowed rapid development and evaluation



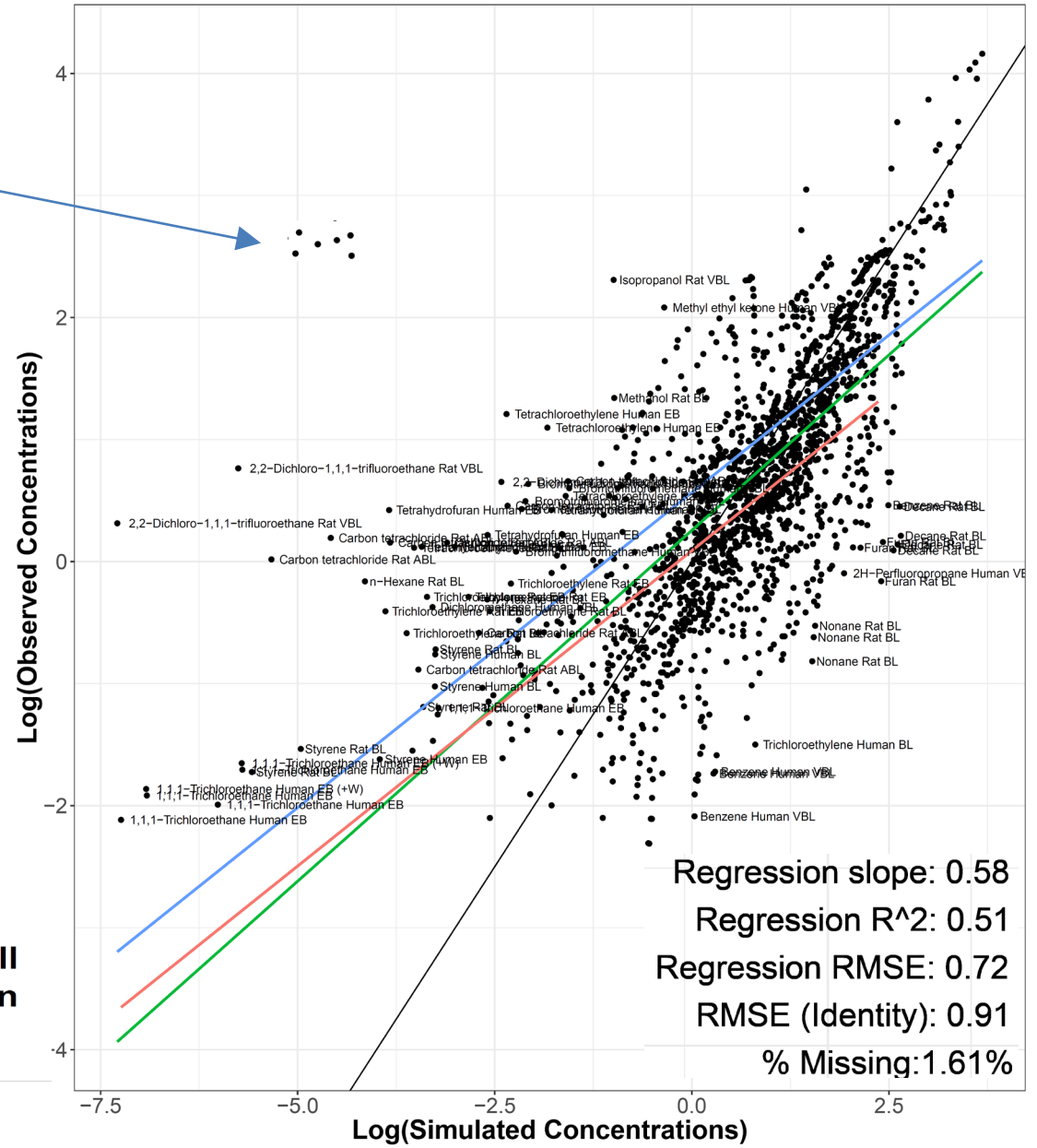


Linakis et al. (in preparation)

Generic Gas Inhalation Model

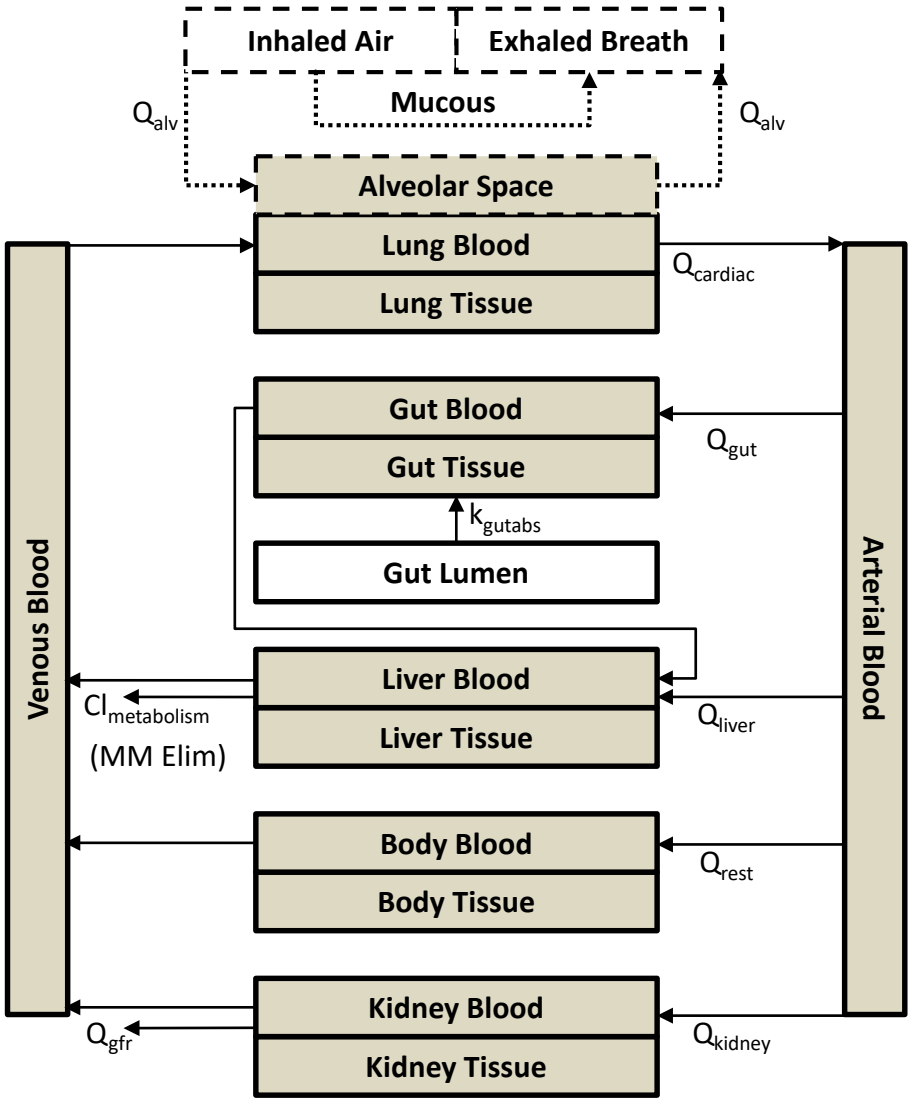


Generic model also helped data curation (units on axis in paper were wrong)



Linakis et al. (in preparation)

