EPA
United States
Environmental Protection
Agency

# Monte Carlo for variability simulation and uncertainty

Caroline L. Ring

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

**Office of Research and Development**
Center for Computational Toxicology and Exposure

**September 16, 2022**

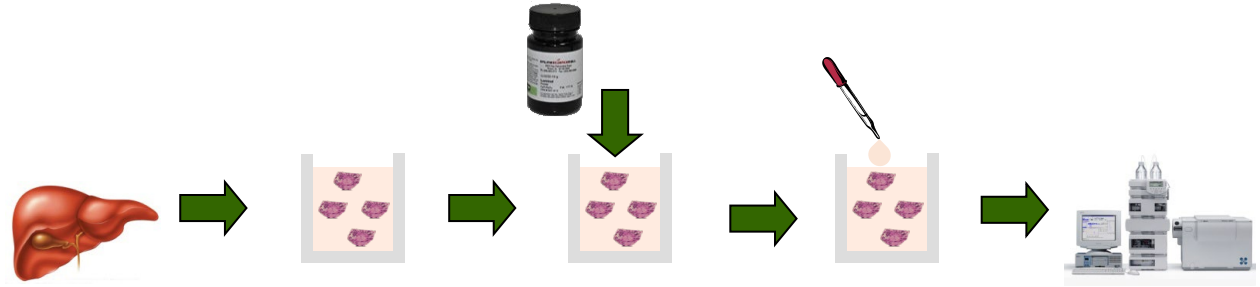
# Review: HTTK model parameters

| **Chemical-specific parameters** | |
|---|---|
| Intrinsic hepatic clearance rate (CLint) | Measured in HT *in vitro* assays (Rotroff *et al.* 2010; Wetmore *et al.* 2012, 2014, 2015; Wambaugh *et al.* 2019) or predicted *in silico* (Sipes *et al.* 2017) |
| Fraction unbound to plasma protein (Fup) | |
| Tissue:blood partition coefficients (for compartmental models) | Predict from phys-chem properties and tissue properties (Pearce et al., 2017) |
| **Physiological parameters** | |
| Tissue masses (including body weight) | Gathered from data available in the published literature [Wambaugh et al. 2015; Pearce et al. 2017a] |
| Tissue blood flows | |
| Glomerular filtration rate (passive renal clearance) | |
| Hepatocellularity | |

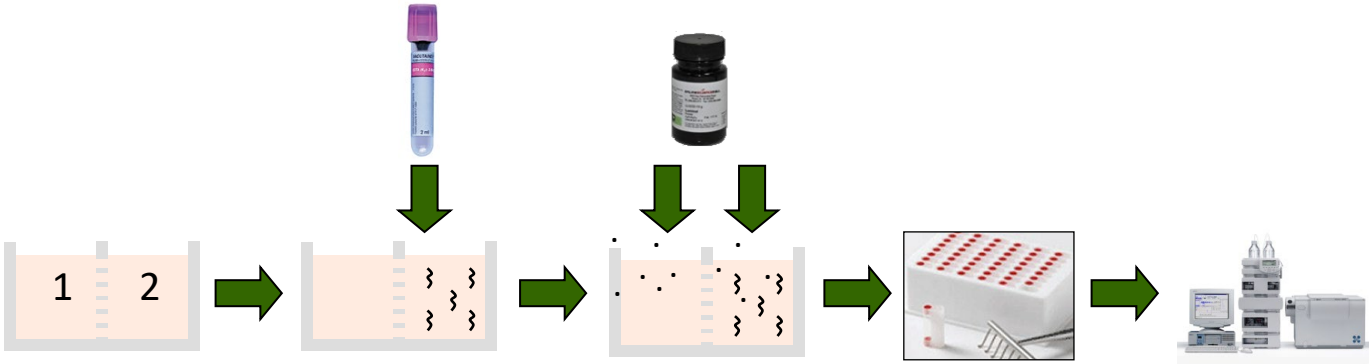
# Chemical-specific parameters measured *in vitro* carry measurement uncertainty

| Chemical-specific parameters | |
|---|---|
| Intrinsic hepatic clearance rate (CLint) | Measured in HT *in vitro* assays (Rotroff *et al.* 2010; Wetmore *et al.* 2012, 2014, 2015; Wambaugh *et al.* 2019) |
| Fraction unbound to plasma protein (Fup) | |

CLint: Cryo-preserved hepatocyte suspension
Shibata *et al.* (2002)



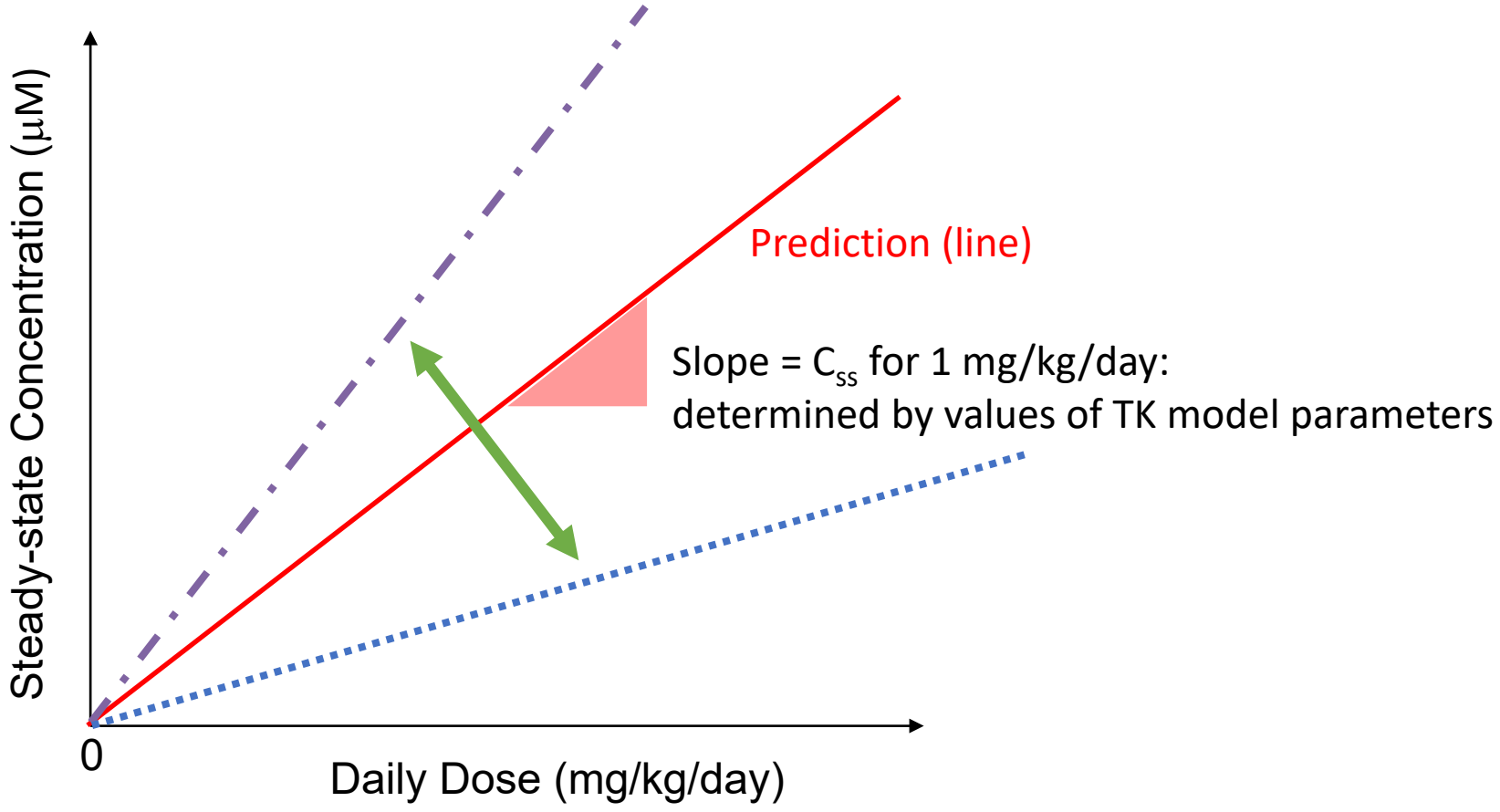
Fup: Rapid Equilibrium Dialysis (RED)
Waters *et al.* (2008)



# Parameters represent biology — so they have population variability

| Chemical-specific parameters | |
|---|---|
| Intrinsic hepatic clearance rate (CLint) | Represent chemical-body interactions — vary with individual genetics, environmental factors, age, etc. |
| Fraction unbound to plasma protein (Fup) | |
| Tissue:blood partition coefficients (for compartmental models) | |
| **Physiological parameters** | |
| Tissue masses (including body weight) | Represent physiology — vary with individual genetics, environmental factors, age, etc. |
| Tissue blood flows | |
| Glomerular filtration rate (passive renal clearance) | |
| Hepatocellularity | |

# HTTK model parameters determine the slope relating Css to daily dose – need to propagate both uncertainty & variability




Wambaugh et al. (2019)

EPA United States Environmental Protection Agency

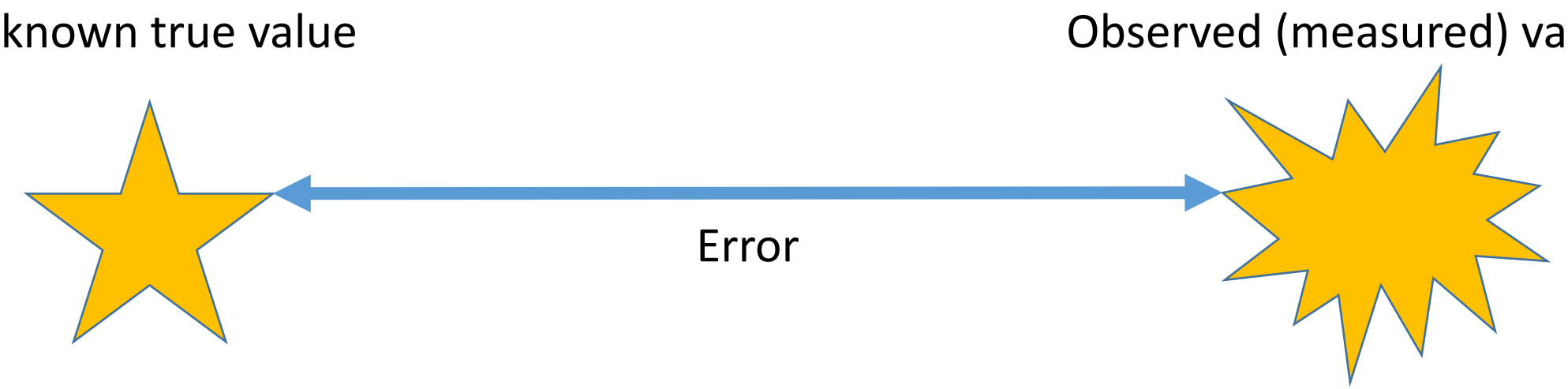
# Approach to uncertainty & variability: Monte Carlo

- Characterize **uncertainty** and **variability** of TK parameters in terms of **probability distributions**
- **Draw samples from these distributions**: “simulated population”
- **Evaluate HTTK model** for each “simulated individual” in the “simulated population”
- Characterize **resulting distribution** of HTTK model predictions

EPA United States Environmental Protection Agency

# Characterizing key uncertainty in chemical-specific TK parameters

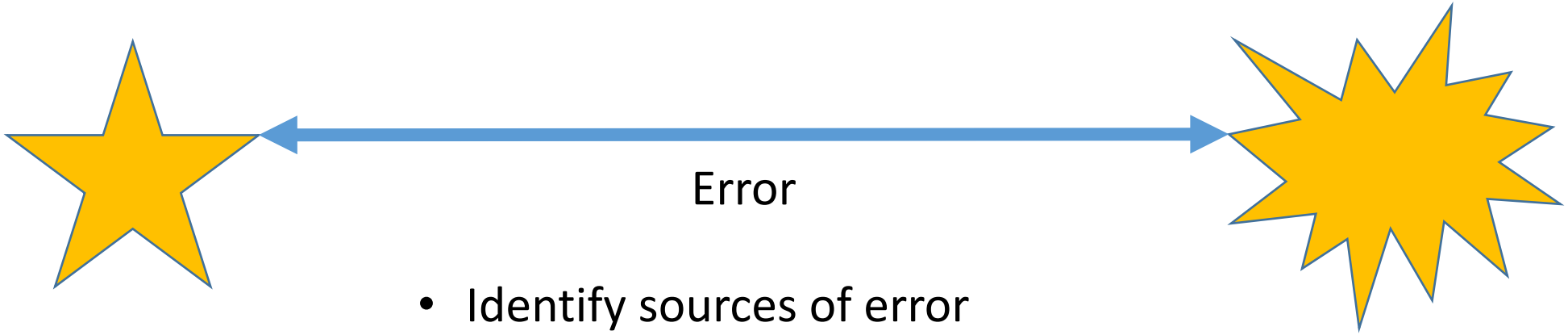
# Bayesian approach to uncertainty quantification



