



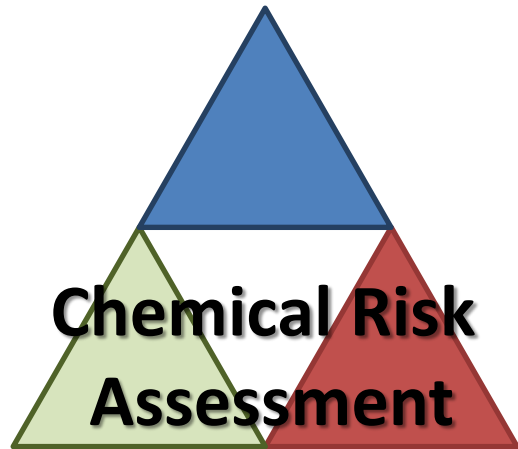
Bioactivity: Exposure Ratio Example
**Comparing NAM-based PODs to Estimates
of Human Daily Intake**

NAMs Training Workshop

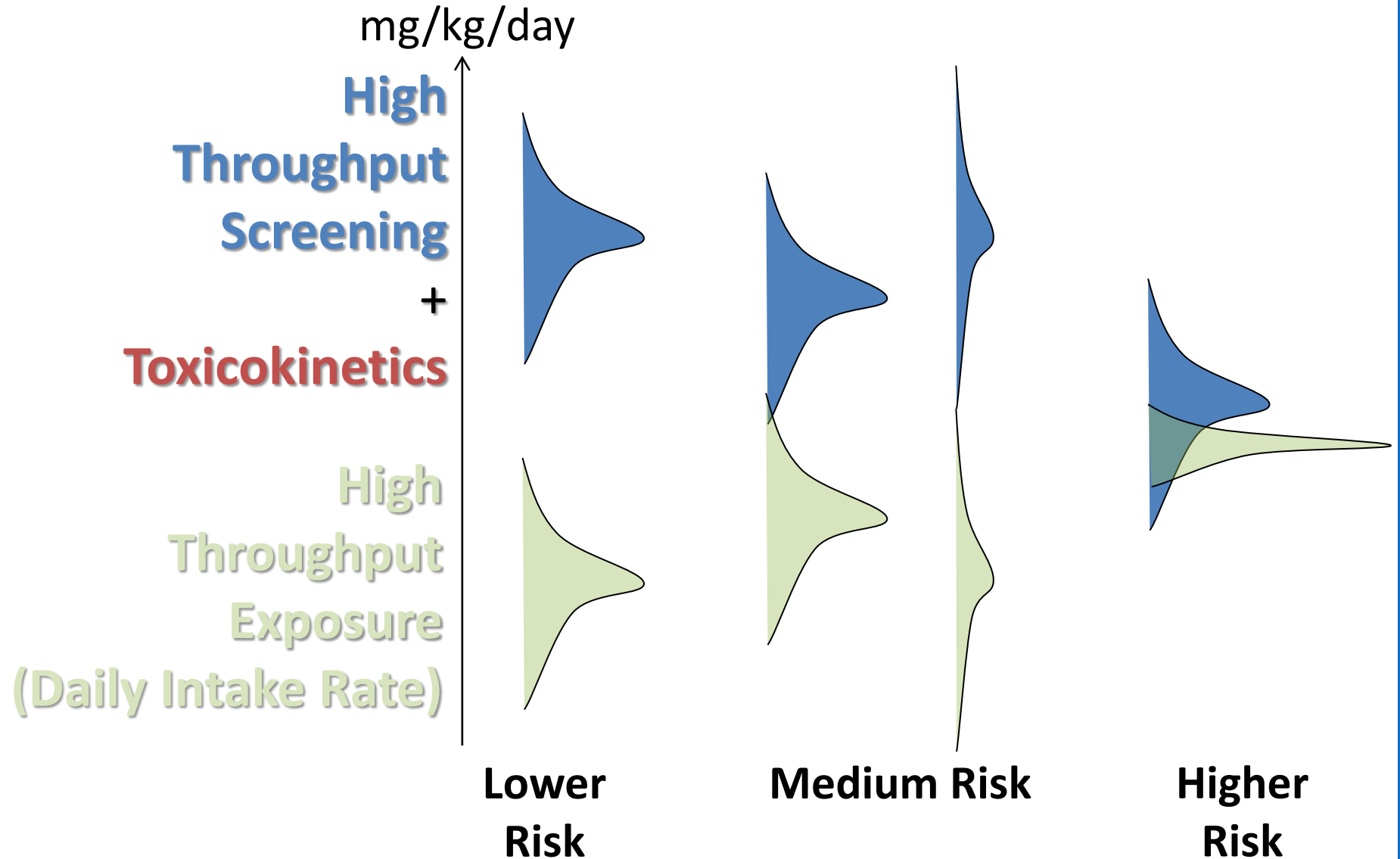
April 24th – 25th, 2024

John Wambaugh

Bioactivity : Exposure Ratios (BERs)