Generic Gas Inhalation Model



- Correct
- Used 4h exposure instead of 2h
- Used mg/m3 dose units instead of ppm

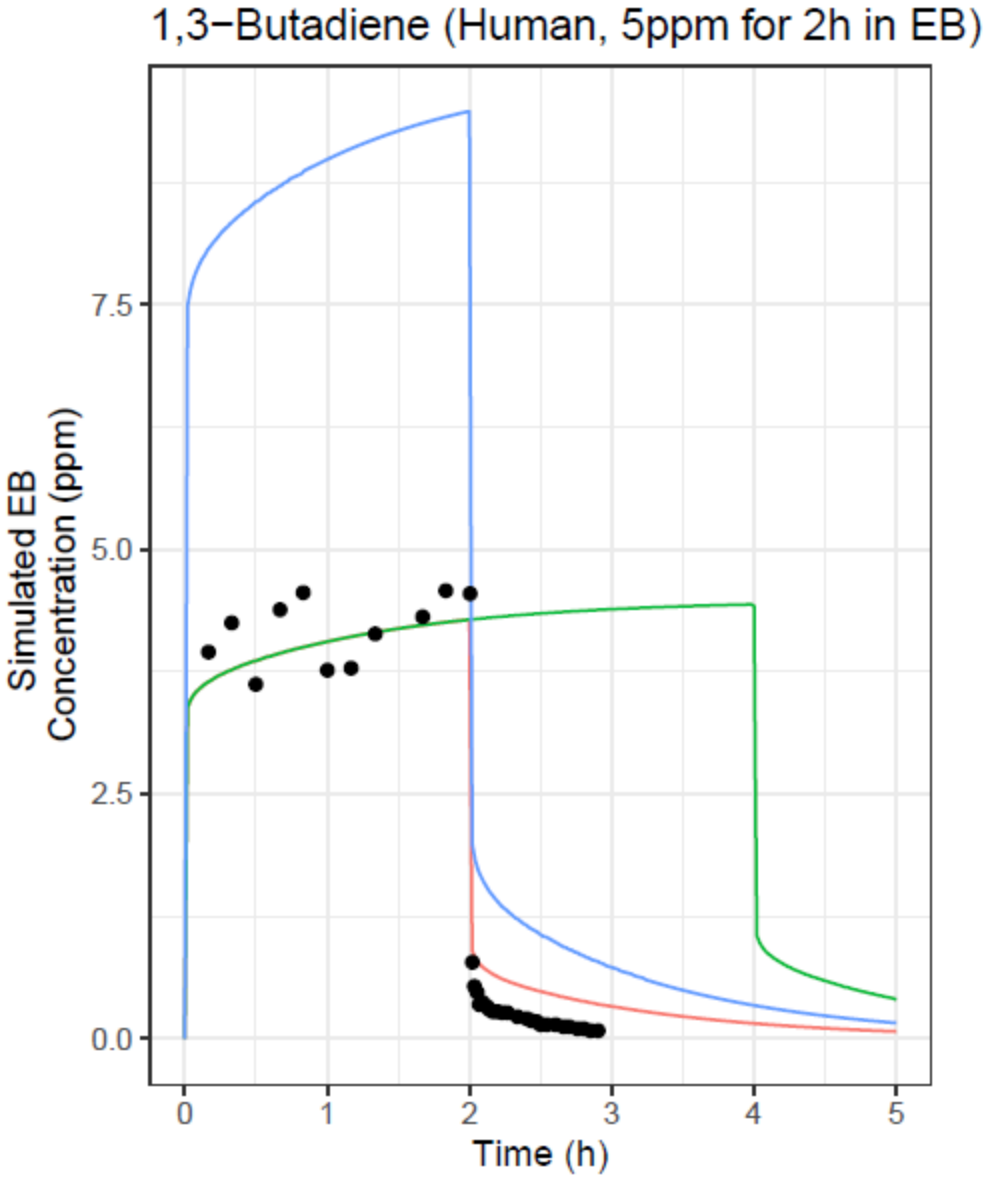
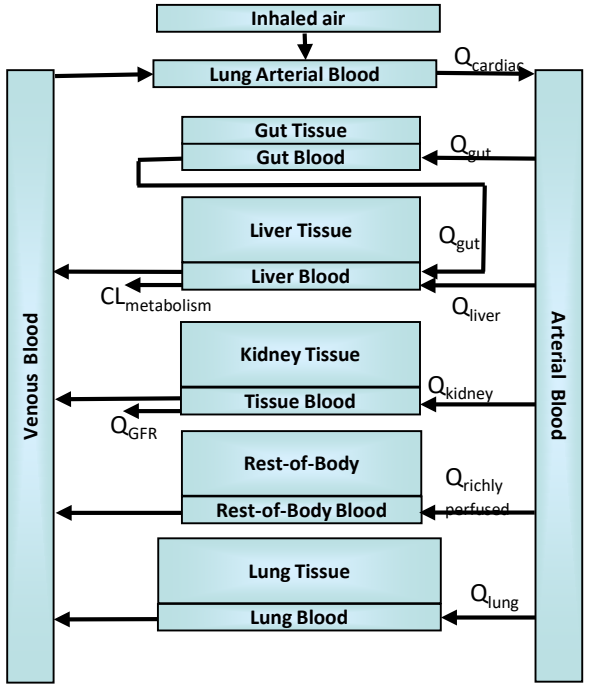


Figure from Matt Linakis (USAFSAM)

New HT-PBTK Models

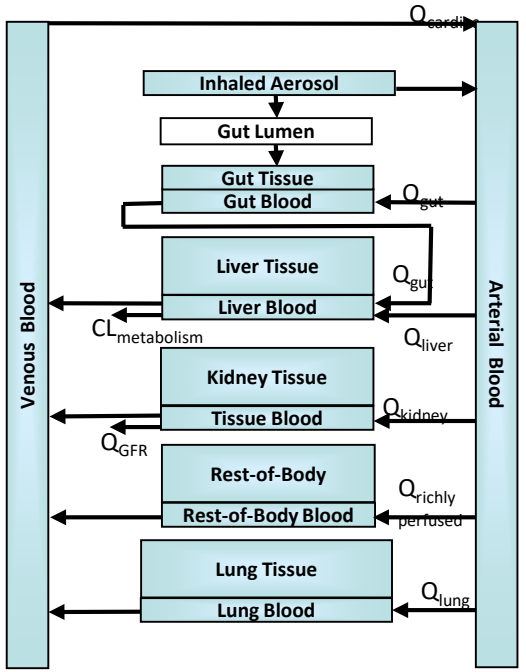
Gas Inhalation Exposure Route

EPA, USAF



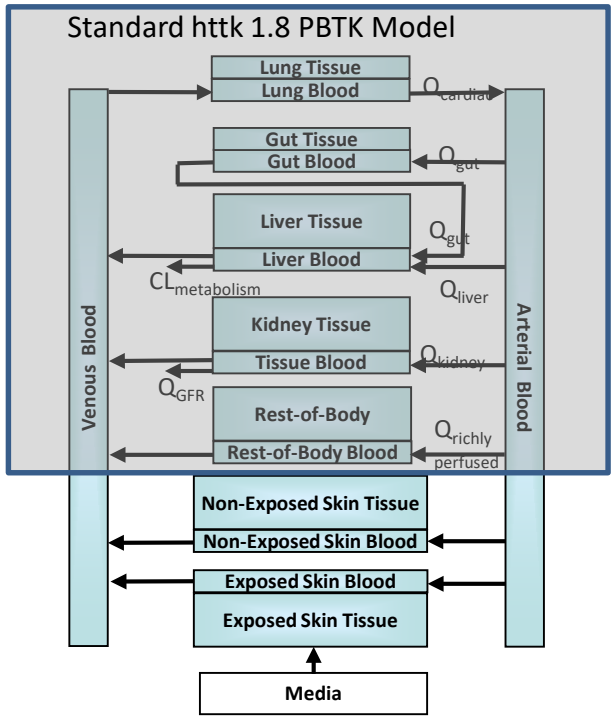
Aerosol Inhalation Exposure Route (with APEX model)