# Bayesian approach to uncertainty quantification

Unknown true value

Observed (measured) value

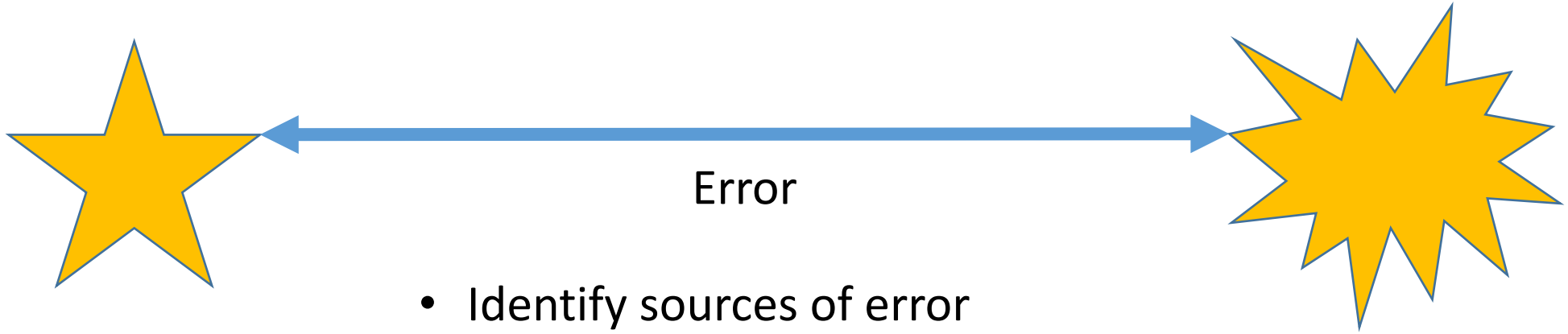


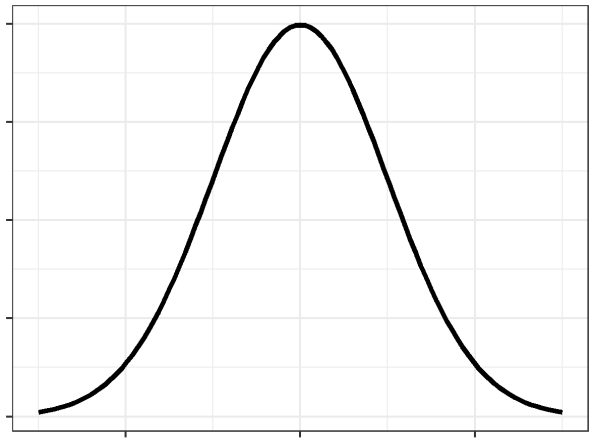

- Identify sources of error
- Develop mathematical model of error

# Bayesian approach to uncertainty quantification

Unknown true value

Observed (measured) value



- Identify sources of error
- Develop mathematical model of error

**Bayesian inference:**
Find a *distribution* of possible true values that could have produced the observed values, under this error model

EPA United States Environmental Protection Agency

# Uncertainty in Fup

# Understanding sources of error in Fup: How to measure *in vitro* using Rapid Equilibrium Dialysis (RED)

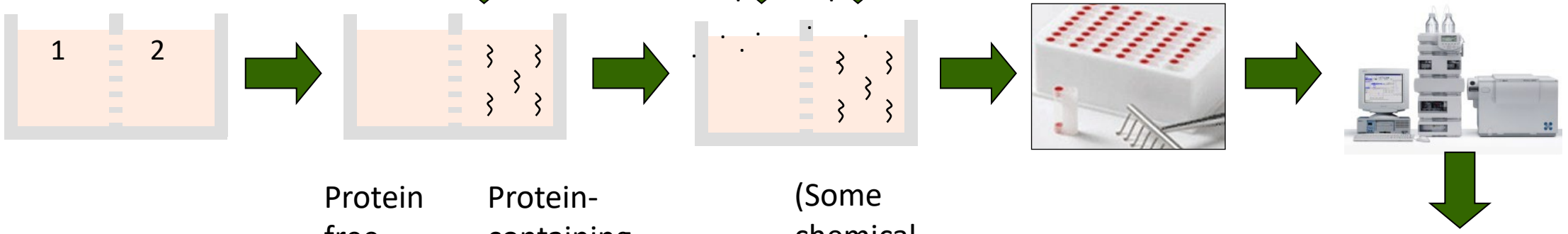
Semi-permeable membrane (chemical can pass, but not protein)

Add plasma to one side

Add chemical to both sides

Let equilibrate (until **unbound** conc. equal on both sides)

Use mass spectrometry to measure **total** chemical conc. from each side




Protein free side

Protein-containing side

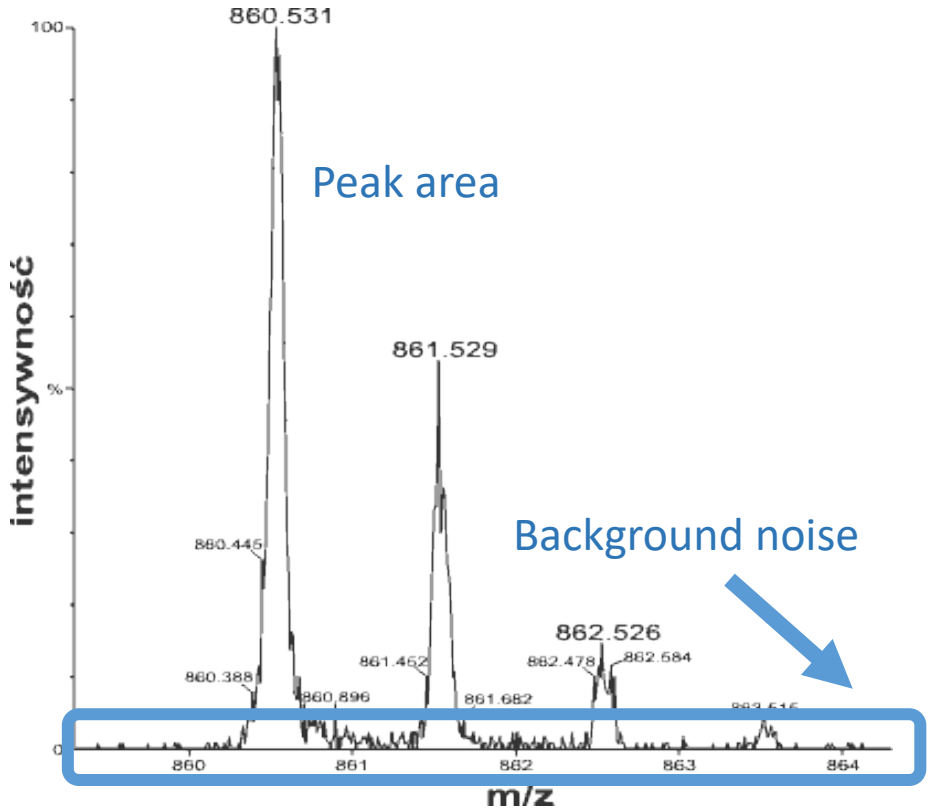
(Some chemical will bind to protein)

$$F_{up} = \frac{\text{Protein-free conc.}}{\text{Protein-containing conc.}}$$

Waters et al. (2008); Rotroff et al. (2010); Wambaugh et al. (2019)

# Source of measurement uncertainty: Mass spectrometry = uncertainty in measured chemical concentrations

- Instrument calibration
- Limit of quantification (LOQ)




https://commons.wikimedia.org/wiki/File:ObwiedniaPeptydu.gif (GPL)

Wambaugh et al. (2019)

Calibration: Peak area vs. concentration

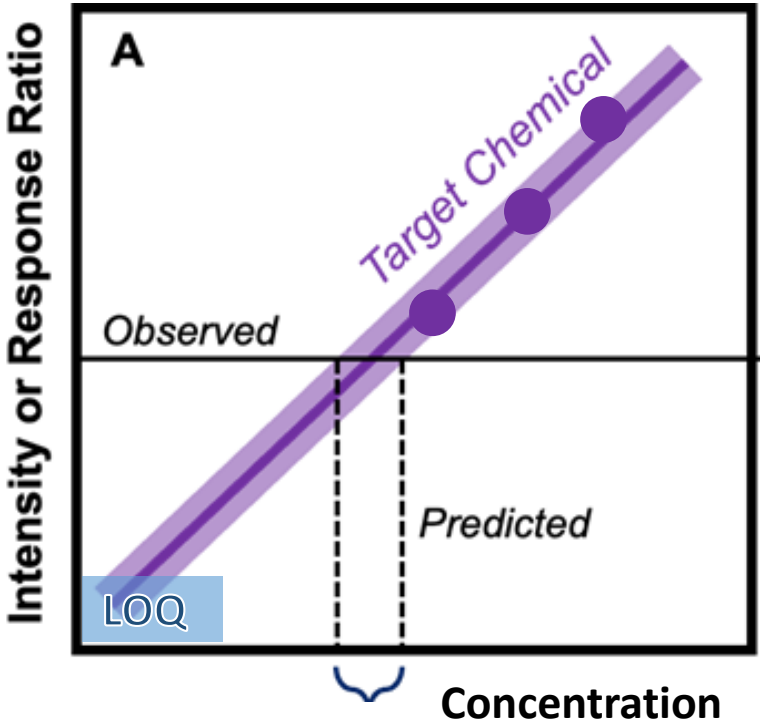
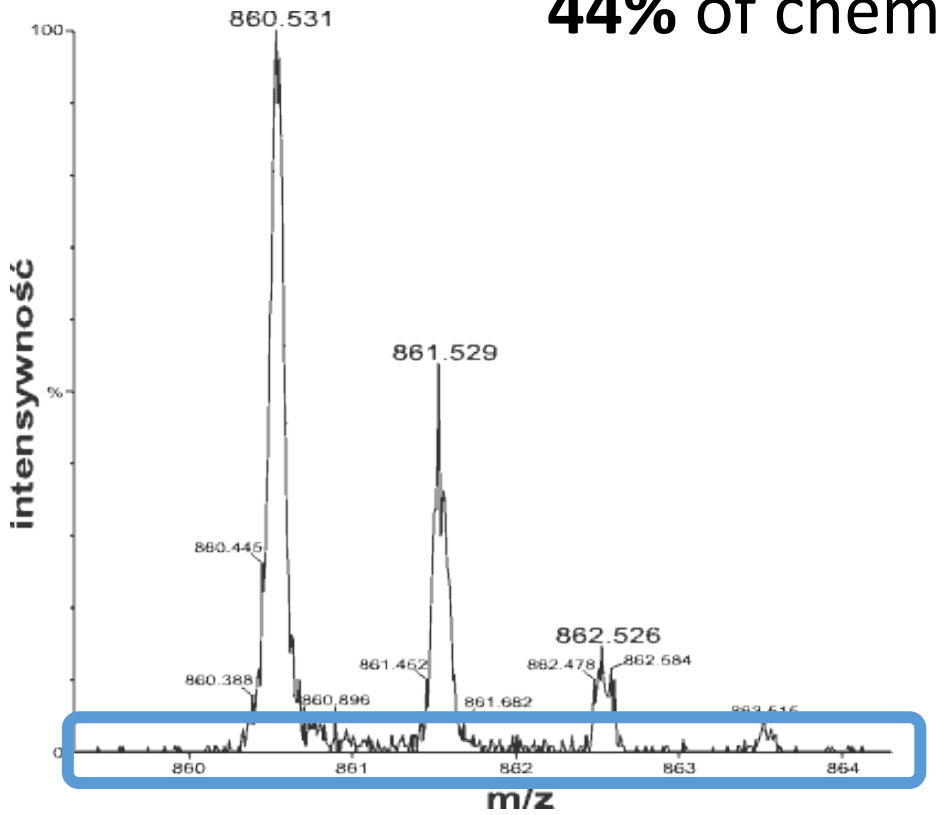



Figure adapted from Fisher et al. (2022)

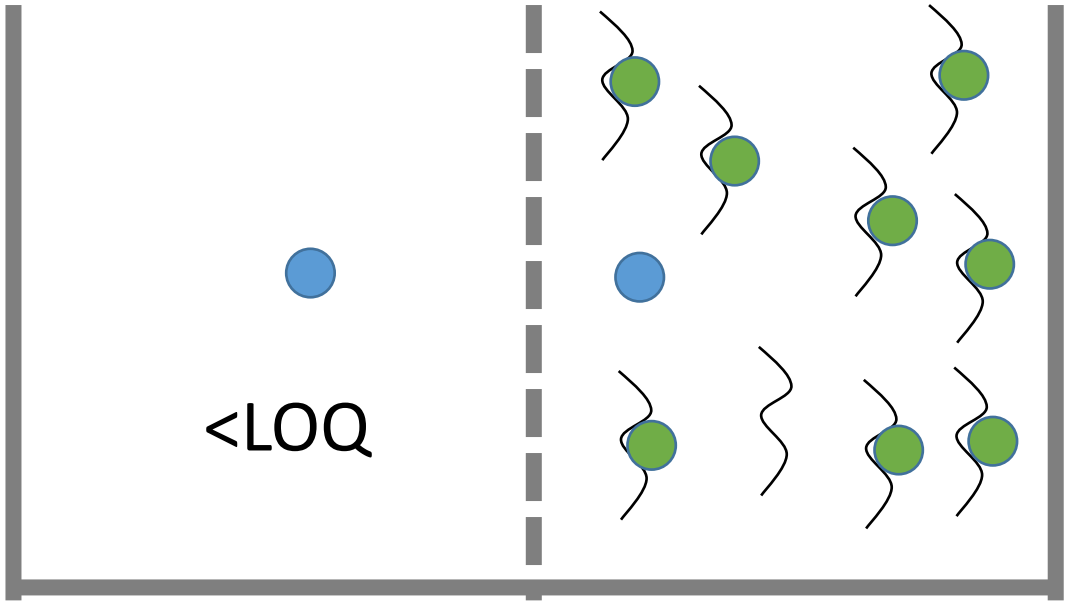
# LOQ is a problem in the RED assay for highly-bound chemicals