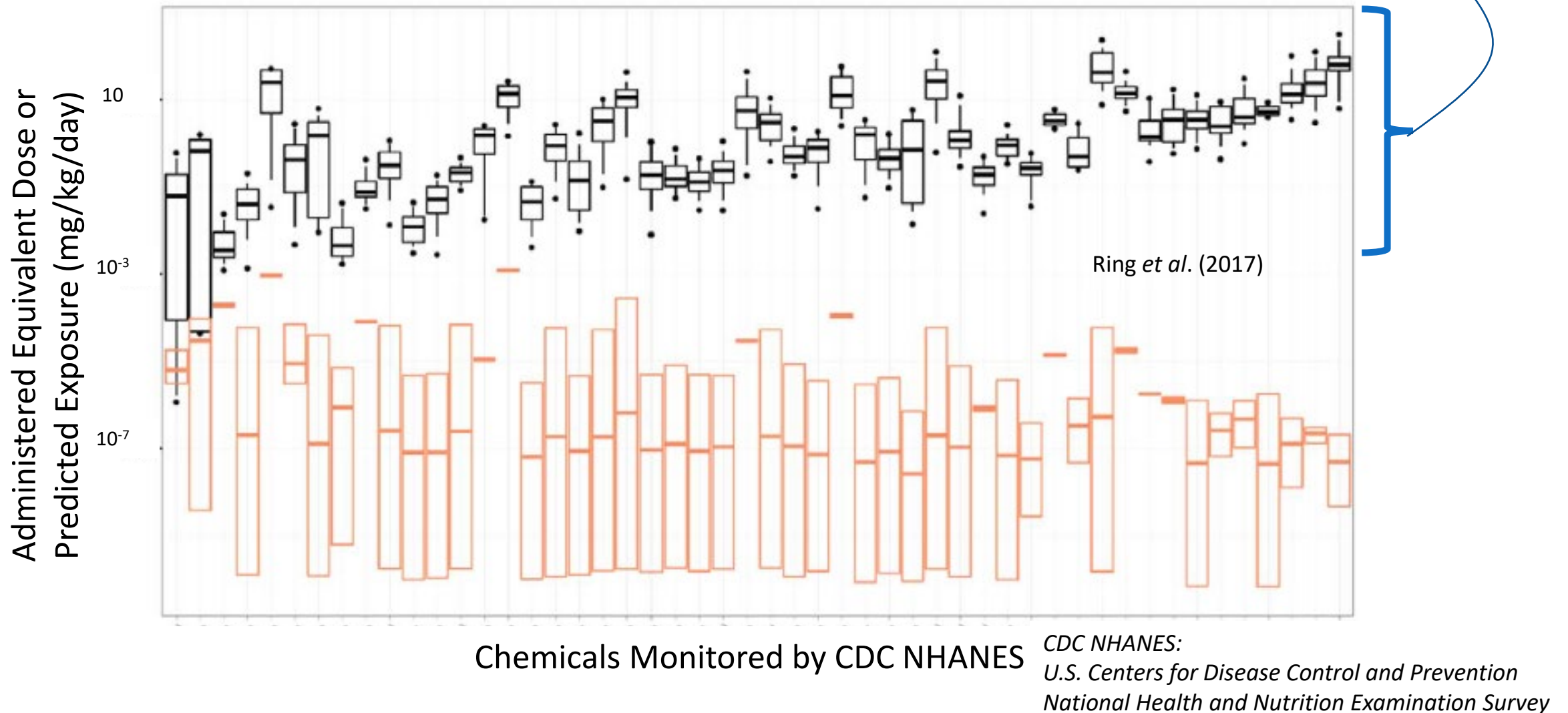


NRC, 1983



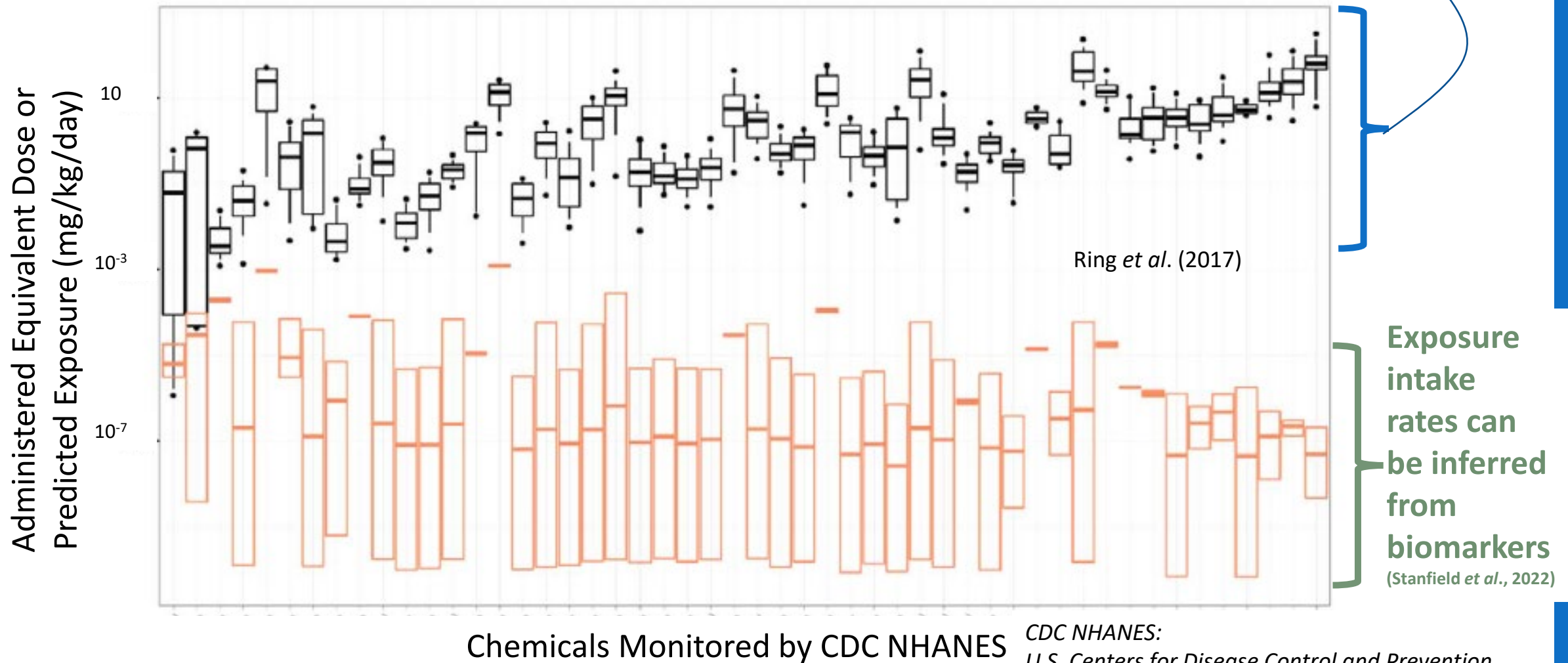
BERs Allow Chemical Prioritization

In Vitro Screening + IVIVE can estimate doses needed to cause bioactivity (Wetmore *et al.*, 2015b)



BERs Allow Chemical Prioritization

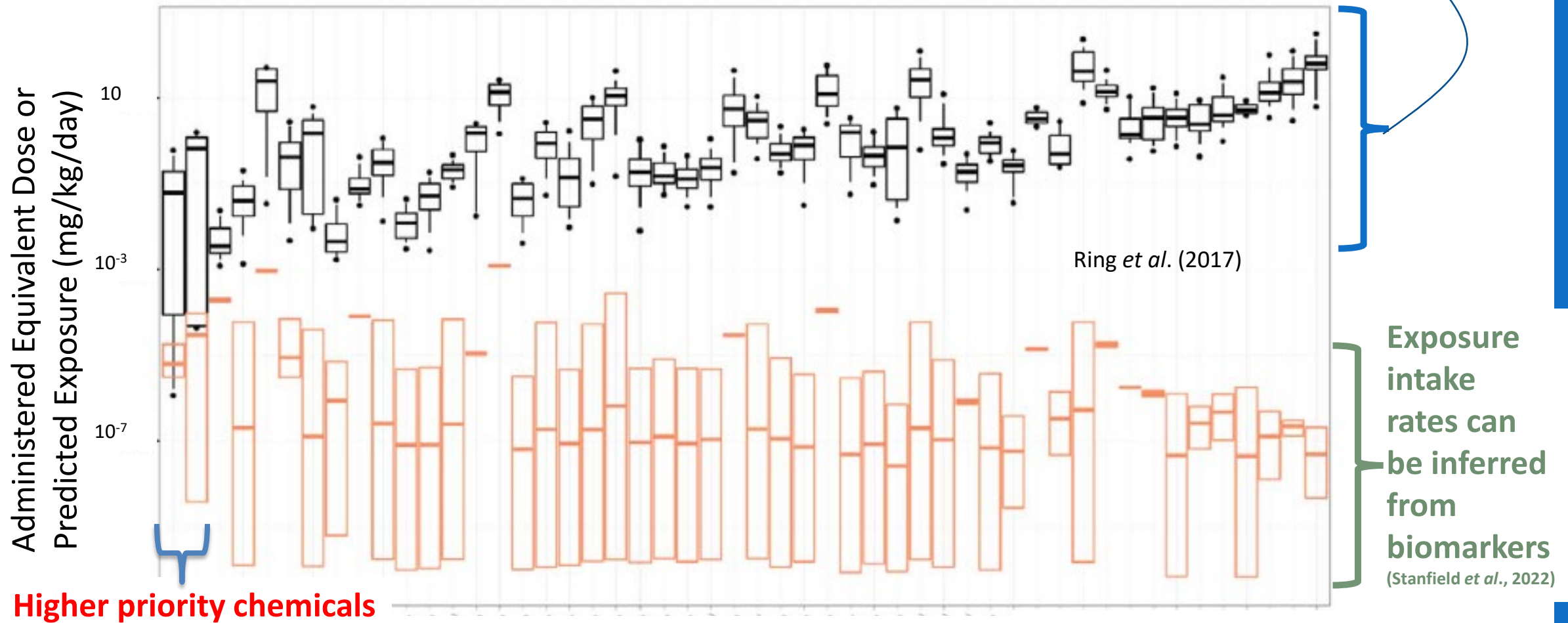
In Vitro Screening + IVIVE can estimate doses needed to cause bioactivity (Wetmore *et al.*, 2015b)



CDC NHANES:
U.S. Centers for Disease Control and Prevention
National Health and Nutrition Examination Survey

BERs Allow Chemical Prioritization

In Vitro Screening + IVIVE can estimate doses needed to cause bioactivity (Wetmore *et al.*, 2015b)



CDC NHANES:
U.S. Centers for Disease Control and Prevention
National Health and Nutrition Examination Survey

Most Chemicals Do Not Have TK Data

- We need chemical-specific toxicokinetics (TK) for *in vitro-in vivo* extrapolation (IVIVE) (Rotroff et al., 2010), **but:**
 - Most non-pharmaceutical chemicals – for example, flame retardants, plasticizers, pesticides, solvents – do not have human *in vivo* TK data
 - Non-pesticidal chemicals are unlikely to have any *in vivo* TK data, even from animals