EPA, USAF



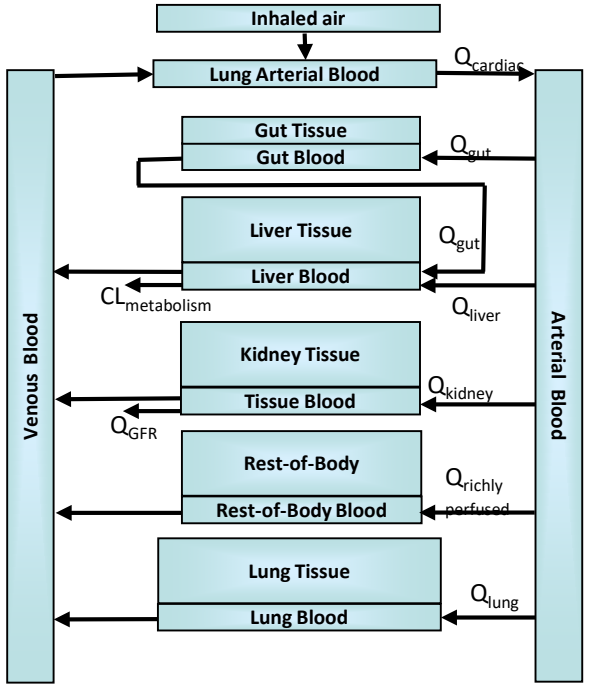
Dermal Exposure Route

EPA, Unilever, INERIS

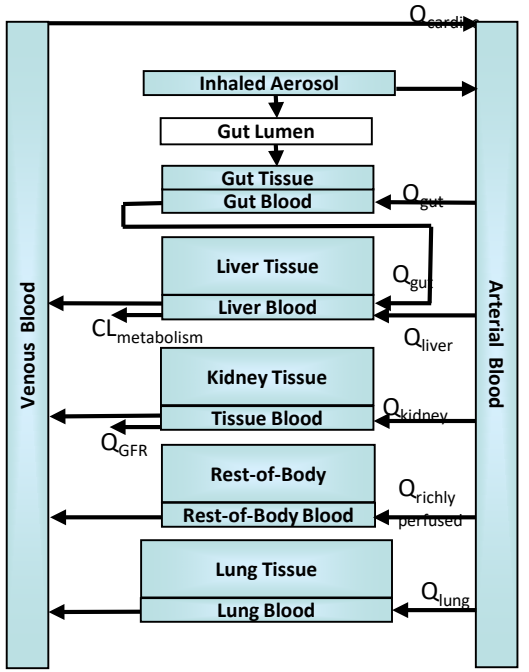


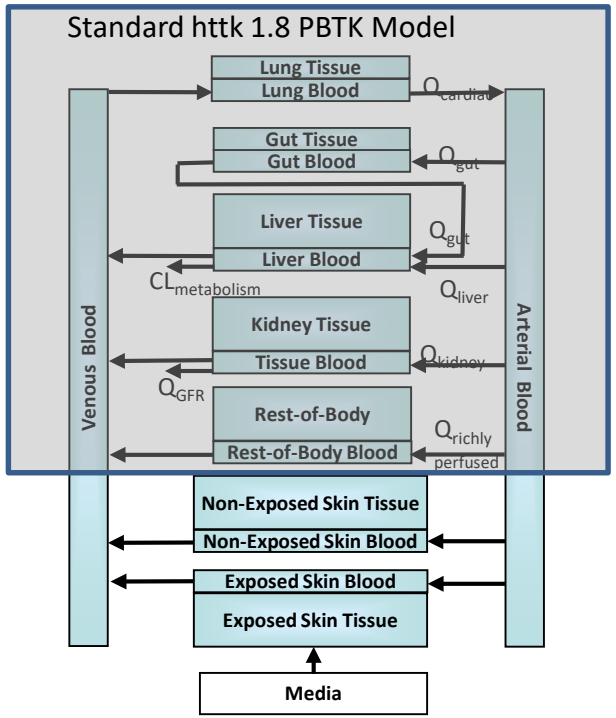
New HT-PBTK Models

Gas Inhalation Exposure Route
EPA, USAFSAM



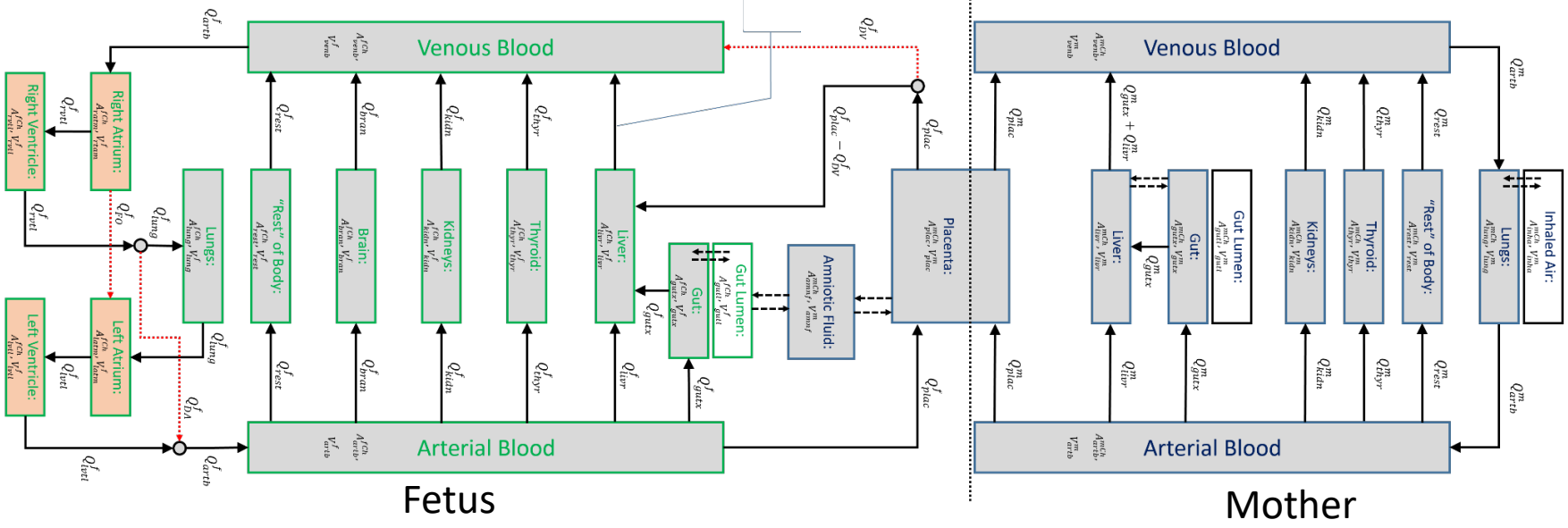
Aerosol Inhalation Exposure Route (with APEX model)
EPA, USAFSAM