**44%** of chemicals in Wambaugh et al. (2019)

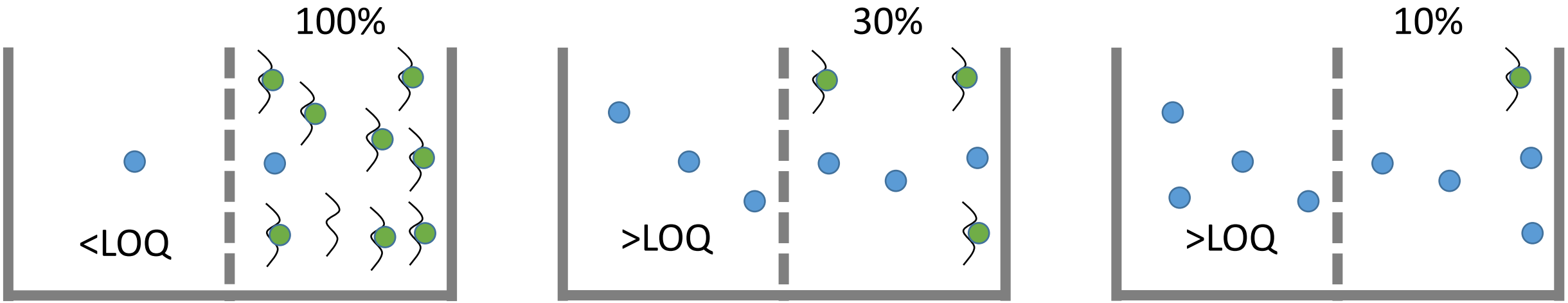



https://commons.wikimedia.org/wiki/File:ObwiedniaPeptydu.gif
(GPL)

$$F_{up} = \frac{\text{Protein—free conc.}}{\text{Protein—containing conc.}}$$



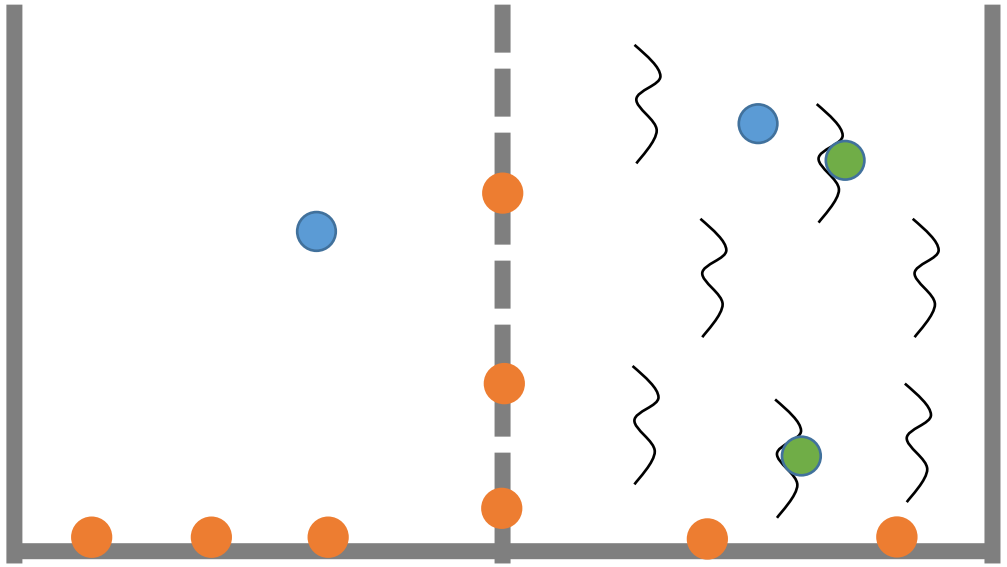
# Approach to <LOQ problem:
# Repeat RED assay with varying amounts of protein




Estimate dissociation constant $K_d$
(strength of binding affinity between chemical and protein)

Wambaugh et al. (2019)

# Additional source of uncertainty: Non-specific chemical binding to membrane or walls



$$F_{up} = \frac{\text{Protein—free conc.}}{\text{Protein—containing conc.}}$$

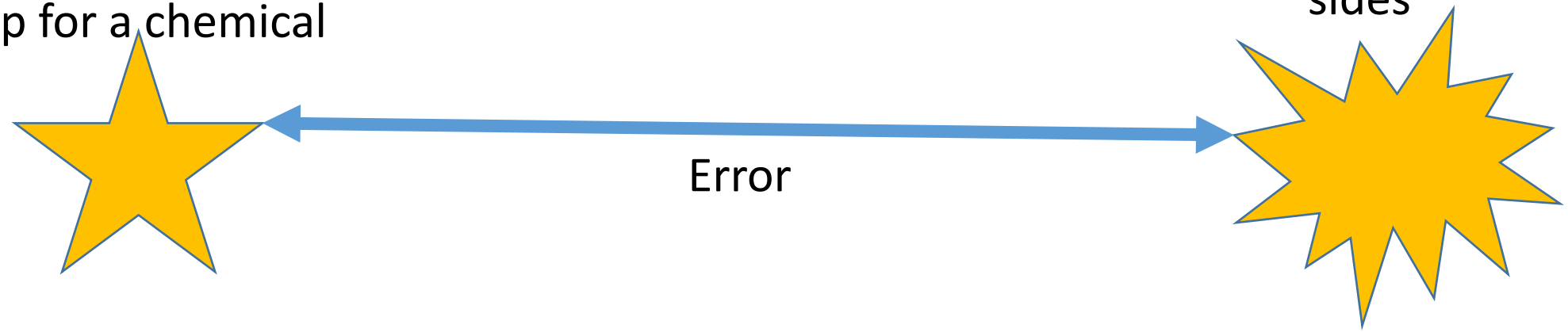
?

Wambaugh et al. (2019)

# Bayesian inference model for Fup uncertainty

Unknown true value:
Fup for a chemical

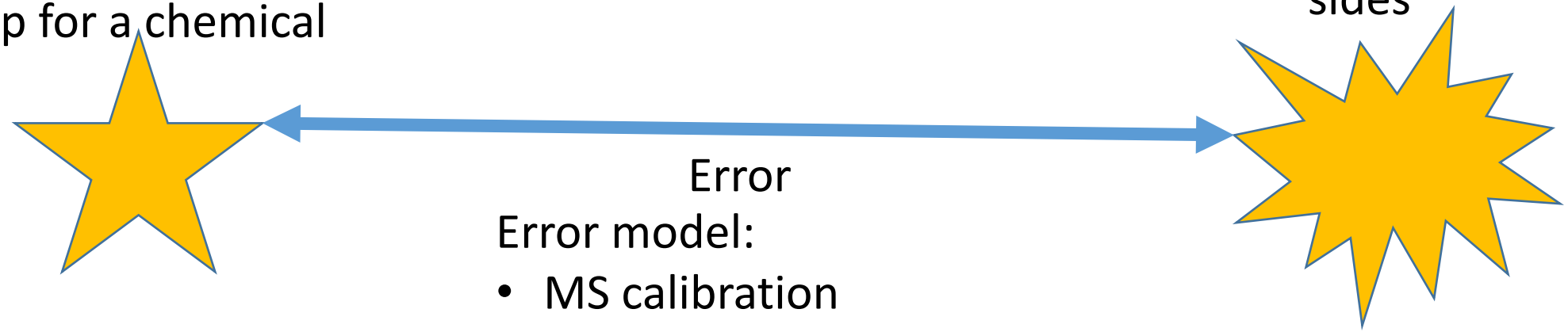
Observed (measured) value:
MS peak areas for protein-free and protein-containing sides




Wambaugh et al. (2019)

# Bayesian inference model for Fup uncertainty

Unknown true value:
Fup for a chemical



Observed (measured) value:
MS peak areas for protein-free and protein-containing sides

Error

Error model:

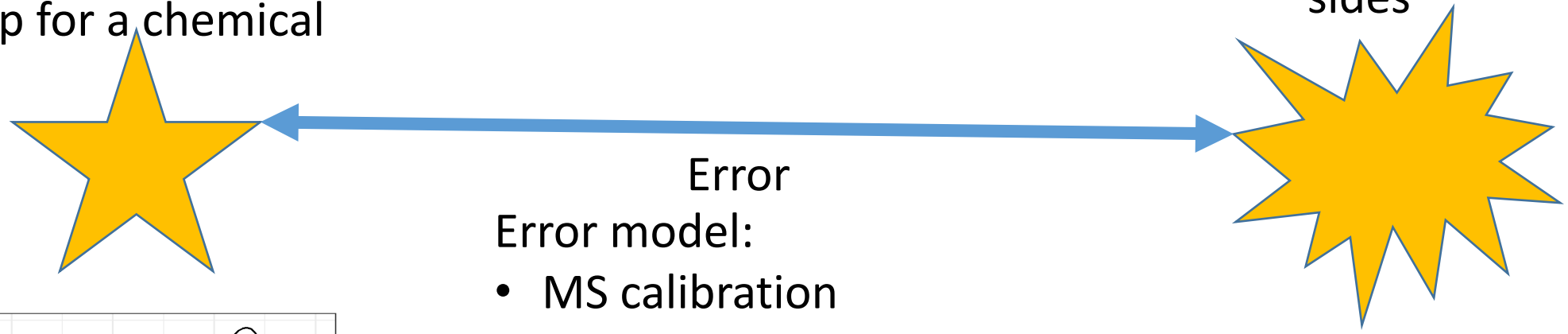
- MS calibration
- MS LOQ
- Dissociation constant $K_d$
- Non-specific binding

Wambaugh et al. (2019)

# Bayesian inference model for Fup uncertainty

Unknown true value:
Fup for a chemical

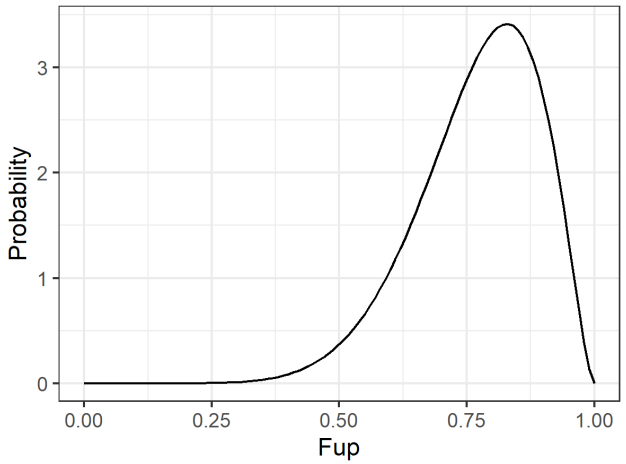
Observed (measured) value:
MS peak areas for protein-free and protein-containing sides



Error

Error model:

- MS calibration
- MS LOQ
- Dissociation constant $K_d$
- Non-specific binding



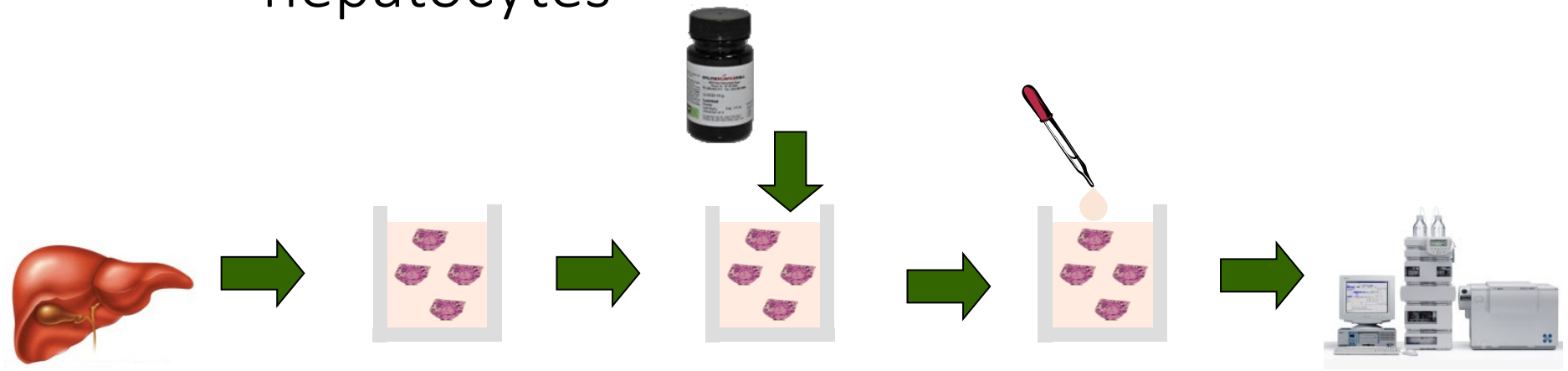

Wambaugh et al. (2019)