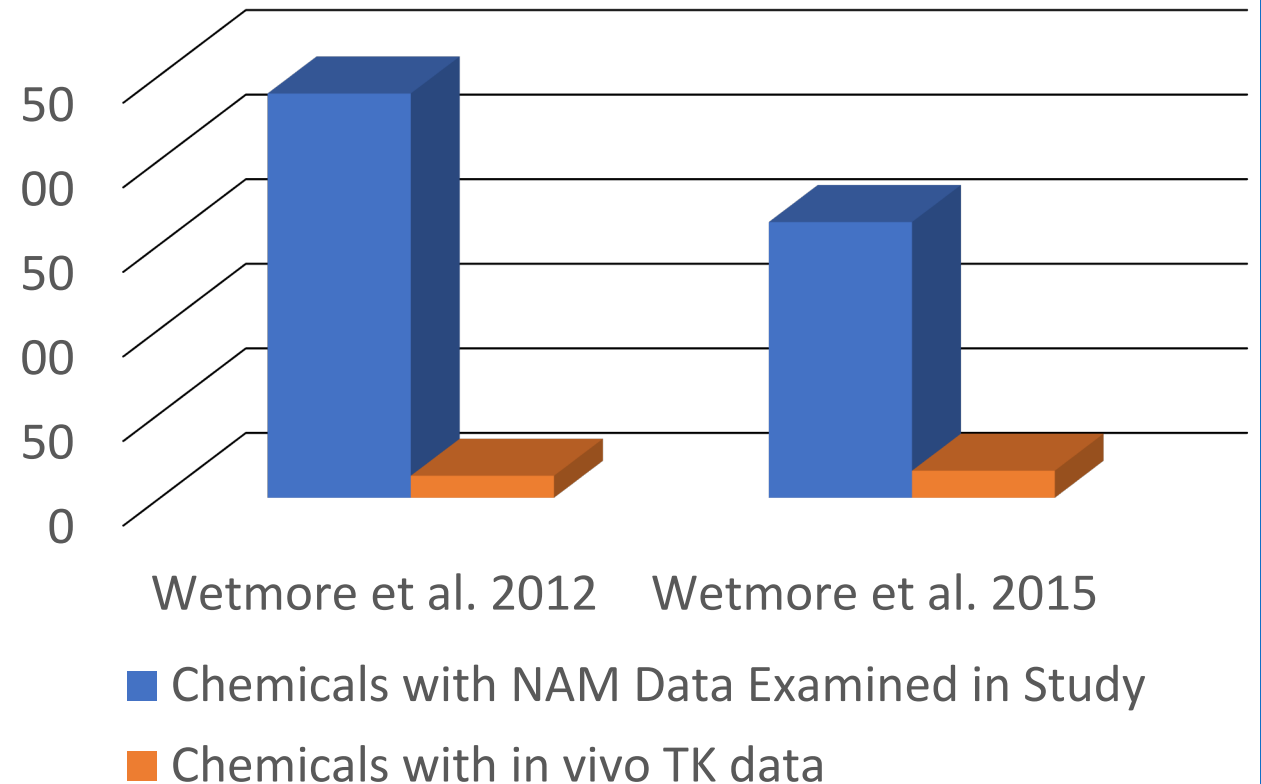
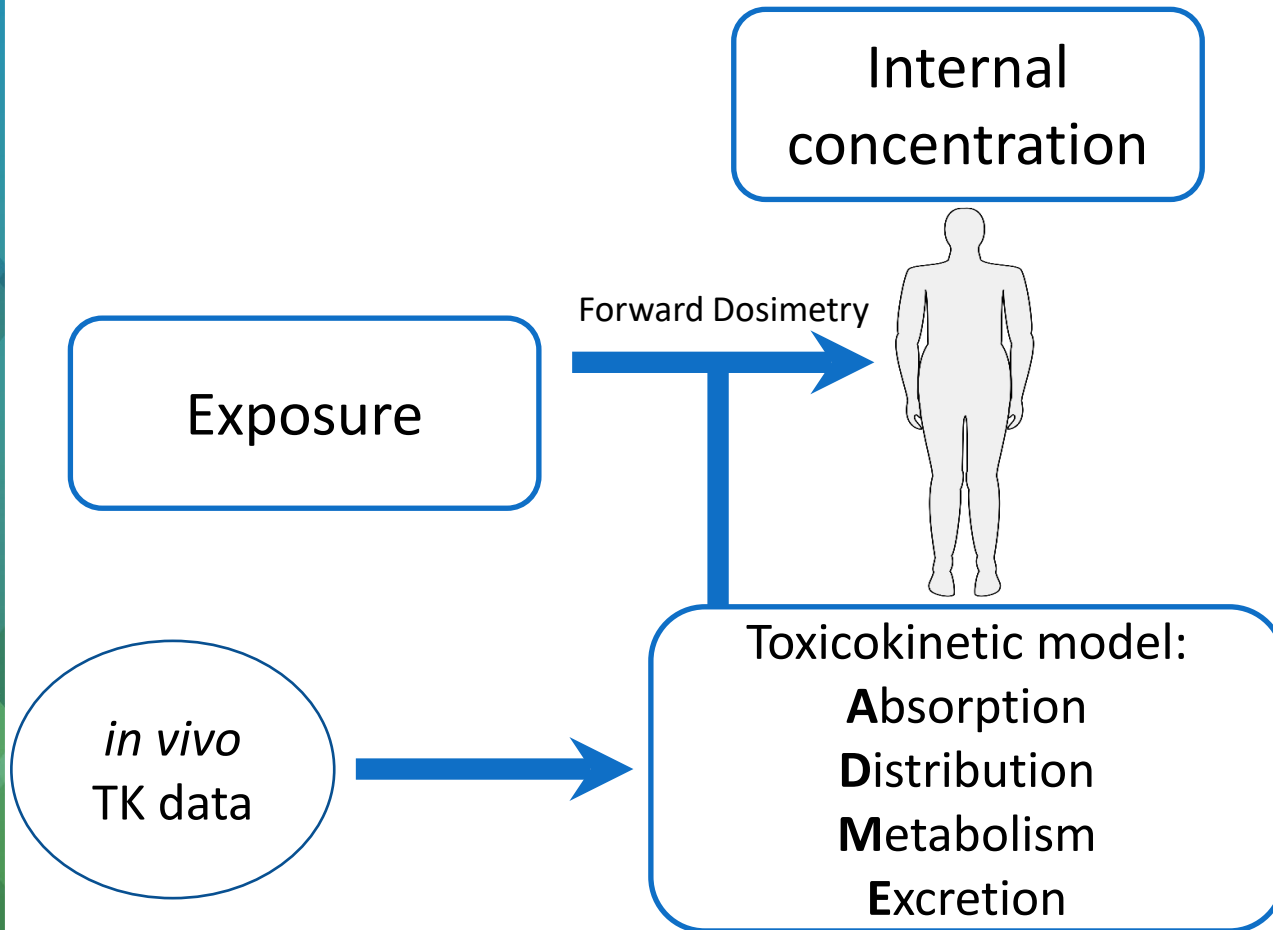


Figure modified from Bell et al. (2018)

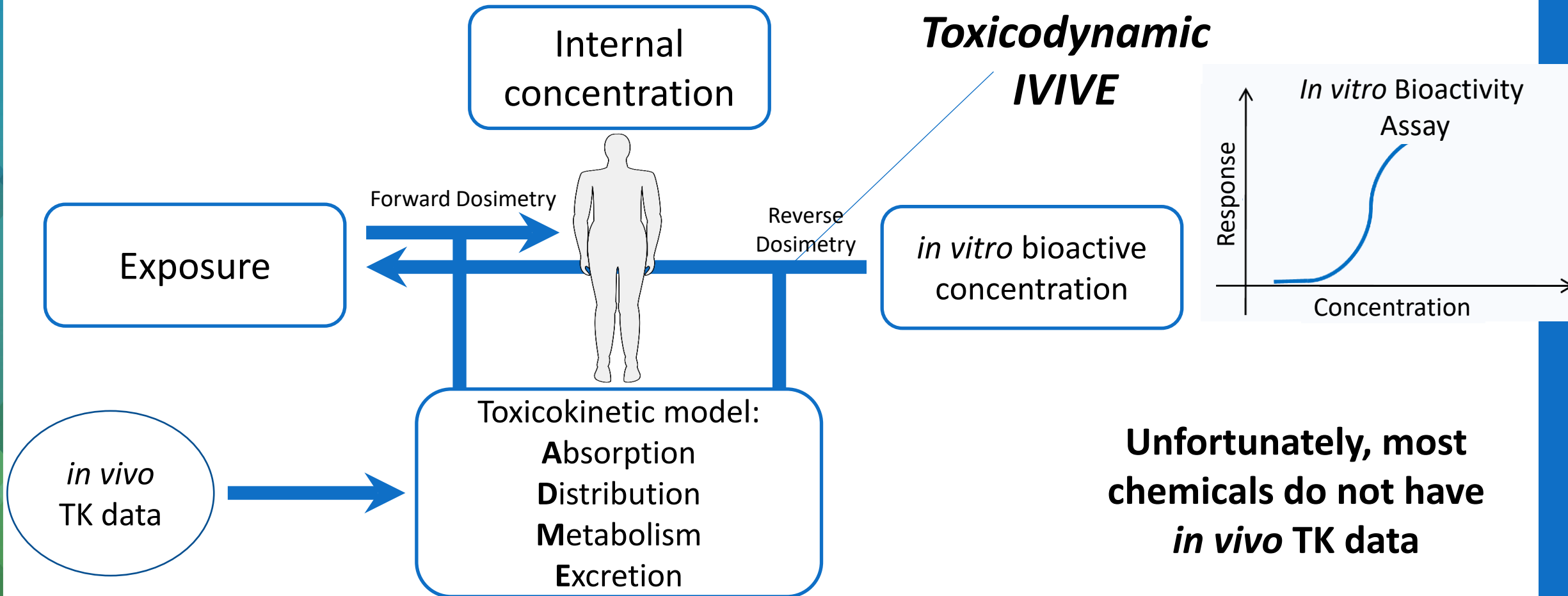
Toxicokinetics

- Toxicokinetics describes the absorption, distribution, metabolism, and excretion of a chemical by the body:
 - Chemical-specific
 - Links exposure with internal concentrations