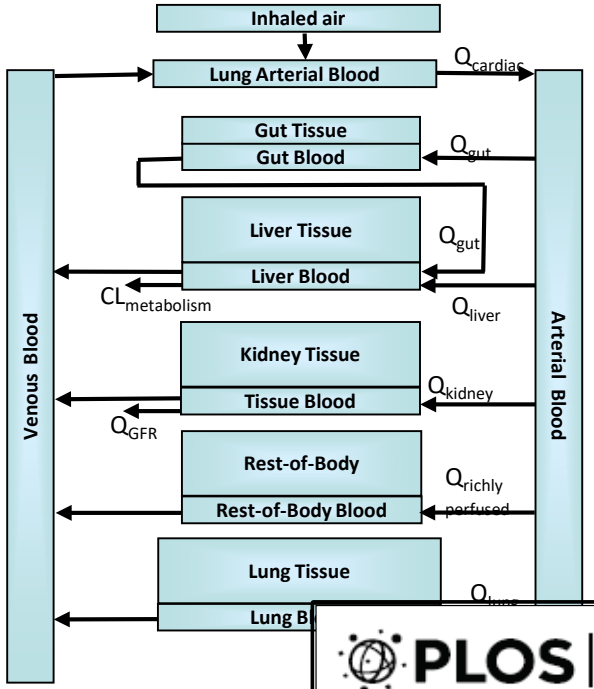
Dermal Exposure Route
EPA, Unilever, INERIS

Human Gestational Model
EPA, FDA

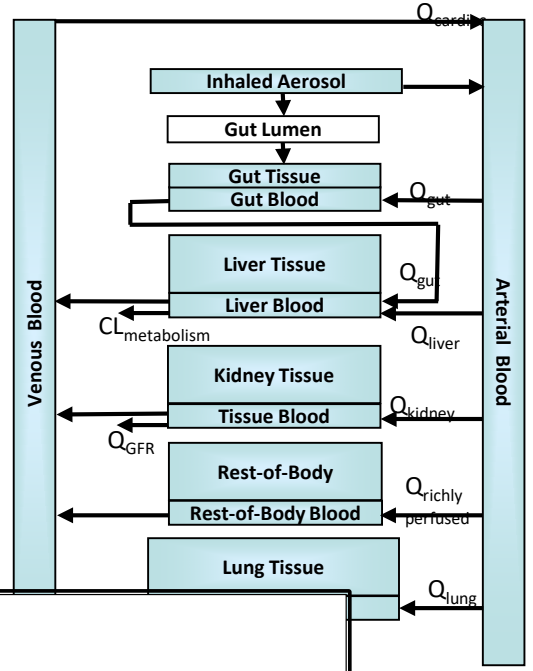


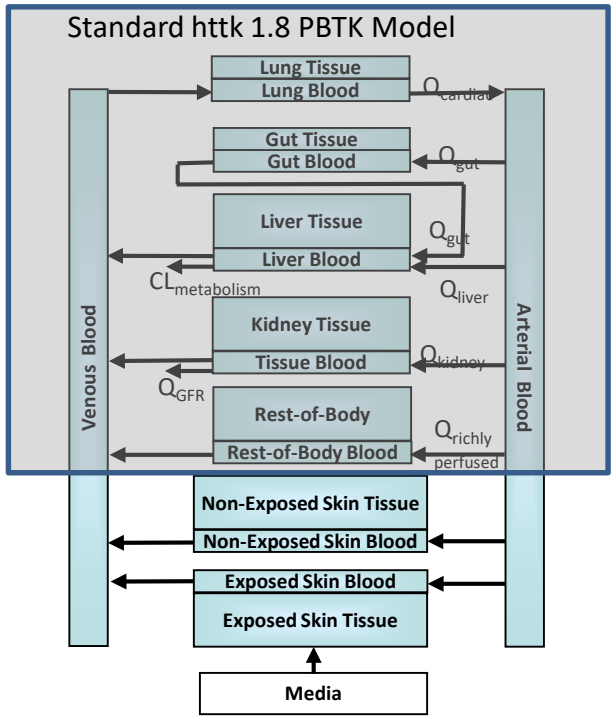
New HT-PBTK Models

Gas Inhalation Exposure Route
EPA, USAFSAM

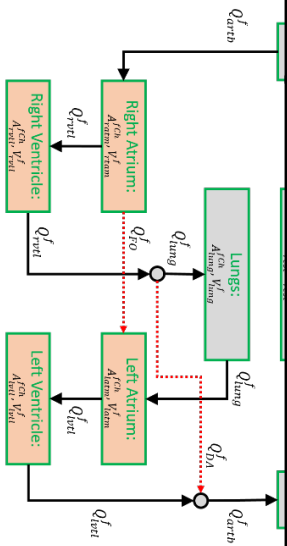


Aerosol Inhalation Exposure Route (with APEX model)
EPA, USAFSAM





Dermal Exposure Route
EPA, Unilever, INERIS



PLOS ONE

RESEARCH ARTICLE

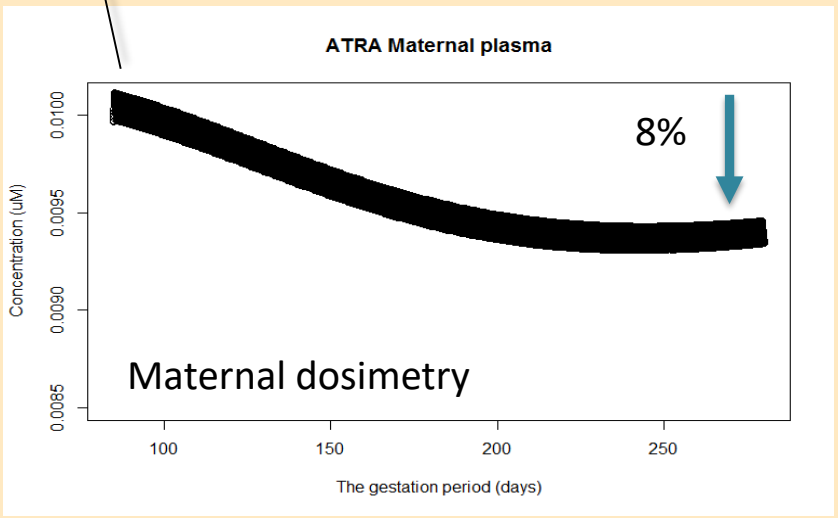
Empirical models for anatomical and physiological changes in a human mother and fetus during pregnancy and gestation