**Result:** *Distribution* of possible Fup values for a chemical, compatible with measurements & error model

EPA United States Environmental Protection Agency

# Uncertainty in CLint

# CLint: How to measure *in vitro* using pooled human hepatocytes

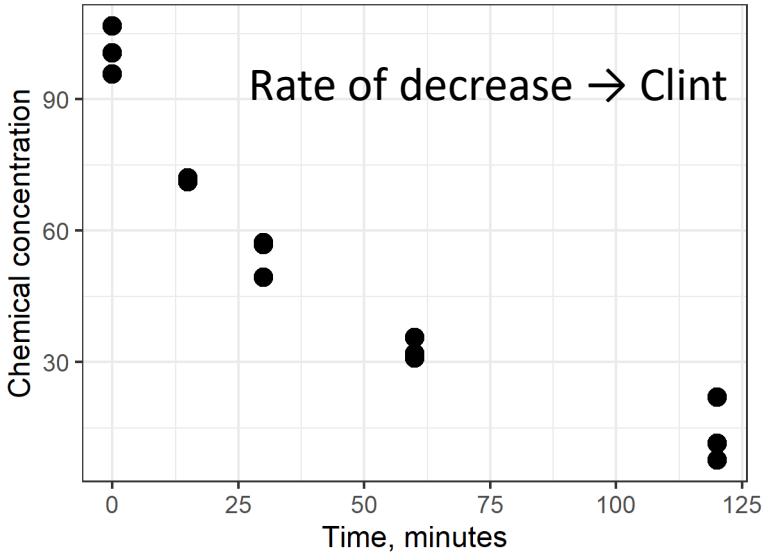


Culture donated human hepatocytes from 10 adult volunteers

Add known amount of chemical

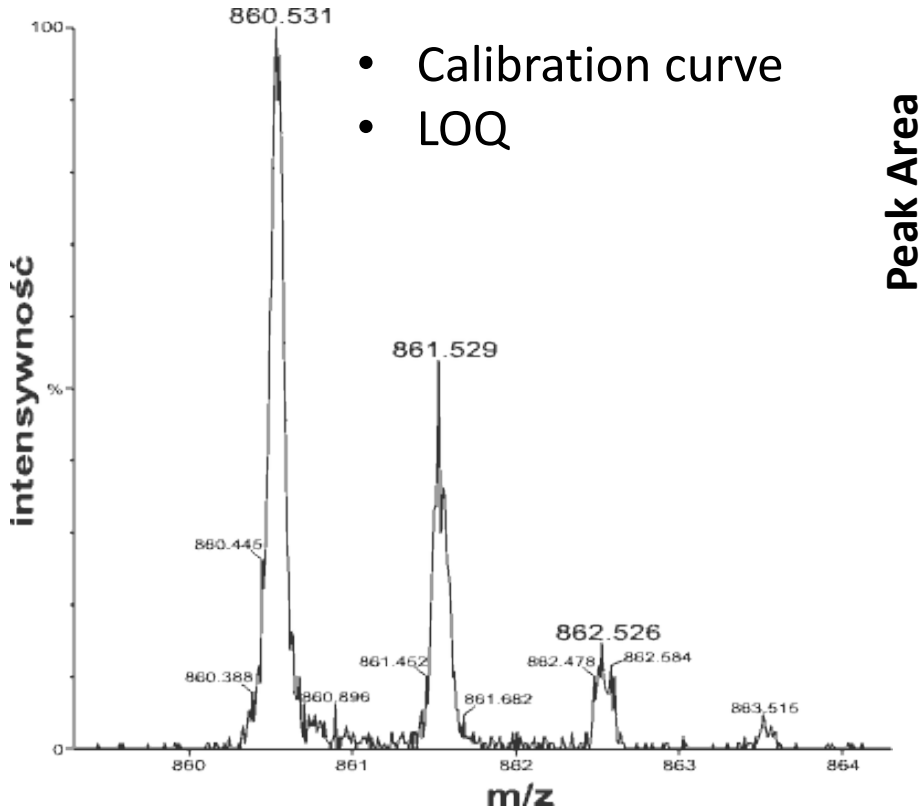
Measure chemical concentration remaining at 0, 15, 30, 60, and 120 minutes

CLint can be estimated from fitting a decaying exponential to concentration vs. time




Rotroff et al. (2010); Wetmore et al. (2012, 2015); Wambaugh et al. (2019)

# Mass spec uncertainties also apply to Clint




- Calibration curve
- LOQ

https://commons.wikimedia.org/wiki/File:ObwiedniaPeptydu.gif
(GPL)

Calibration: Area vs. concentration

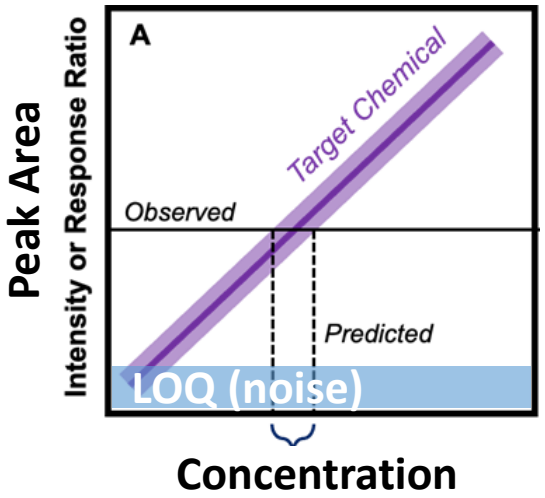
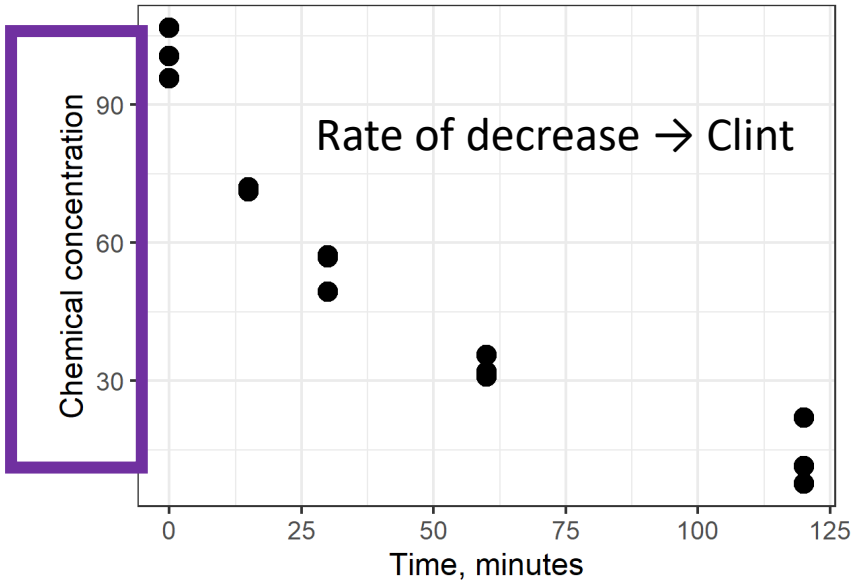



Figure adapted from Fisher et al. (2022)

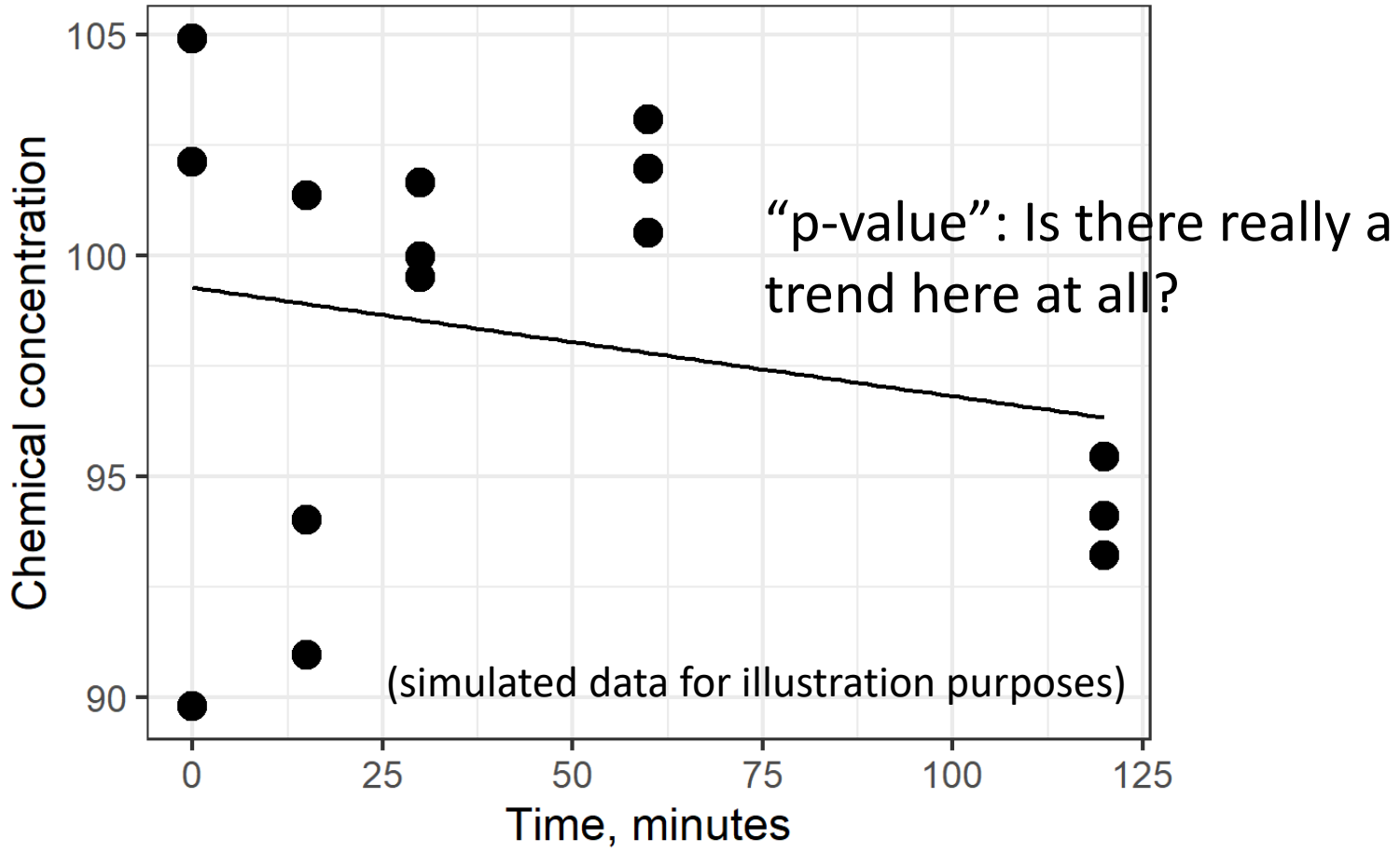
Result: Uncertainty in chemical conc.