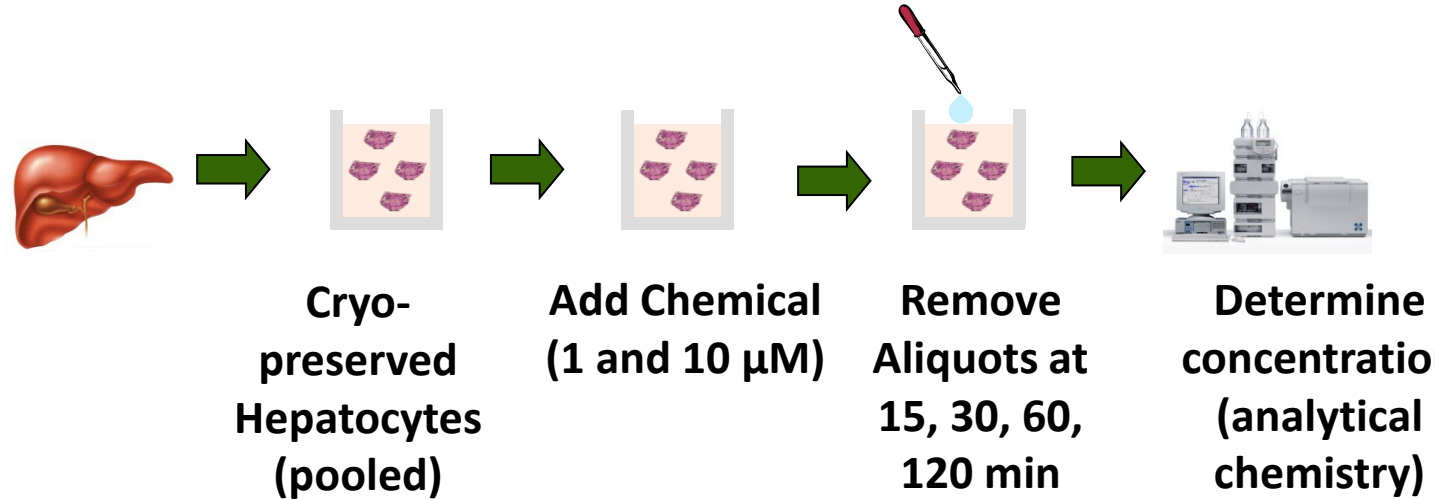
In Vitro-In Vivo Extrapolation (IVIVE)

- Translation of *in vitro* high throughput screening requires chemical-specific toxicokinetic models
 - Needed for anywhere from dozens to thousands of chemicals

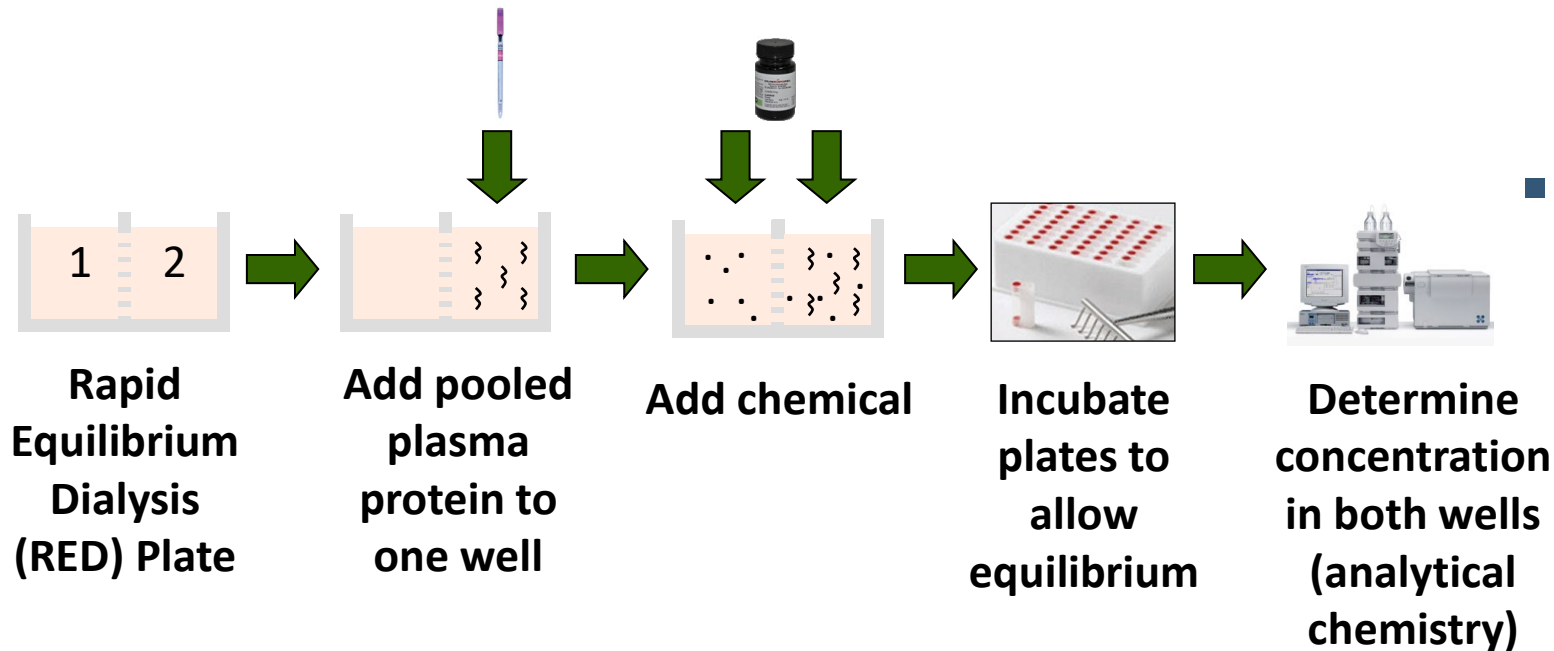


High Throughput Toxicokinetics (HTTK) for IVIVE

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



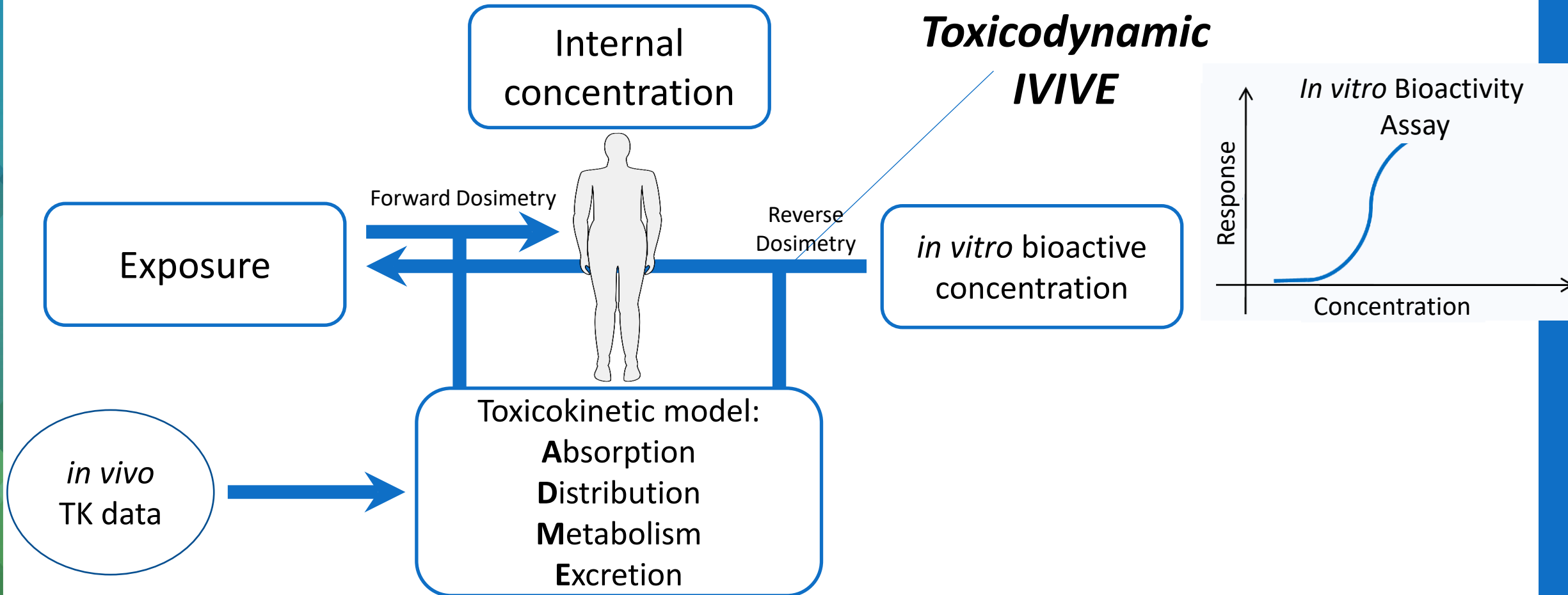
Rapid Equilibrium Dialysis (RED)
Waters *et al.* (2008)