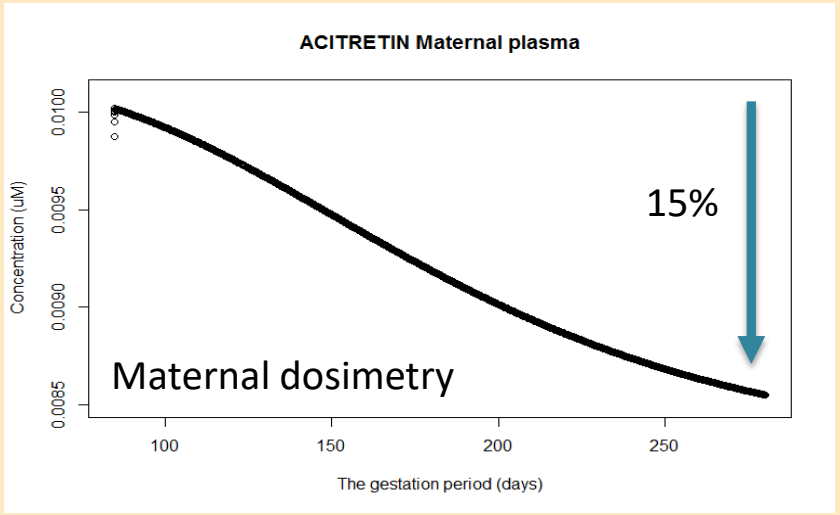
Dustin F. Kapraun[1]*, John F. Wambaugh[2], R. Woodrow Setzer[2], Richard S. Judson[2]

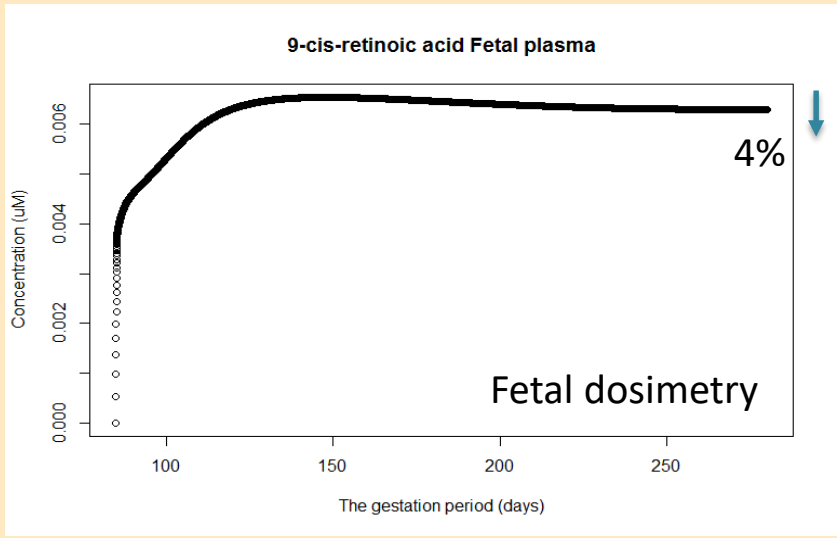
1 National Center for Environmental Assessment, US Environmental Protection Agency, Research Triangle Park, North Carolina, United States of America, 2 National Center for Computational Toxicology, US Environmental Protection Agency, Research Triangle Park, North Carolina, United States of America

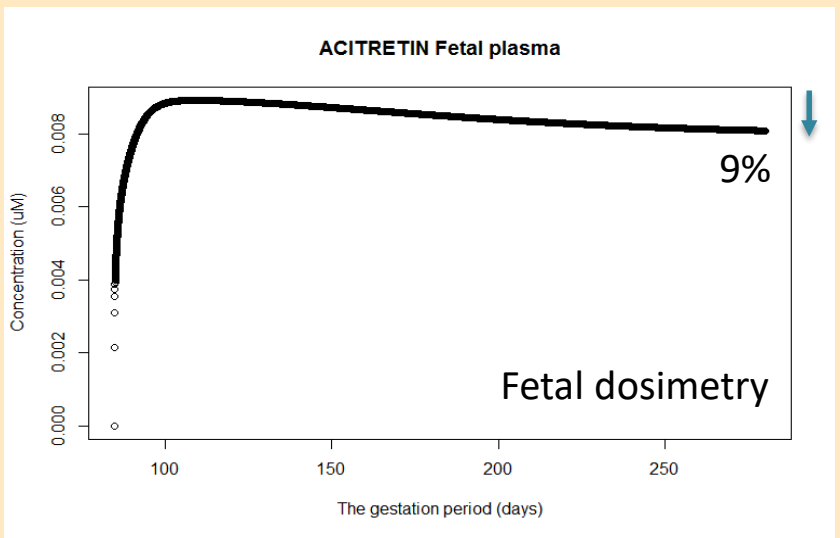
Maternal/Fetal HTTK Model Predictions for Retinoid Analogues:

Normalized initial plasma concentration for each retinoid analogue





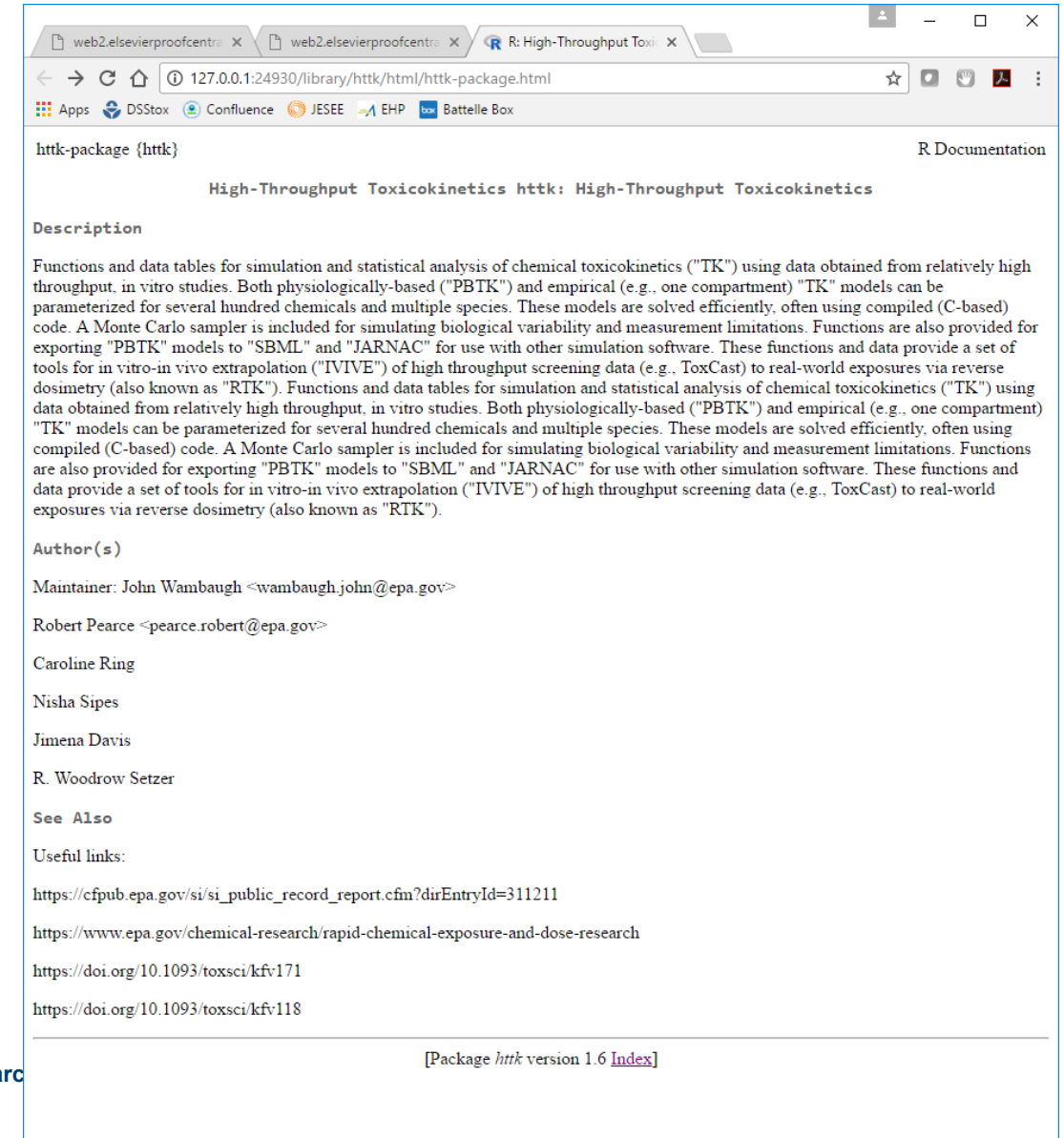




- Decrease in maternal plasma concentrations for retinoid analogues ranged from 8-15%
- Decrease in Fetal plasma concentrations for retinoid analogues ranged from 4-9%