Concentration vs. time



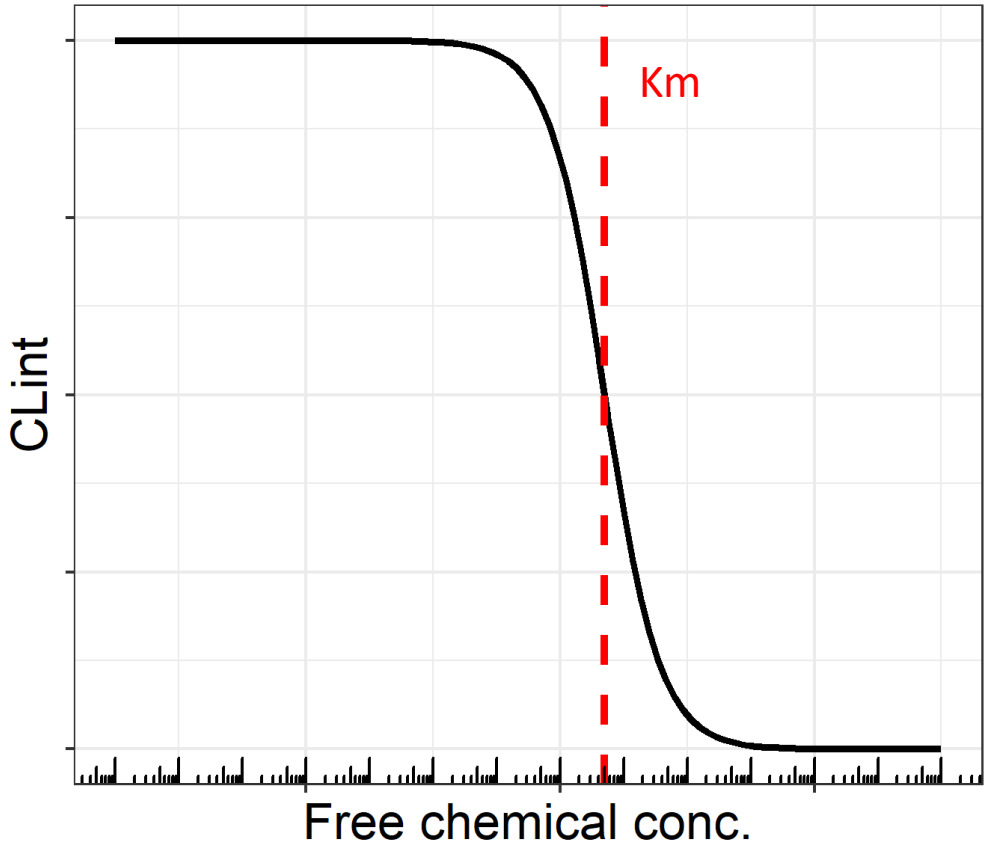

Wambaugh et al. (2019)

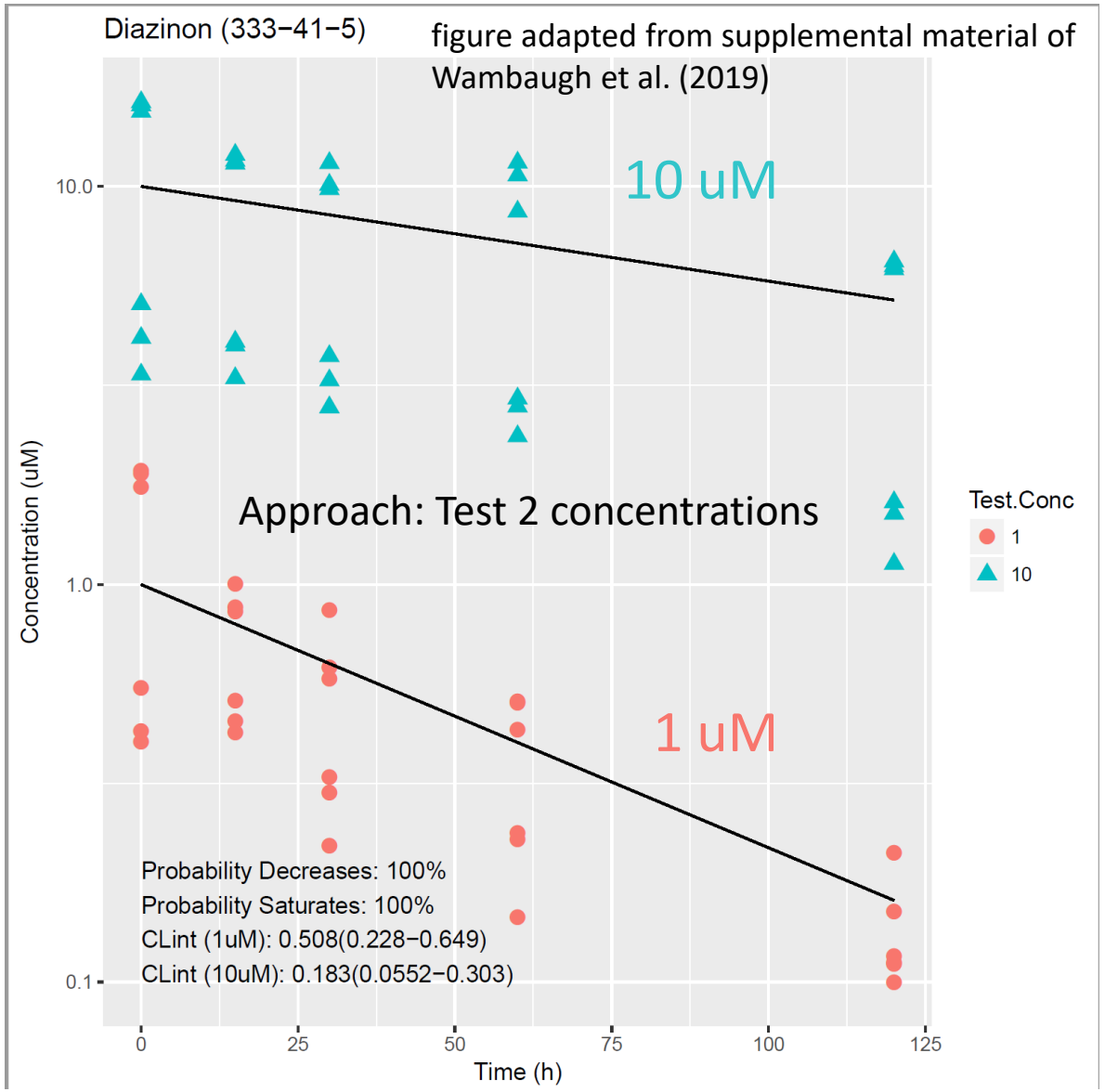
# Additional uncertainty source: Is chemical really metabolized at all?




Wambaugh et al. (2019)

# Additional uncertainty source: Saturable metabolism

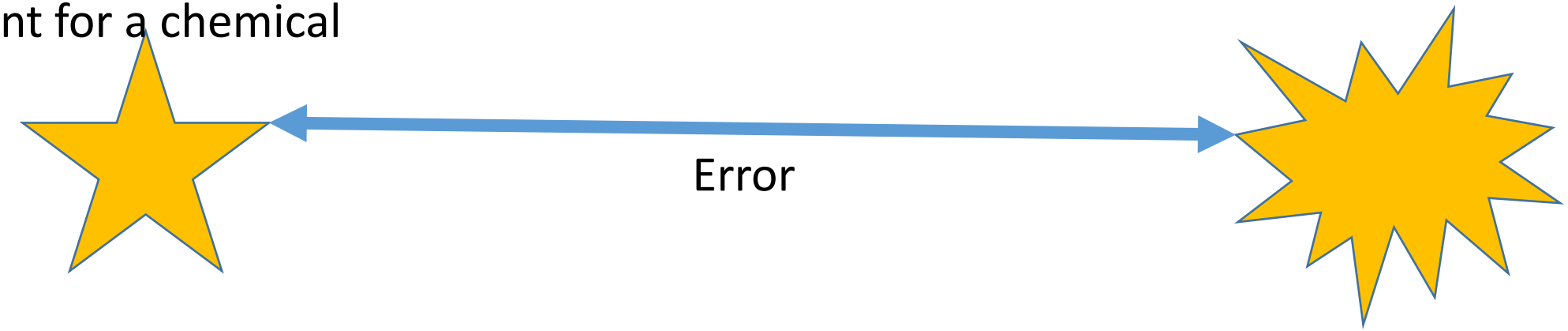


# Bayesian inference model for Clint uncertainty

Observed (measured) value:
MS peak areas at 5 time points

Unknown true value:
Clint for a chemical

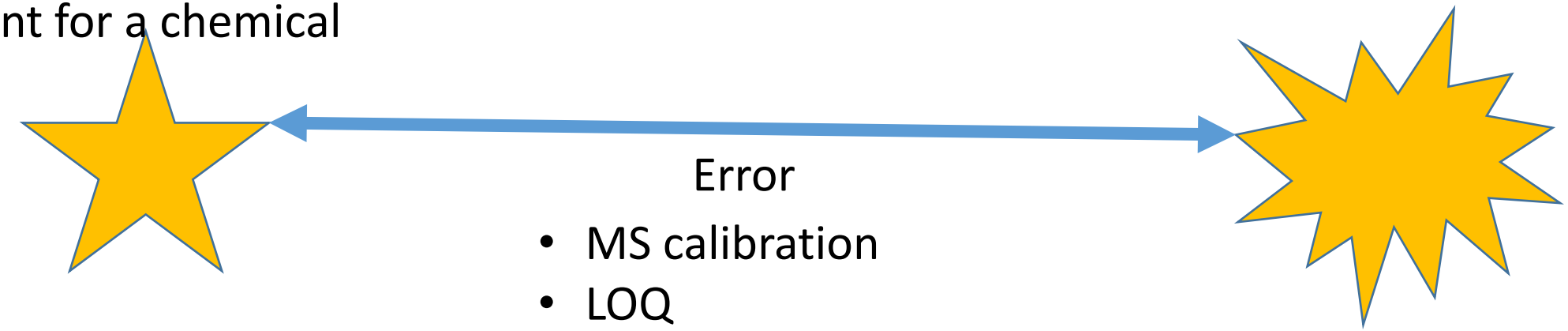



Wambaugh et al. (2019)

# Bayesian inference model for Clint uncertainty

Observed (measured) value:
MS peak areas at 5 time points

Unknown true value:
Clint for a chemical



Error

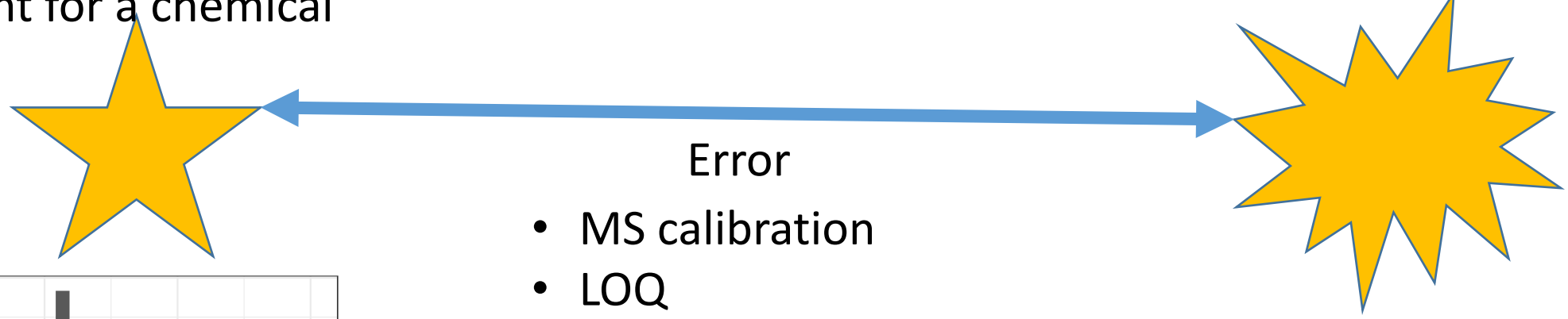
- MS calibration
- LOQ
- Probability of no metabolism
- Probability of saturation

Wambaugh et al. (2019)

# Bayesian inference model for Clint uncertainty

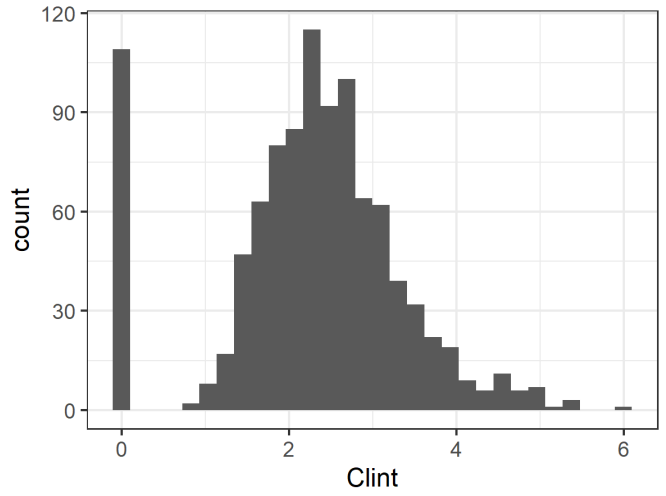
Observed (measured) value:
MS peak areas at 5 time points

Unknown true value:
Clint for a chemical



Error

- MS calibration
- LOQ
- Probability of no metabolism
- Probability of saturation




Wambaugh et al. (2019)

**Result:** *Distribution* of possible Clint values for a chemical, compatible with measurements & error model

EPA
United States
Environmental Protection
Agency

# Characterizing variability: HTTK-Pop for human TK variability

EPA United States Environmental Protection Agency

# HTTK physiological parameters

| Physiological parameters |
| --- |
| Tissue masses (including body weight) |
| Tissue blood flows |
| Glomerular filtration rate (passive renal clearance) |
| Hepatocellularity |

# Data source for population physiology: CDC NHANES


CDC NHANES = Centers for Disease Control National Health and Nutrition Examination Survey

Large, representative, ongoing survey of US population: demographics, body measures, medical examination data….

**NHANES does measure:**

Sex
Age
Height
Weight
Serum creatinine

**NHANES does not measure:**

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

Ring et al. (2017)

# Correlated Monte Carlo approach to simulating population variability in physiology: **HTTK-Pop**

*Sample* NHANES measured quantities for actual NHANES individuals (capturing covariance):

- Sex
- Age
- Height
- Weight
- Serum creatinine

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

*Predict* physiological TK quantities (as used by generic TK model) for each individual:

- Tissue masses
- Tissue blood flows
- GFR (kidney function)
- Hepatocellularity

(Similar approach used in SimCYP [Jamei et al. 2009], GastroPlus, PopGen [McNally et al. 2014], P3M [Price et al. 2003], physB [Bosgra et al. 2012], etc.)