- Most chemicals do not have TK data – we use *in vitro* HTTK methods adapted from pharma to fill gaps
- In drug development, predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)
- Chemical-specific data are steadily being generated by ORD laboratories (Barbara Wetmore), EPA contractors, and collaborators

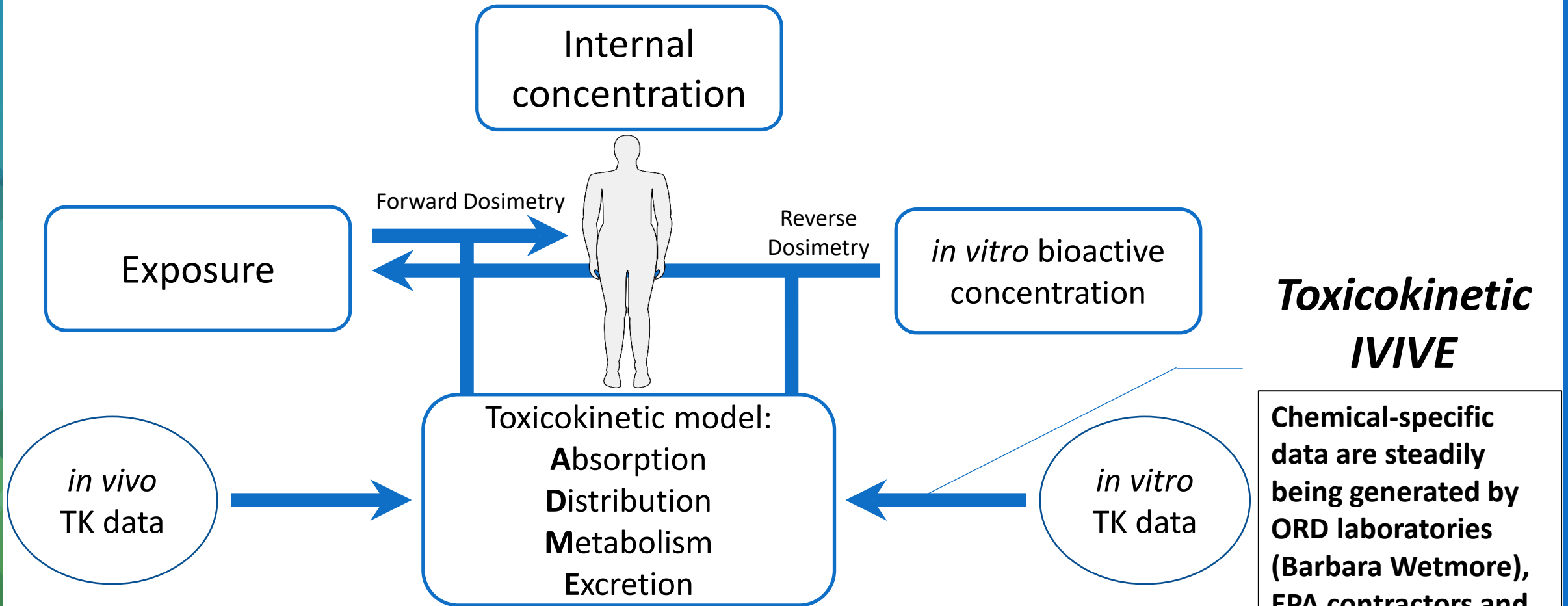
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In Vitro-In Vivo Extrapolation (IVIVE)

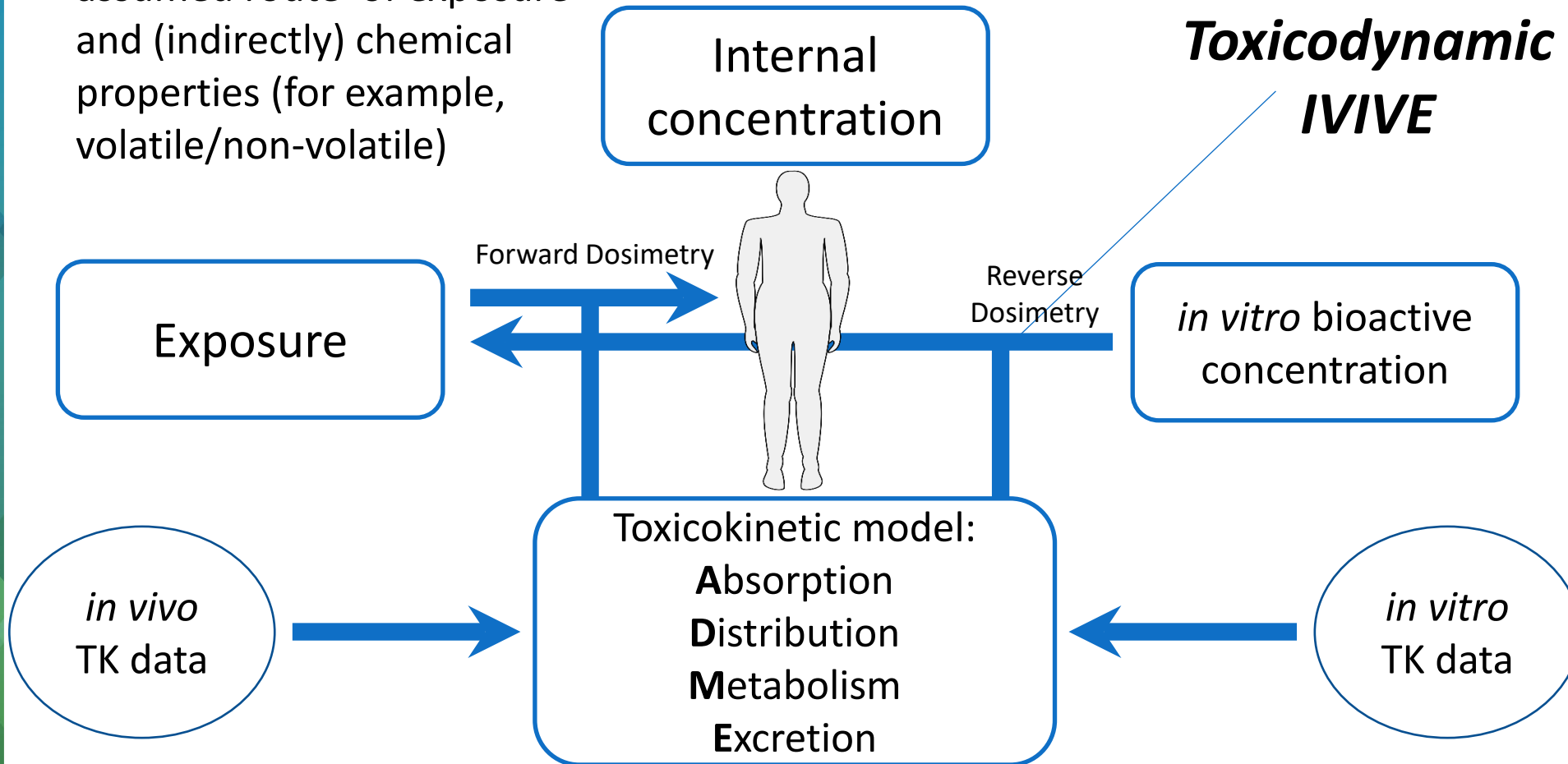
- Translation of *in vitro* high throughput screening requires chemical-specific toxicokinetic models
 - Needed for anywhere from dozens to thousands of chemicals



Breen et al. (2021)

In Vitro-In Vivo Extrapolation (IVIVE)

- IVIVE estimates equivalent doses that depend on the assumed route of exposure and (indirectly) chemical properties (for example, volatile/non-volatile)



IVIVE by Scaling Factor

- There are many approaches to IVIVE, but we choose a relatively simple one:
- We make various assumptions that allow conversion of an *in vitro* concentration $[X]$ (μM) into an **administered equivalent dose** (AED) with units of mg/kg body weight/day:

$$\text{AED} = F_{IVIVE} \times [X]$$

- **AED** is the **external dose rate** that would be needed to **produce a given steady-state plasma concentration**
- F_{IVIVE} is a scaling factor that varies by chemical