HTTK is (mostly) Documented

Within R: type “help(httk)”



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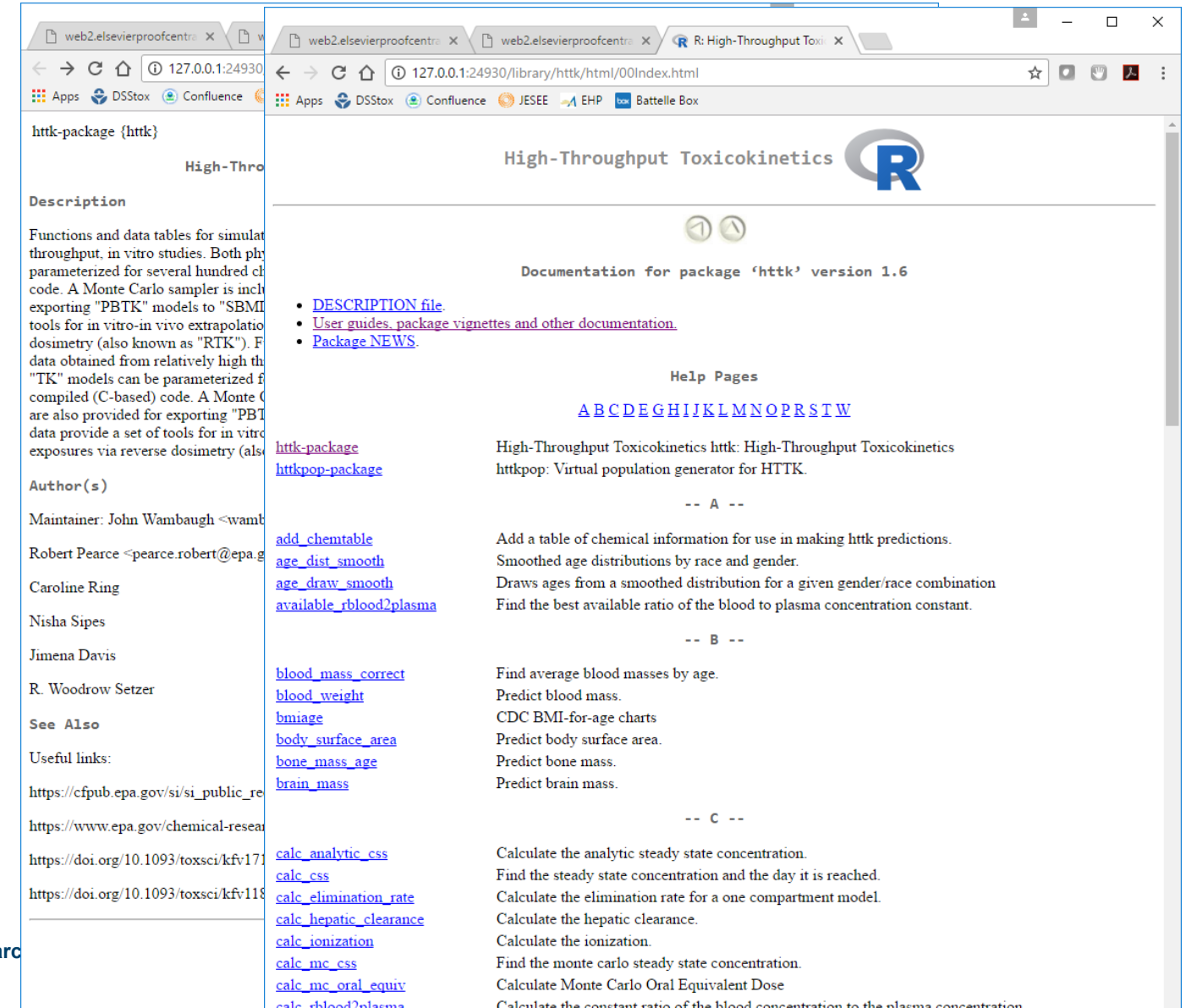

httk-package {httk}

High-Thro

Description

Functions and data tables for simulat
throughput, in vitro studies. Both phy
parameterized for several hundred ch
code. A Monte Carlo sampler is incl
exporting "PBTK" models to "SBMI
tools for in vitro-in vivo extrapolatio
dosimetry (also known as "RTK"). F
data obtained from relatively high th
"TK" models can be parameterized f
compiled (C-based) code. A Monte
are also provided for exporting "PBT
data provide a set of tools for in vitr
exposures via reverse dosimetry (als

Author(s)

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Robert Pearce <pearce.robert@epa.g

Caroline Ring

Nisha Sipes

Jimena Davis

R. Woodrow Setzer

See Also

Useful links:

https://cfpub.epa.gov/si/si_public_re

https://www.epa.gov/chemical-resea

https://doi.org/10.1093/toxsci/kfv17

https://doi.org/10.1093/toxsci/kfv118

127.0.0.1:24930/library/httk/html/00Index.html

Apps DSStox Confluence JESEE EHP Battelle Box

High-Throughput Toxicokinetics

Documentation for package ‘httk’ version 1.6

- DESCRIPTION file.
- User guides, package vignettes and other documentation.
- Package NEWS.