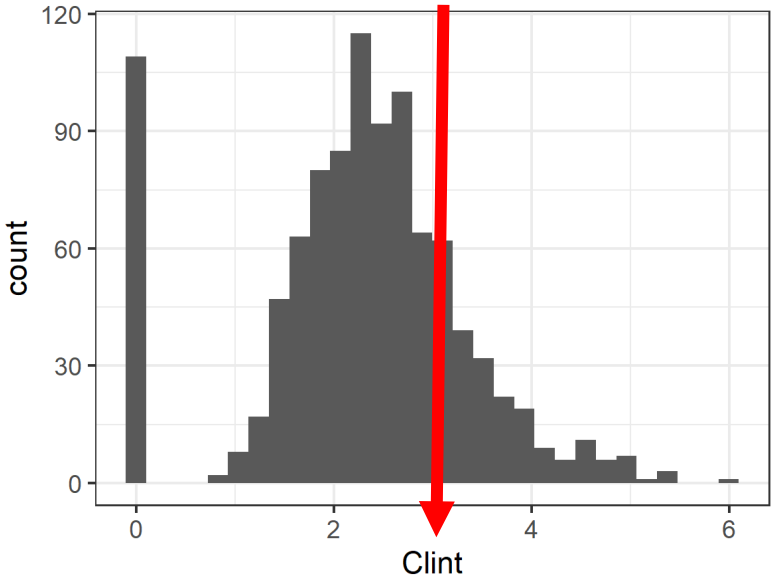
Ring *et al*. (2017)

# Chemical-specific parameters have both uncertainty and variability

| Chemical-specific parameters | |
|---|---|
| Intrinsic hepatic clearance rate (CLint) | Carry uncertainty from *in vitro* measurements<br><br>**Also** have population variability: represent chemical-body interactions — vary with individual genetics, environmental factors, age, etc. |
| Fraction unbound to plasma protein (Fup) | |

# Chemical-specific TK parameters: Two-stage Monte Carlo approach to modeling both *measurement uncertainty* and *population variability*

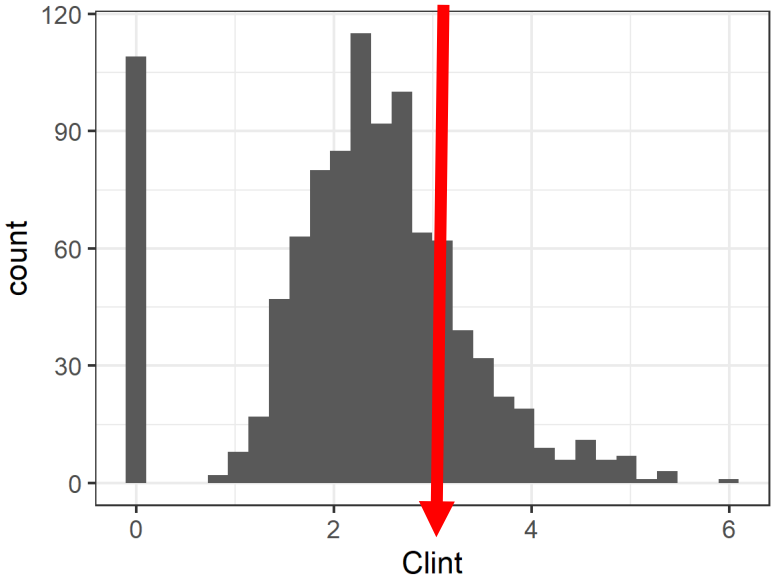
Step 1: Draw 1 sample from **uncertainty** distribution and treat as "population average" value



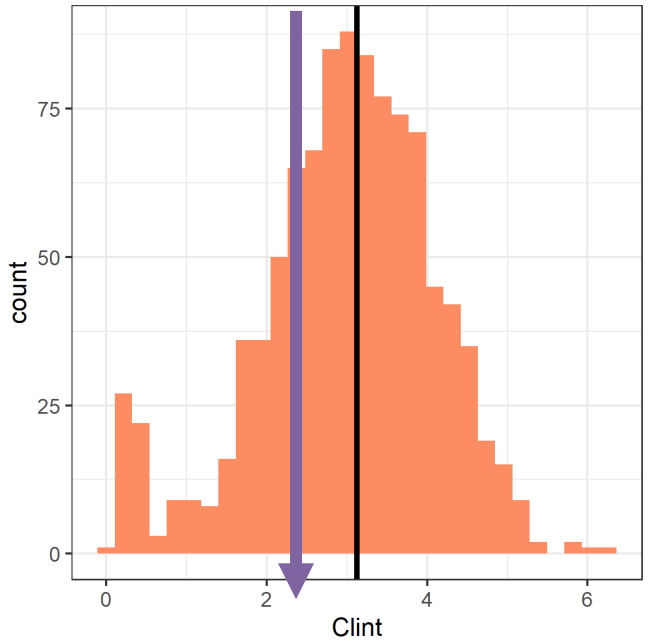

Wambaugh et al. (2019)

# Chemical-specific TK parameters: Two-stage Monte Carlo approach to modeling both *measurement uncertainty* and *population variability*

Step 1: Draw 1 sample from **uncertainty** distribution and treat as "population average" value




Step 2: Assume **population variability** (30% CV) around the sampled "population average" value from Step 1, and draw 1 sample

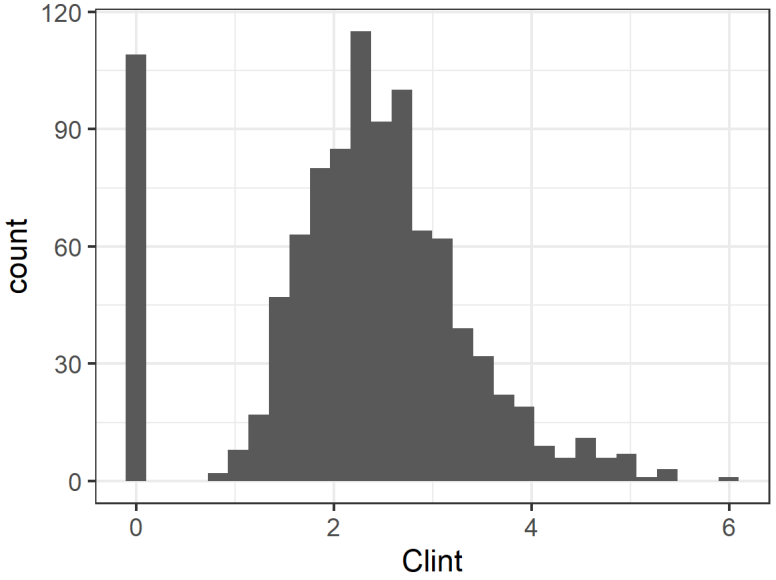



For CLint: Add 5% "poor metabolizers" (10% of original pop. average)

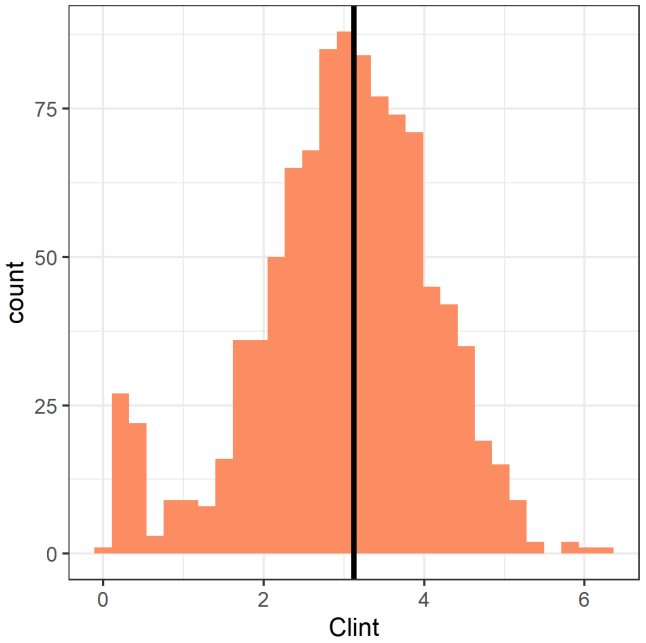
Wambaugh et al. (2019)

# Chemical-specific TK parameters: Two-stage Monte Carlo approach to modeling both *measurement uncertainty* and *population variability*

Step 1: Draw 1 sample from **uncertainty** distribution and treat as "population average" value

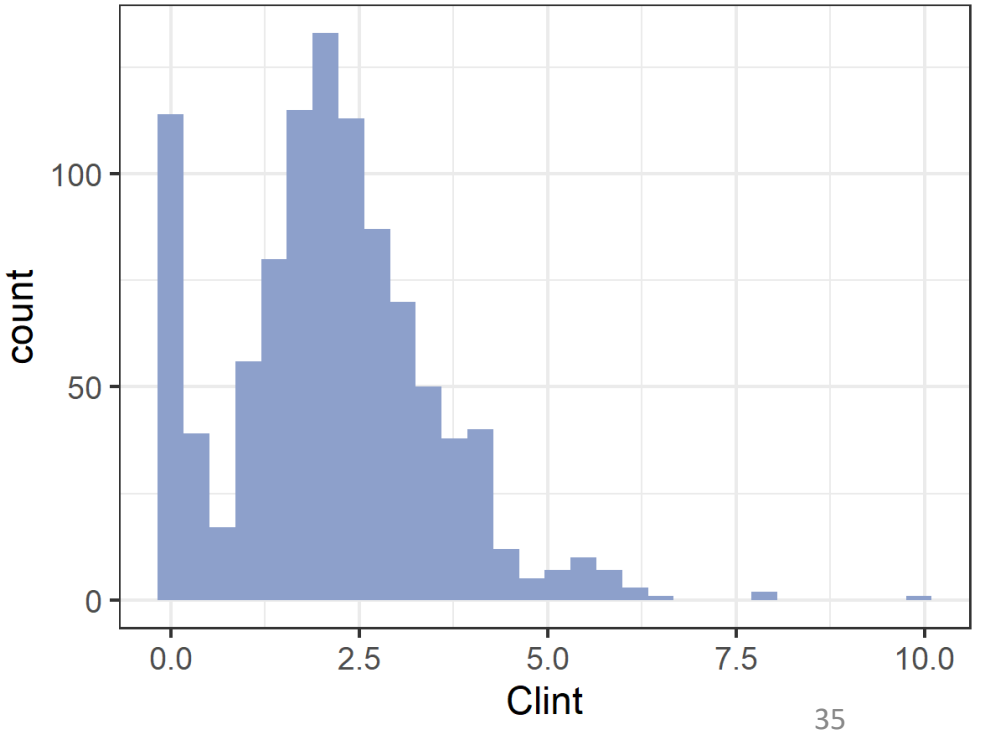



Step 2: Assume **population variability** (30% CV) around the sampled "population average" value from Step 1, and draw 1 sample




For CLint: Add 5% "poor metabolizers" (10% of original pop. average)

Repeat Steps 1 and 2 for each simulated individual to get sampled values that include **both uncertainty & variability**

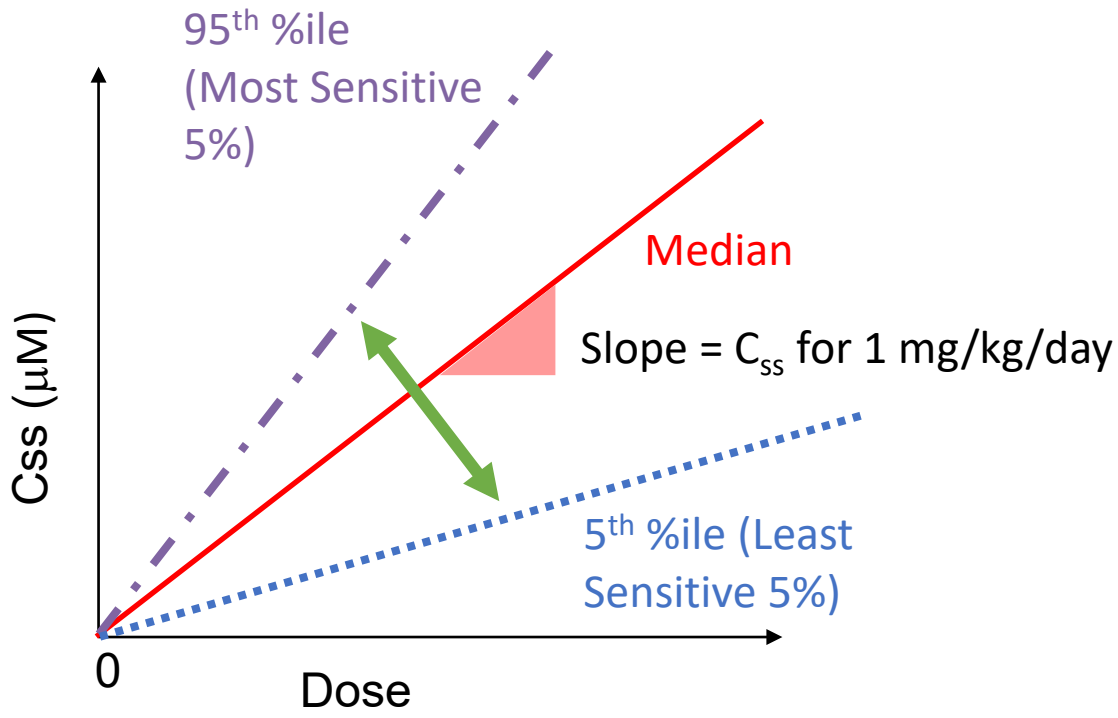



Wambaugh et al. (2019)