HTTK can predict F_{IVIVE}

IVIVE by Scaling Factor

- For a given chemical, $F_{IVIVE} = 1 / C_{ss,95}$
- $C_{ss,95}$ is the steady-state plasma concentration resulting from a 1 mg/kg/day exposure
- HTKK can predict $C_{ss,95}$ using “reverse dosimetry” IVIVE (Tan et al., 2007)

$$AED_{95} = \frac{[X]}{C_{ss,95}}$$

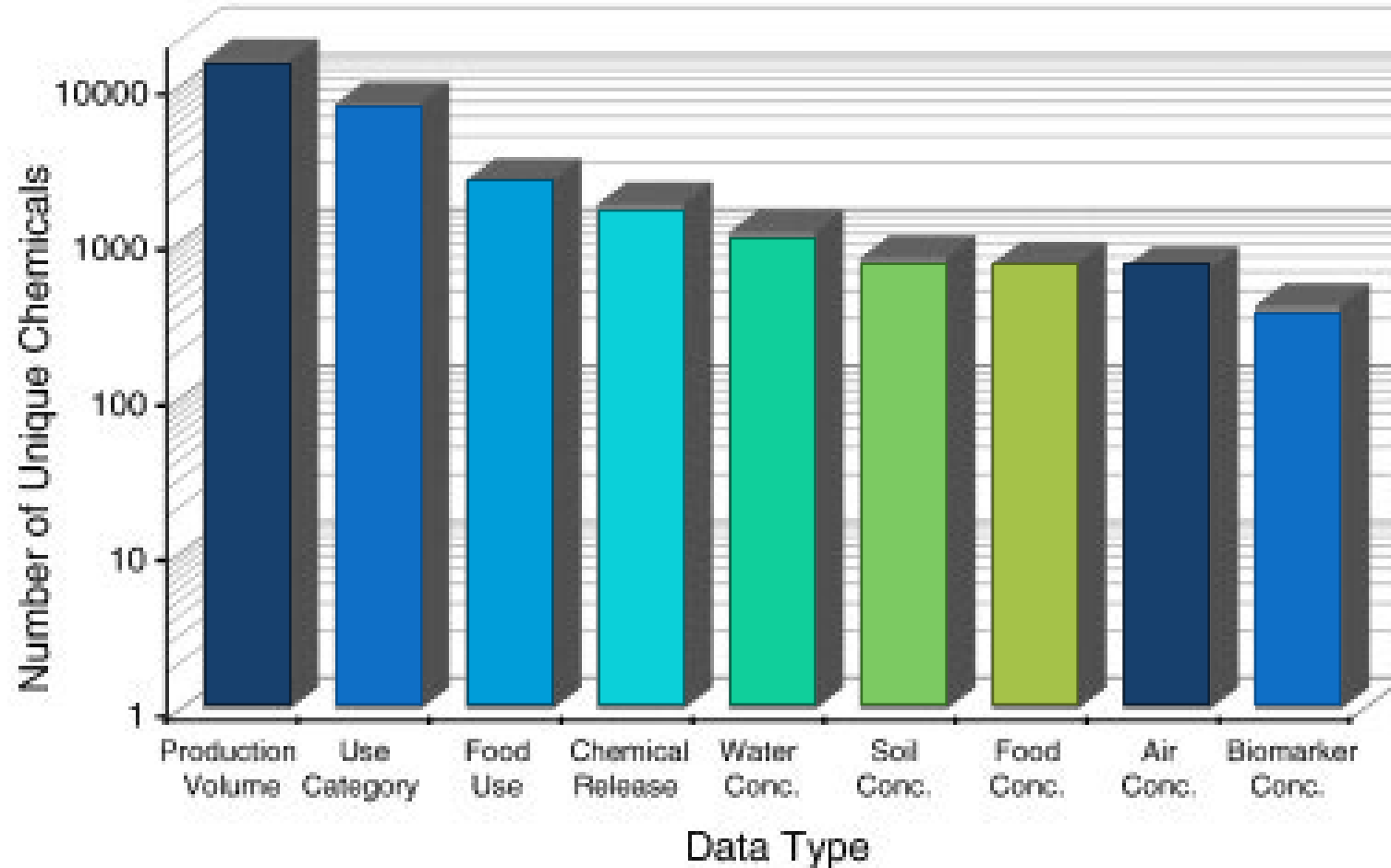
- The “95” refers to the upper 95th percentile – due to human variability and measurement uncertainty there are a range of possible C_{ss} values
- All of this assumes that the individuals have enough time to come to “steady-state” with respect to their daily exposures

Don't forget:

$$\mu M = 1000 \frac{1 \text{ mg}}{MW} \frac{1}{L}$$

Limited Available Data for Exposure Estimation

Most chemicals lack public exposure-related data beyond production volume (Egeghy et al., 2012)



New approach methods for exposure address these gaps

Fit-for-Purpose Exposure Modeling Frameworks

- All models vary in complexity and data needed to describe chemical exposure
- High throughput exposure (HTE) models can handle many chemicals with minimal descriptive information
- HTE models can provide rough but quantitative estimates of exposure

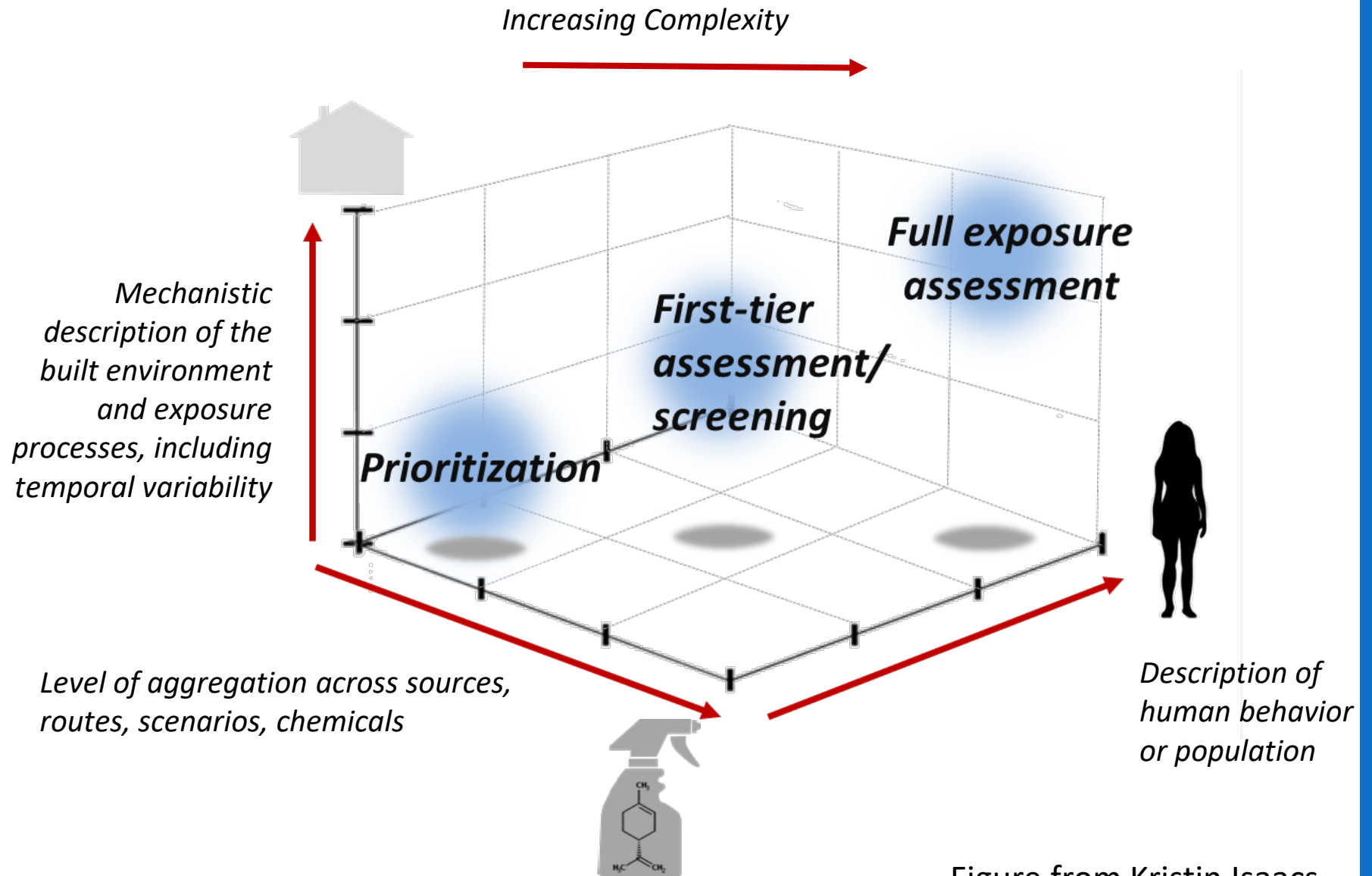
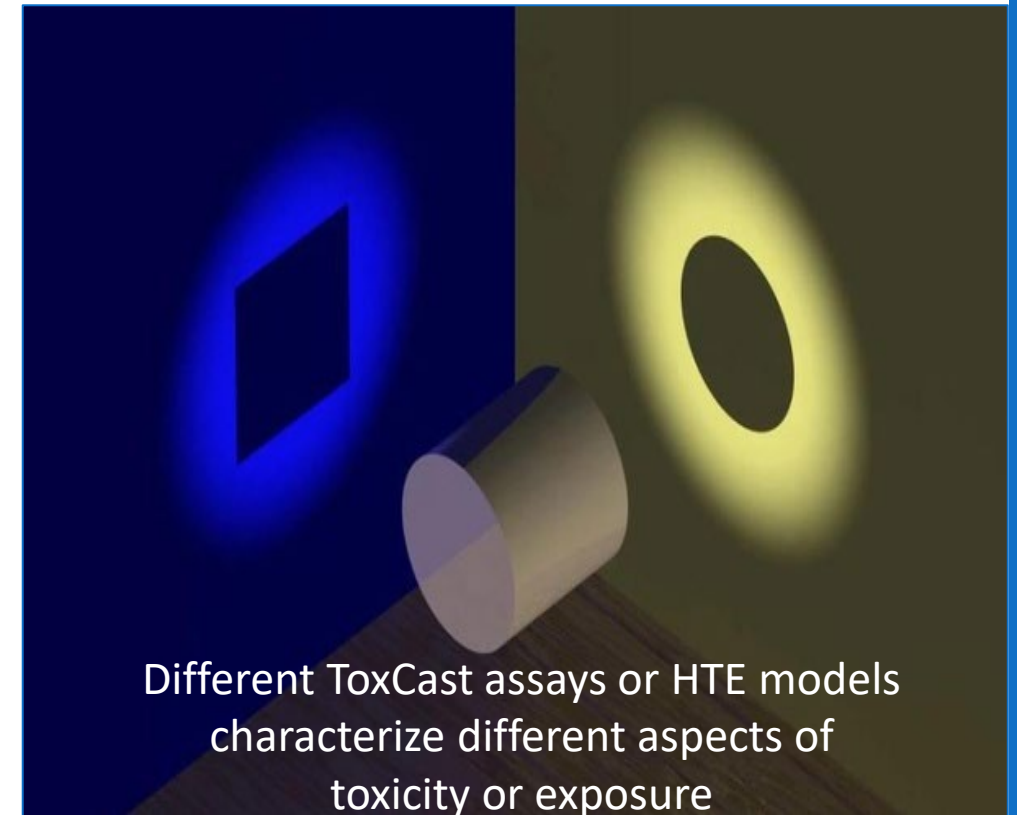