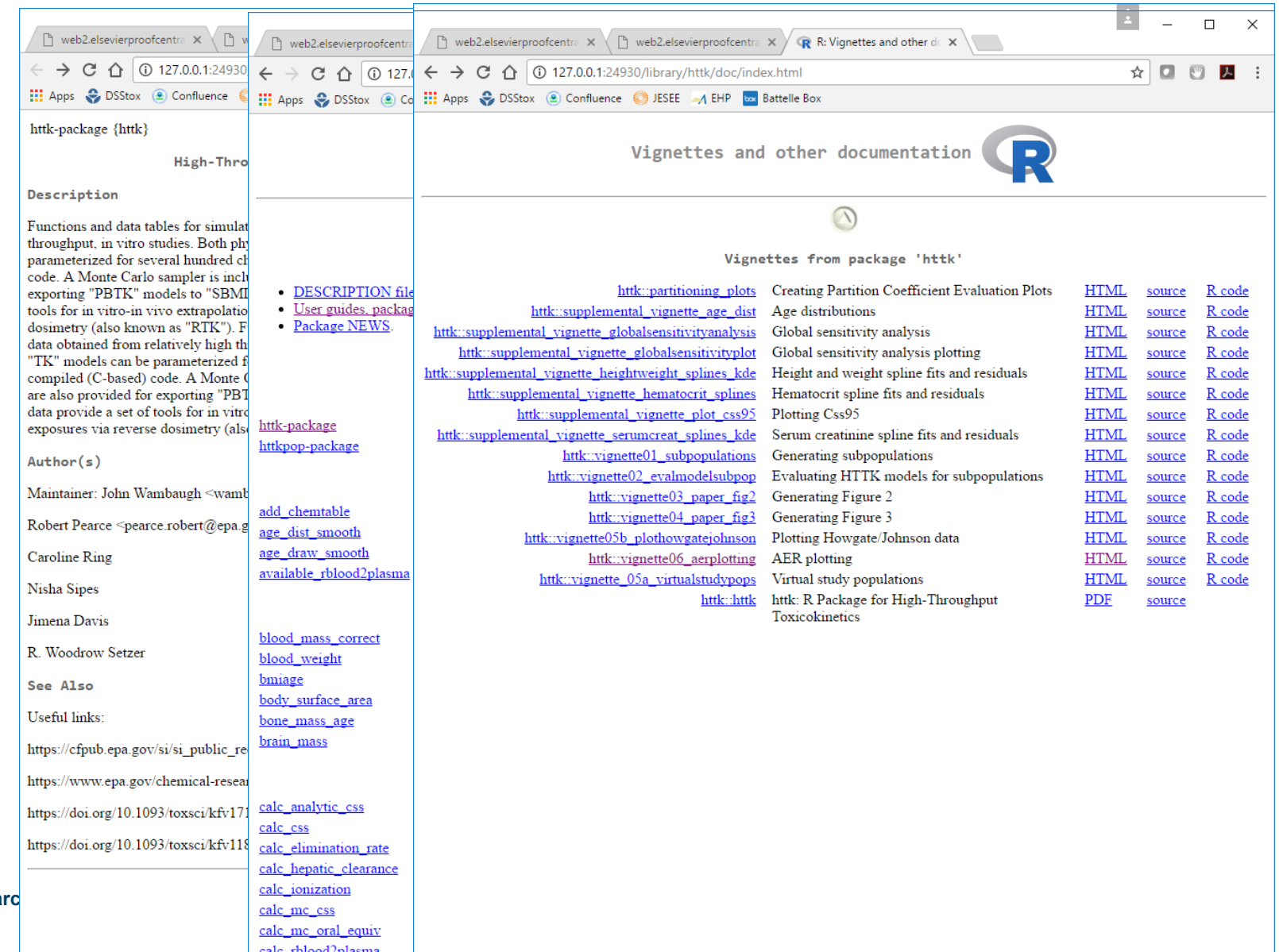
Help Pages

A B C D E G H I J K L M N O P R S T W

httk-package	High-Throughput Toxicokinetics httk: High-Throughput Toxicokinetics
httkpop-package	httkpop: Virtual population generator for HTTK.
	-- A --
add_chemtable	Add a table of chemical information for use in making httk predictions.
age_dist_smooth	Smoothed age distributions by race and gender.
age_draw_smooth	Draws ages from a smoothed distribution for a given gender/race combination
available_rblood2plasma	Find the best available ratio of the blood to plasma concentration constant.
	-- B --
blood_mass_correct	Find average blood masses by age.
blood_weight	Predict blood mass.
bmiage	CDC BMI-for-age charts
body_surface_area	Predict body surface area.
bone_mass_age	Predict bone mass.
brain_mass	Predict brain mass.
	-- C --
calc_analytic_css	Calculate the analytic steady state concentration.
calc_css	Find the steady state concentration and the day it is reached.
calc_elimination_rate	Calculate the elimination rate for a one compartment model.
calc_hepatic_clearance	Calculate the hepatic clearance.
calc_ionization	Calculate the ionization.
calc_mc_css	Find the monte carlo steady state concentration.
calc_mc_oral_equiv	Calculate Monte Carlo Oral Equivalent Dose

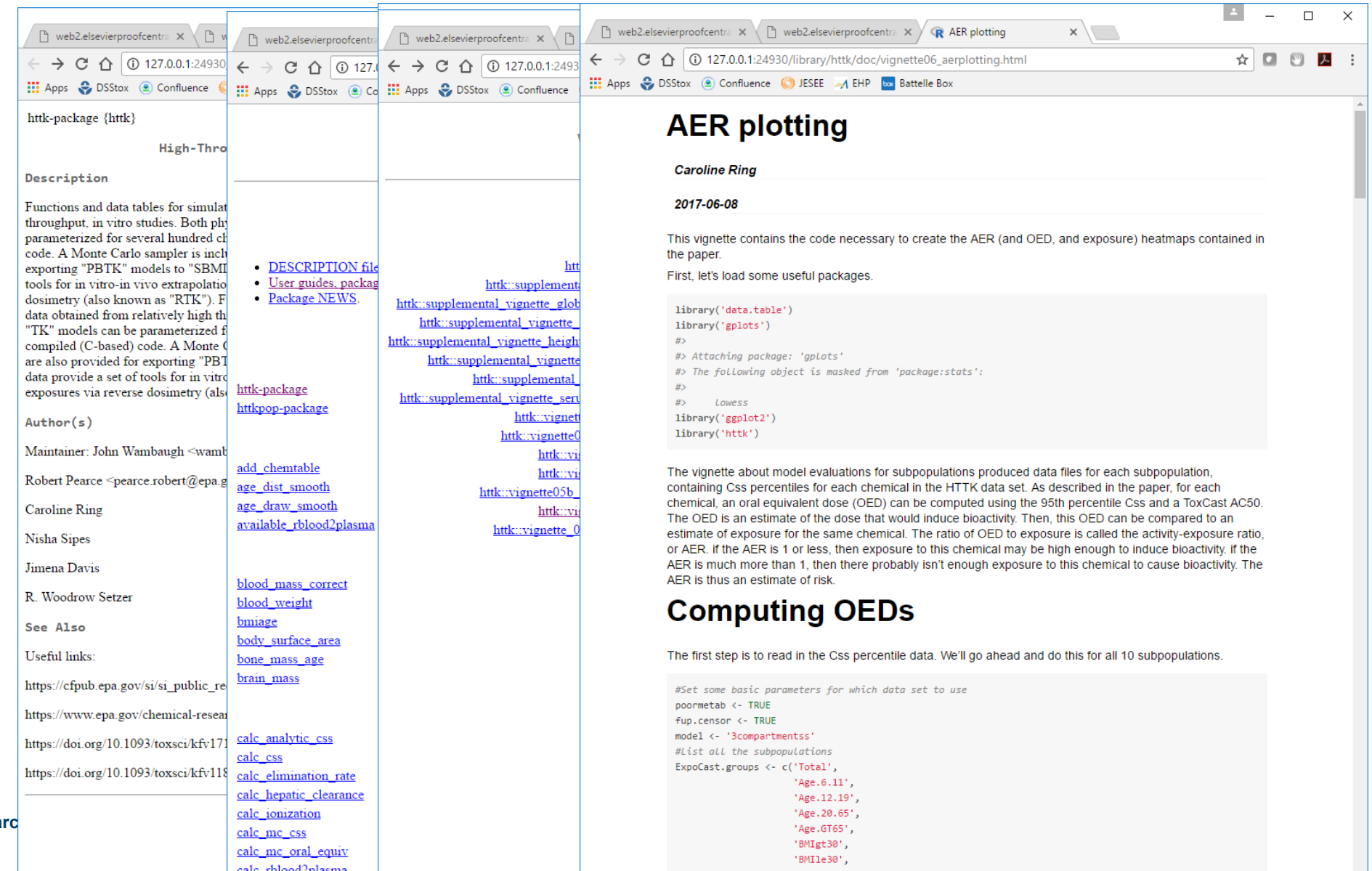
HTTK is (mostly) Documented

Within R: type “help(httk)”



HTTK is (mostly) Documented

Within R: type “help(httk)”



Does My Chemical Have HTTK Data?

```
> 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")
```

Is a chemical available?