# Putting it all together: A table of HTTK model parameters for each "simulated individual" in a "simulated population" for a given chemical

| SEQN | Demographics | | Body measures | | Tissue volumes | Blood flows | GFR | Hepatocellularity | Fup | Clint |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sex | Age | Ht | Wt | | | | | | |
| 67184 | M | 42 | 171 | 55 | […] | […] | […] | […] | […] | […] |
| 52034 | M | 0.5 | 73 | 9 | […] | […] | […] | […] | […] | […] |
| 64847 | F | 11 | 154 | 47 | […] | […] | […] | […] | […] | […] |
| 51787 | F | 22 | 166 | 87 | […] | […] | […] | […] | […] | […] |
| 49889 | M | 9 | 147 | 50 | […] | […] | […] | […] | […] | […] |
| 64606 | F | 59 | 169 | 115 | […] | […] | […] | […] | […] | […] |
| 45549 | F | 50 | 165 | 80 | […] | […] | […] | […] | […] | […] |
| […] | […] | […] | […] | […] | […] | […] | […] | […] | […] | […] |

# Evaluate TK model for each "simulated individual" = distribution of Css-dose slopes

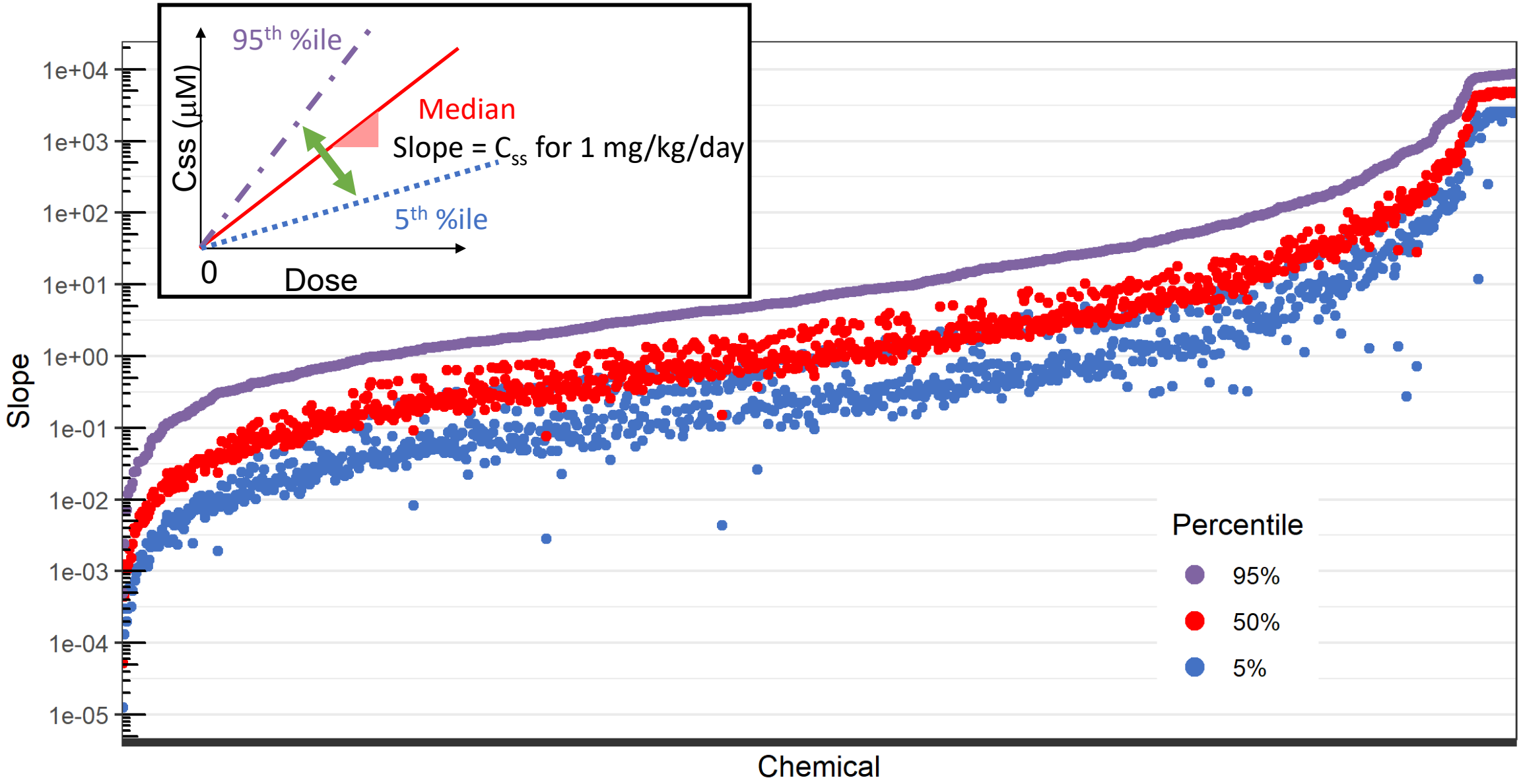


# httk R package automates this Monte Carlo sampling & model evaluation process

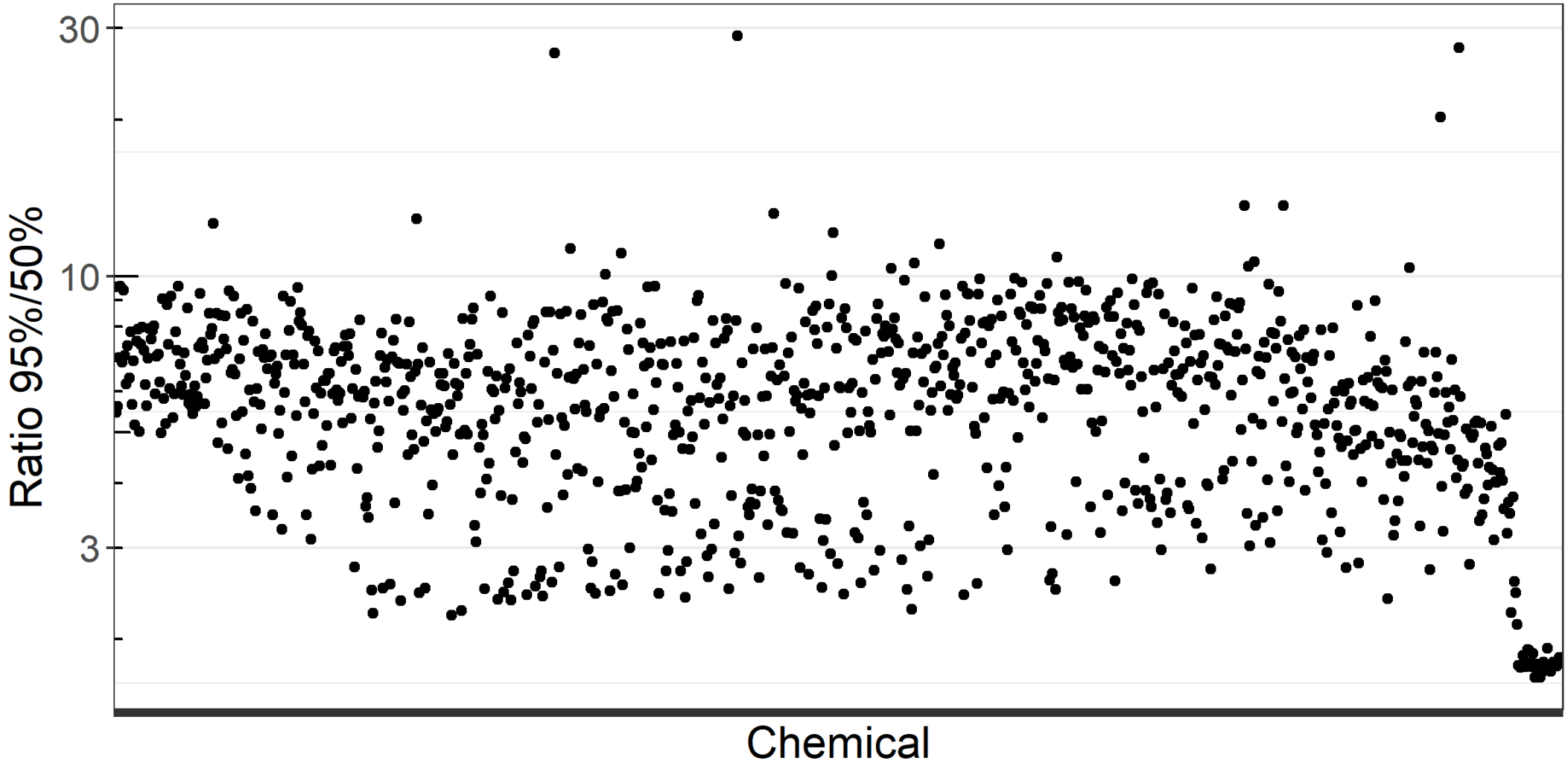
```
> library(httk)
> set.seed(42)
> #Css for 1 mg/kg/day = slope
 calc_mc_css(chem.name="benzo(a)pyrene",
             which.quantile = c(0.95, 0.5, 0.05))

Human plasma concentration returned in mg/L units
for 0.95 0.5 0.05 quantile.
   95%    50%     5%
68.510 13.070  3.742
```