Figure from Kristin Isaacs

High Throughput Exposure: Analogy to *In Vitro* Screening

- EPA's Toxicity Forecasting (ToxCast) high throughput testing project:
 - >3000 chemicals tested to date
 - Each ToxCast assay-endpoint has the potential to capture *an aspect* of chemical biology – more than 1000 to date
 - No one assay gives the whole picture
 - Reference chemicals covering diverse mechanisms to establish what different types hazard “look like”
- ExpoCast (Exposure Forecasting):
 - Various HTE models provide the “assays” for different aspects (pathways, chemistries, assumptions) of exposure
 - Monitoring data provides our “reference” exposures
 - We build a probabilistic, consensus prediction using multiple HTE models and other predictors



Individual Model Predictions Available on CompTox Dashboard

SEEM3 Collaboration

Jon Arnot, Deborah H. Bennett, Peter P. Egeghy, Peter Fantke, Lei Huang, Kristin K. Isaacs, Olivier Jolliet, Hyeong-Moo Shin, Katherine A. Phillips, Caroline Ring, R. Woodrow Setzer, John F. Wambaugh, Johnny Westgate



Arnot Research & Consulting



UC DAVIS

UNIVERSITY OF CALIFORNIA



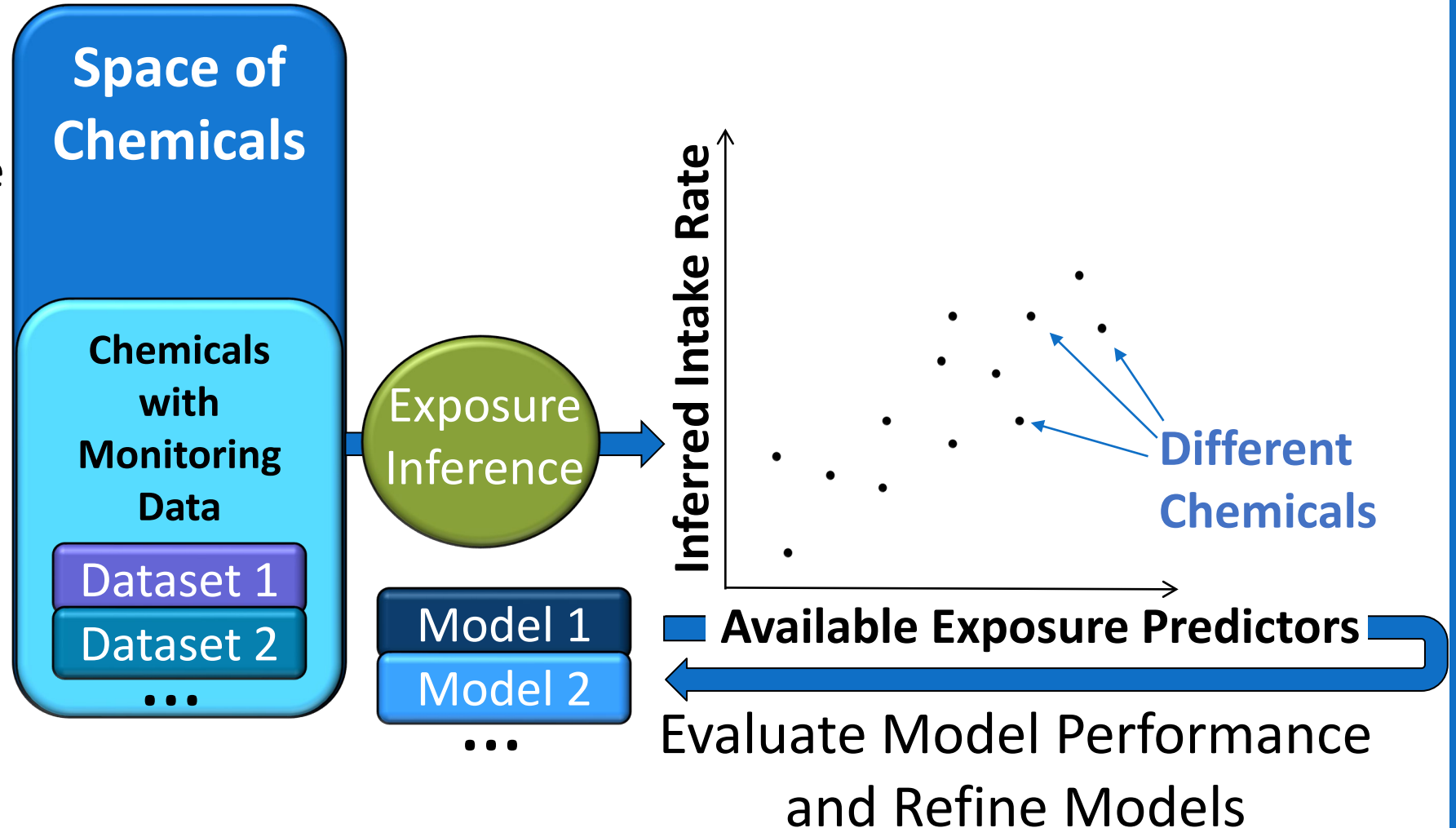
Predictor	Reference(s)	Chemicals Predicted	Pathway(s)
EPA Inventory Update Reporting and Chemical Data Reporting (CDR) (2015)	US EPA (2018)	7856	All
Stockholm Convention of Banned Persistent Organic Pollutants (2017)	Lallas (2001)	248	far field Industrial and Pesticide
EPA Pesticide Reregistration Eligibility Documents (REDs) Exposure Assessments (Through 2015)	Wetmore et al. (2012, 2015)	239	far field Pesticide
United Nations Environment Program and Society for Environmental Toxicology and Chemistry toxicity model (USEtox) Industrial Scenario (2.0)	Rosenbaum et al. (2008)	8167	far field Industrial
USEtox Pesticide Scenario (2.0)	Fantke et al. (2011, 2012, 2016)	940	far field Pesticide
Risk Assessment IDentification And Ranking (RAIDAR) far field (2.02)	Arnot et al. (2008)	8167	far field Pesticide
EPA Stochastic Human Exposure Dose Simulator High Throughput (SHEDS-HT) near field Direct (2017)	Isaacs (2017)	7511	far field Industrial and Pesticide
SHEDS-HT near field Indirect (2017)	Isaacs (2017)	1119	Residential
Fugacity-based INdoor Exposure (FINE) (2017)	Bennett et al. (2004), Shin et al. (2012)	645	Residential
RAIDAR-ICE near field (0.803)	Arnot et al., (2014), Zhang et al. (2014)	1221	Residential
USEtox Residential Scenario (2.0)	Jolliet et al. (2015), Huang et al. (2016,2017)	615	Residential
USEtox Dietary Scenario (2.0)	Jolliet et al. (2015), Huang et al. (2016), Ernststoff et al. (2017)	8167	Dietary