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

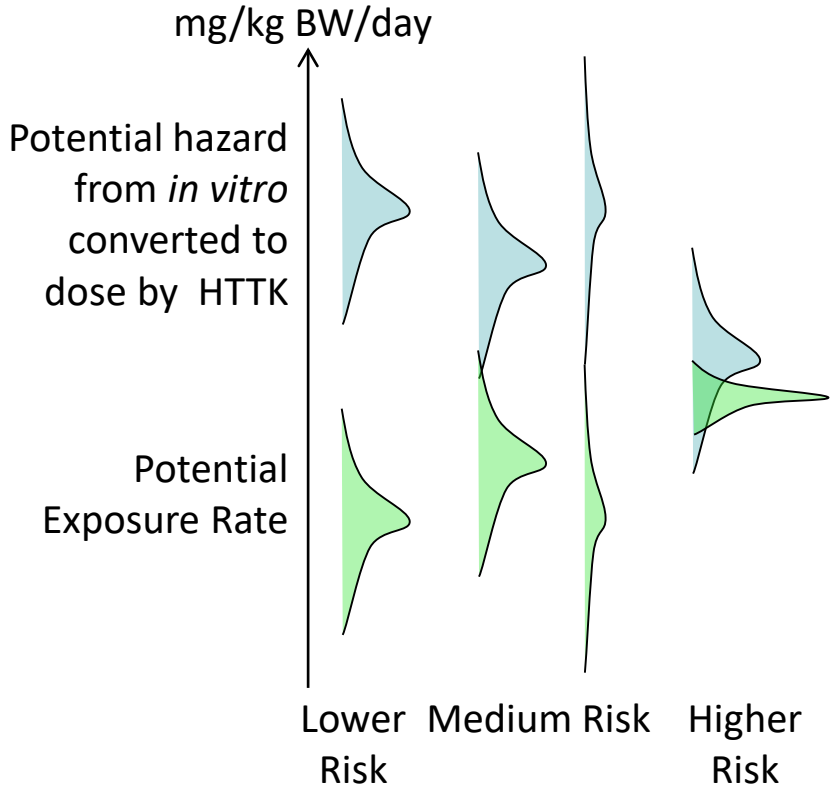
All data on chemicals A, B, C

```
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

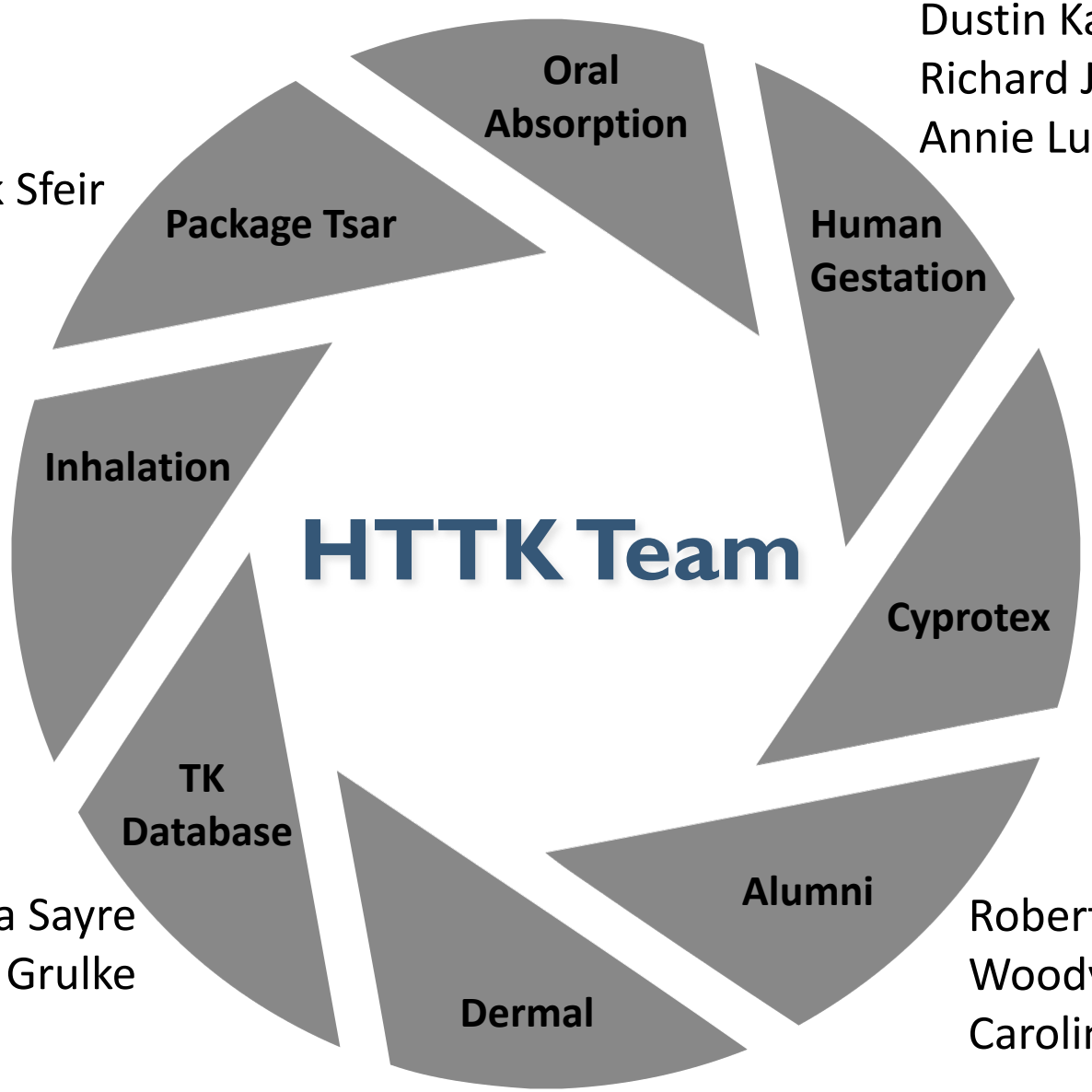
Conclusions

- HTTK allows dosimetric adjustment of high-throughput screening (HTS) data across thousands of chemicals.
- New, chemical-specific *in vitro* experiments have been conducted by Cyprotex, using a revised protocol for measuring protein binding
- Overall, variability contributed more significantly to C_{ss} estimations of the 95th percentile
- Comparison between high throughput toxicokinetics (HTTK) predicted concentrations and in vivo data is a valuable approach for evaluation and establishing confidence
- Recent analyses indicate that some properties (e.g. average and maximum concentration) can be predicted with confidence.
  - A new database of in vivo concentration vs. time data is being developed (Sayre, in preparation)



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

EPA United States Environmental Protection Agency



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Chris Grulke

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Woody Setzer
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Beate Nicol (Unilever)

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EPA United States Environmental Protection Agency

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