# Result: Percentiles of predicted Css vs. dose slope



# Another way to visualize: ratio of 95$^{th}$ percentile to median (roughly, how wide is the Css slope distribution?)



EPA
United States
Environmental Protection
Agency

# Relative contributions of variability & uncertainty

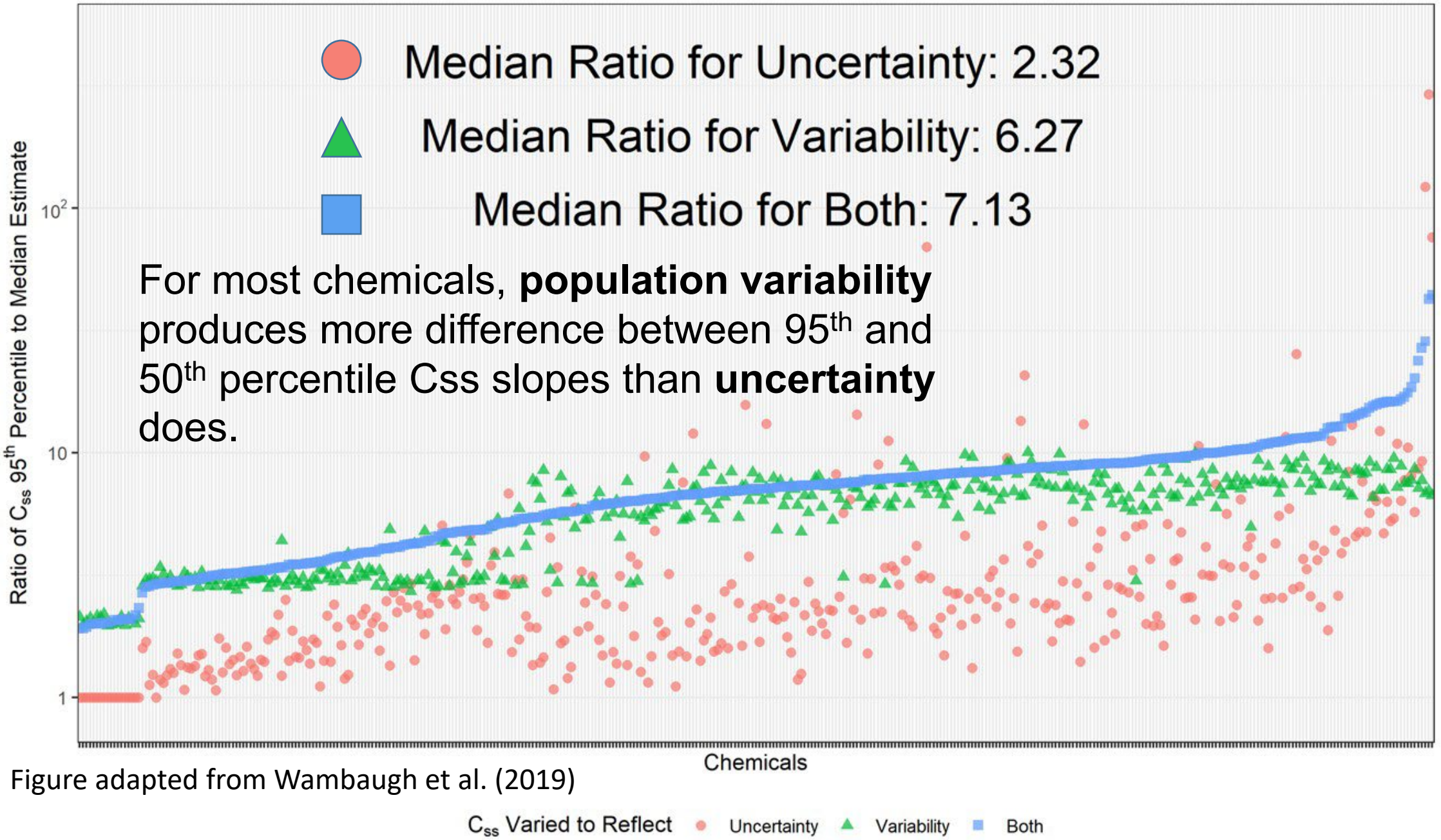
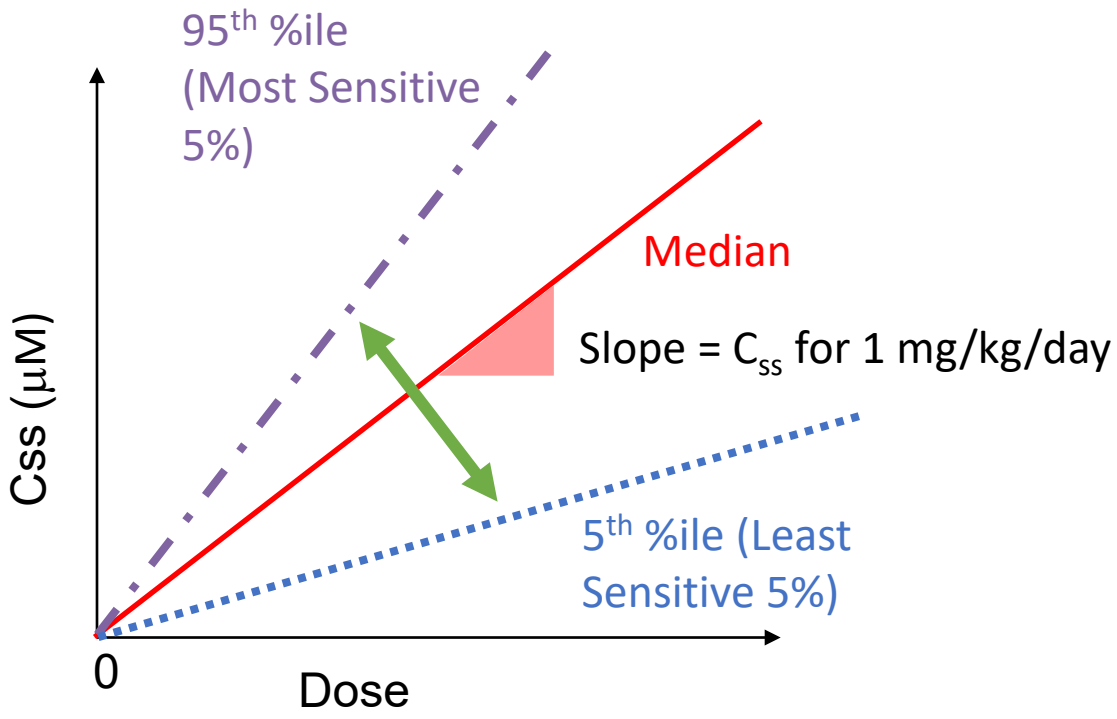



Figure adapted from Wambaugh et al. (2019)

EPA United States Environmental Protection Agency

# Simulating sensitive subpopulations

# Identifying potentially sensitive sub-populations




Who is in the most sensitive portion of the population?

What does this slope distribution look like for kids, for example?

Or people over 65?

To answer this question: Need to model TK variability for specified sub-populations

Ring et al. (2017)

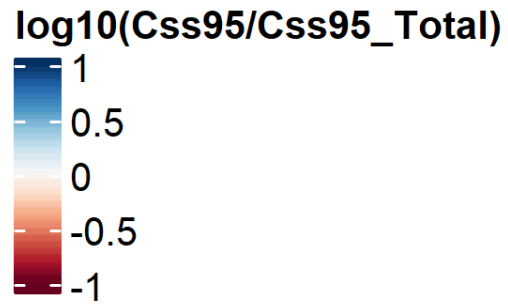
# HTTK-Pop can generate simulated subpopulations with user-specified demographics

Use `httkpop.generate.args` argument to `calc_mc_css()` function: Takes a named list of arguments

| Name of list element | User can specify… | Example | | Default if not specified |
|---|---|---|---|---|
| `agelim_years` | Age limits in years | `c(6,11)` | Ages 6-11 years | All NHANES (0-79 years) |
| `agelim_months` | Age limits in months | `c(0,36)` | Ages 0-36 months | All NHANES (0-79 years) |
| `gendernum` | # of males and females | `list(Male = 1000, Female = 0)` | 1000 males, 0 females | Randomly selected from NHANES |
| `weight_category` | BMI category | `c('Overweight', 'Obese')` | BMI > 25 (overweight & obese) | c('Underweight', 'Normal', 'Overweight', 'Obese') |

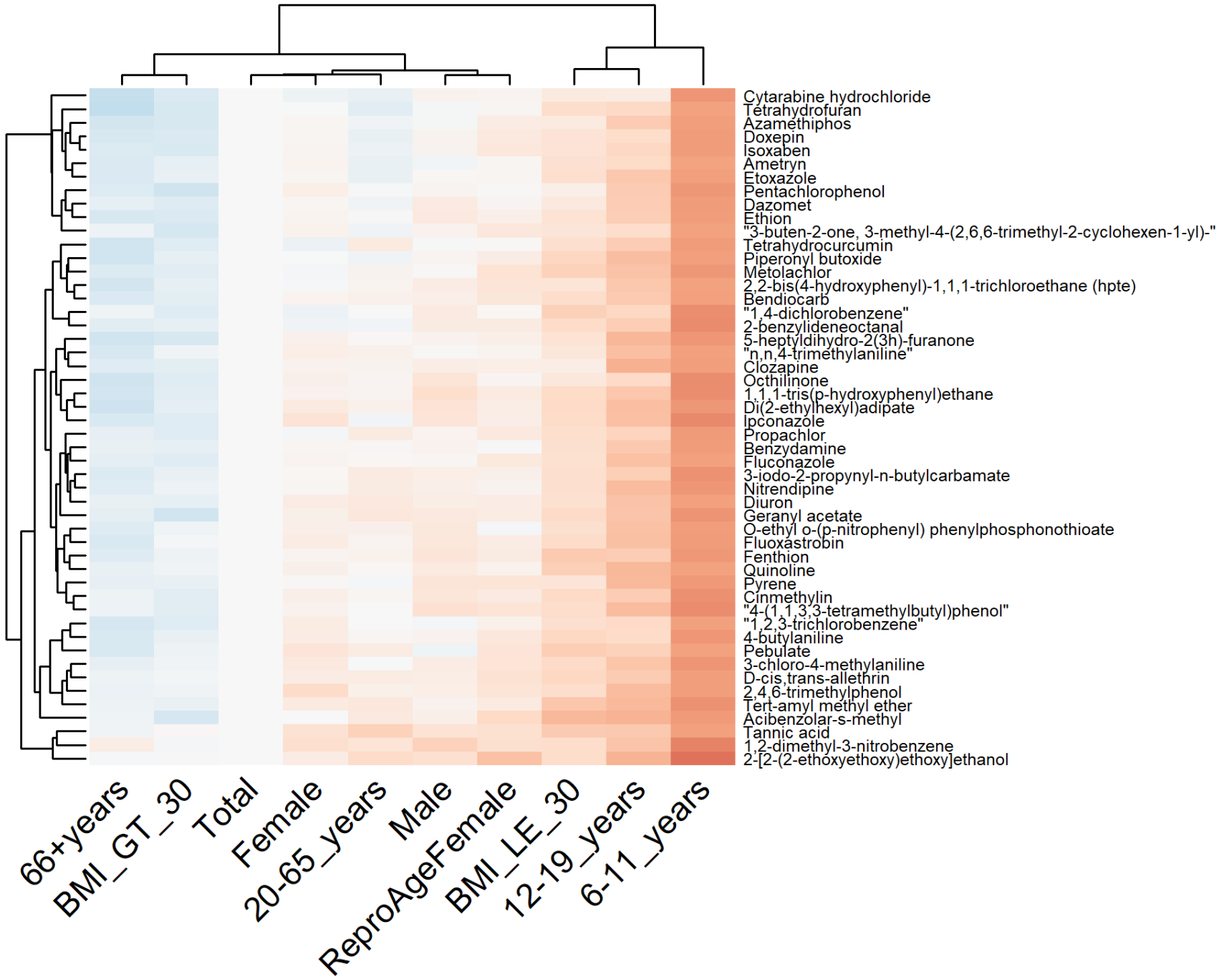
HTTK-Pop generates physiology based on NHANES respondents in the specified demographic groups

# Example of Css95 differences by subpopulation




10 subgroups of interest

Heatmap: Css95 difference (subgroup vs. Total population) for 50 chemicals with largest Css95 difference in *any* subgroup



EPA United States Environmental Protection Agency

# Conclusions

EPA United States Environmental Protection Agency

# Conclusions

- Uncertainty vs. Variability in TK model parameters
  - Measurement uncertainty: Chemical-specific parameters measured *in vitro*
  - Population variability: Physiological & chemical-specific parameters
- Characterizing key uncertainty in chemical-specific TK parameters using Bayesian inference
  - Fraction unbound in plasma protein (Fup)
  - Intrinsic hepatic clearance rate (Clint)
- Characterizing variability: HTTK-Pop for human TK variability
  - Correlated Monte Carlo approach based on CDC NHANES data
- Relative contributions of uncertainty and variability to TK model predictions
  - For most chemicals, population variability has larger effect
- Simulating sensitive subpopulations
  - HTTK-Pop can simulate populations with user-specified demographics

EPA
United States
Environmental Protection
Agency

# Thank you!

Questions?

EPA United States Environmental Protection Agency

# References

1. Rotroff DM, Wetmore BA, Dix DJ, et al. Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening. Toxicological Sciences. 2010;117(2):348-358
2. Wetmore BA, Wambaugh JF, Allen B, et al. Incorporating High-Throughput Exposure Predictions With Dosimetry-Adjusted In Vitro Bioactivity to Inform Chemical Toxicity Testing. Toxicological Sciences. 2015 Nov;148(1):121-36
3. Wambaugh JF, Wetmore BA, Pearce R, Strope C, Goldsmith R, Sluka JP, et al. Toxicokinetic Triage for Environmental Chemicals. Toxicol Sci. 2015;147(1):55-67.
4. Ring CL, Pearce RG, Setzer RW, et al. Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability. Environment International. 2017 2017/09/01/;106:105-118.
5. Shibata Y, Takahashi H, Chiba M, Ishii Y. Prediction of hepatic clearance and availability by cryopreserved human hepatocytes: an application of serum incubation method. Drug Metab Dispos. 2002;30(8):892-6.
6. Waters NJ, Jones R, Williams G, Sohal B. Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding. J Pharm Sci. 2008;97(10):4586-95.
7. Wetmore BA, Wambaugh JF, Ferguson SS, et al. Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicological Sciences. 2012 Jan;125(1):157-74.
8. Wetmore BA. Quantitative in vitro-to-in vivo extrapolation in a high-throughput environment. Toxicology. 2015;332:94-101.

9. Wambaugh JF, Wetmore BA, Ring CL, Nicolas CI, Pearce RG, Honda GS, et al. Assessing Toxicokinetic Uncertainty and Variability in Risk Prioritization. Toxicol Sci. 2019;172(2):235-51.
10. Sipes NS, Wambaugh JF, Pearce R, et al. An Intuitive Approach for Predicting Potential Human Health Risk with the Tox21 10k Library. Environmental Science & Technology. 2017 2017/09/19;51(18):10786-10796.
11. Pearce RG, Setzer RW, Davis JL, Wambaugh JF. Evaluation and calibration of high-throughput predictions of chemical distribution to tissues. J Pharmacokinet Pharmacodyn. 2017b;44(6):549-65.
12. Jamei M, Marciniak S, Feng K, et al. The Simcyp® population-based ADME simulator. Expert Opinion on Drug Metabolism & Toxicology. 2009;5(2):211-223.
13. McNally K, Cotton R, Hogg A, Loizou G. PopGen: A virtual human population generator. Toxicology. 2014;315:70-85.
14. Price PS, Conolly RB, Chaisson CF, Gross EA, Young JS, Mathis ET, et al. Modeling Interindividual Variation in Physiological Factors Used in PBPK Models of Humans. Critical Reviews in Toxicology. 2003;33(5):469-503.
15. Bosgra S, van Eijkeren J, Bos P, Zeilmaker M, Slob W. An improved model to predict physiologically based model parameters and their inter-individual variability from anthropometry. Crit Rev Toxicol. 2012;42(9):751-67.
16. Wetmore BA, Allen B, Clewell HJ, 3rd, et al. Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing. Toxicological Sciences. 2014 Nov;142(1):210-24.