Individual Model Predictions Available on CompTox Dashboard Ring et al., 2018

Evaluating Exposure Models with the SEEM Framework

We use Bayesian methods to incorporate multiple models into consensus predictions for 1000s of chemicals within the **Systematic Empirical Evaluation of Models (SEEM)**

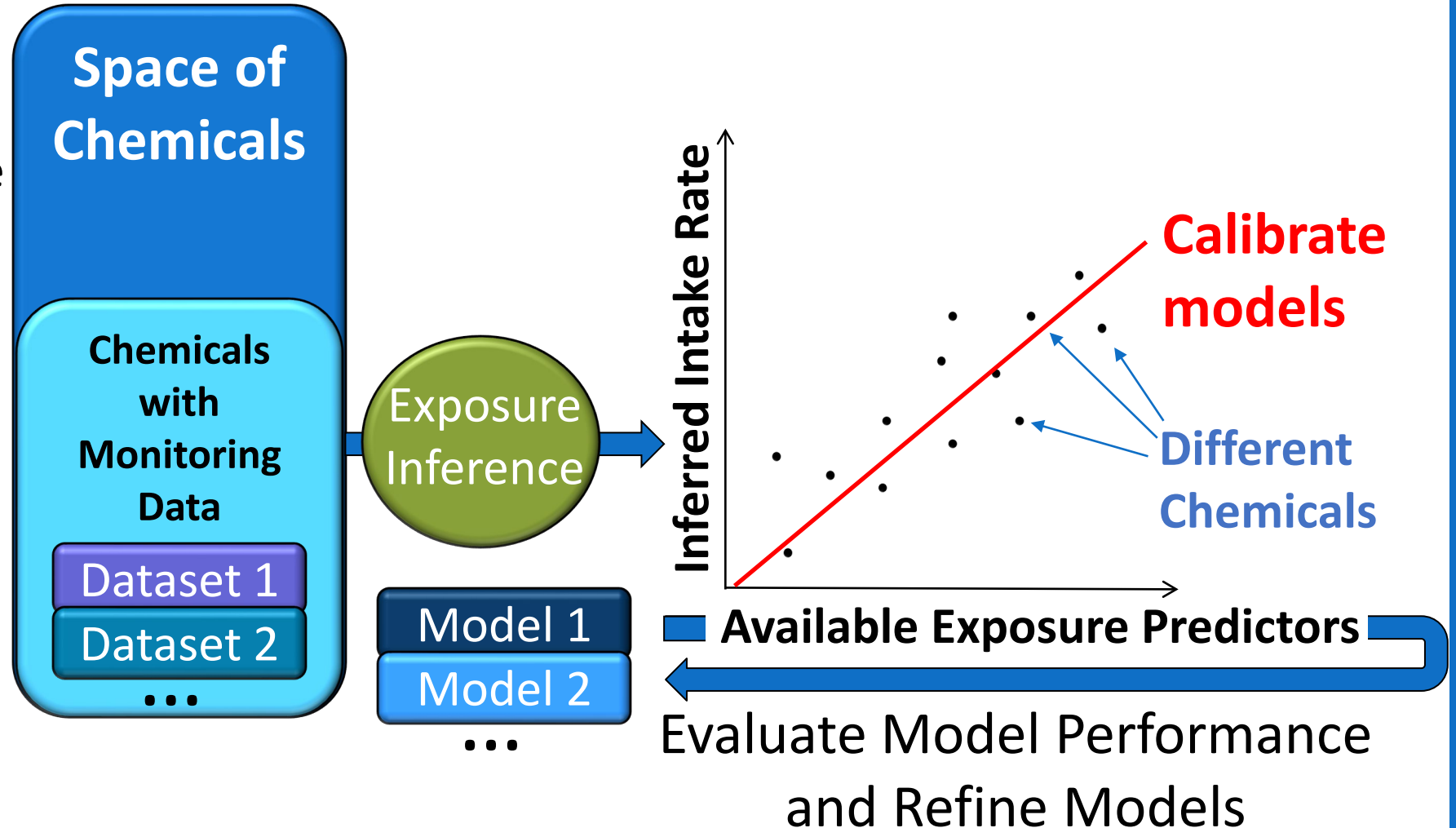
(Wambaugh et al., 2013, 2014; Ring et al., 2018)



Evaluating Exposure Models with the SEEM Framework

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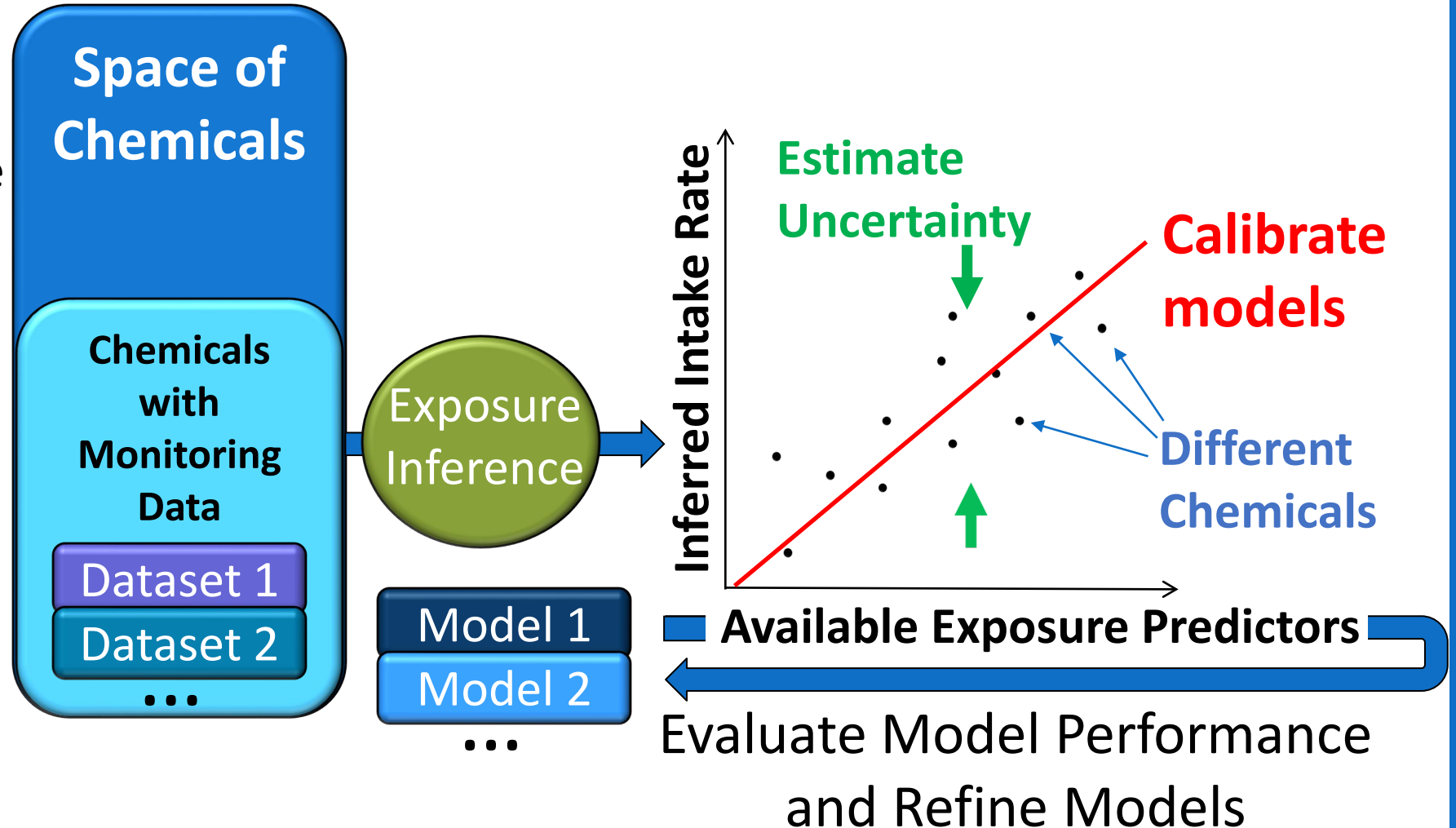
(Wambaugh et al., 2013, 2014; Ring et al., 2018)



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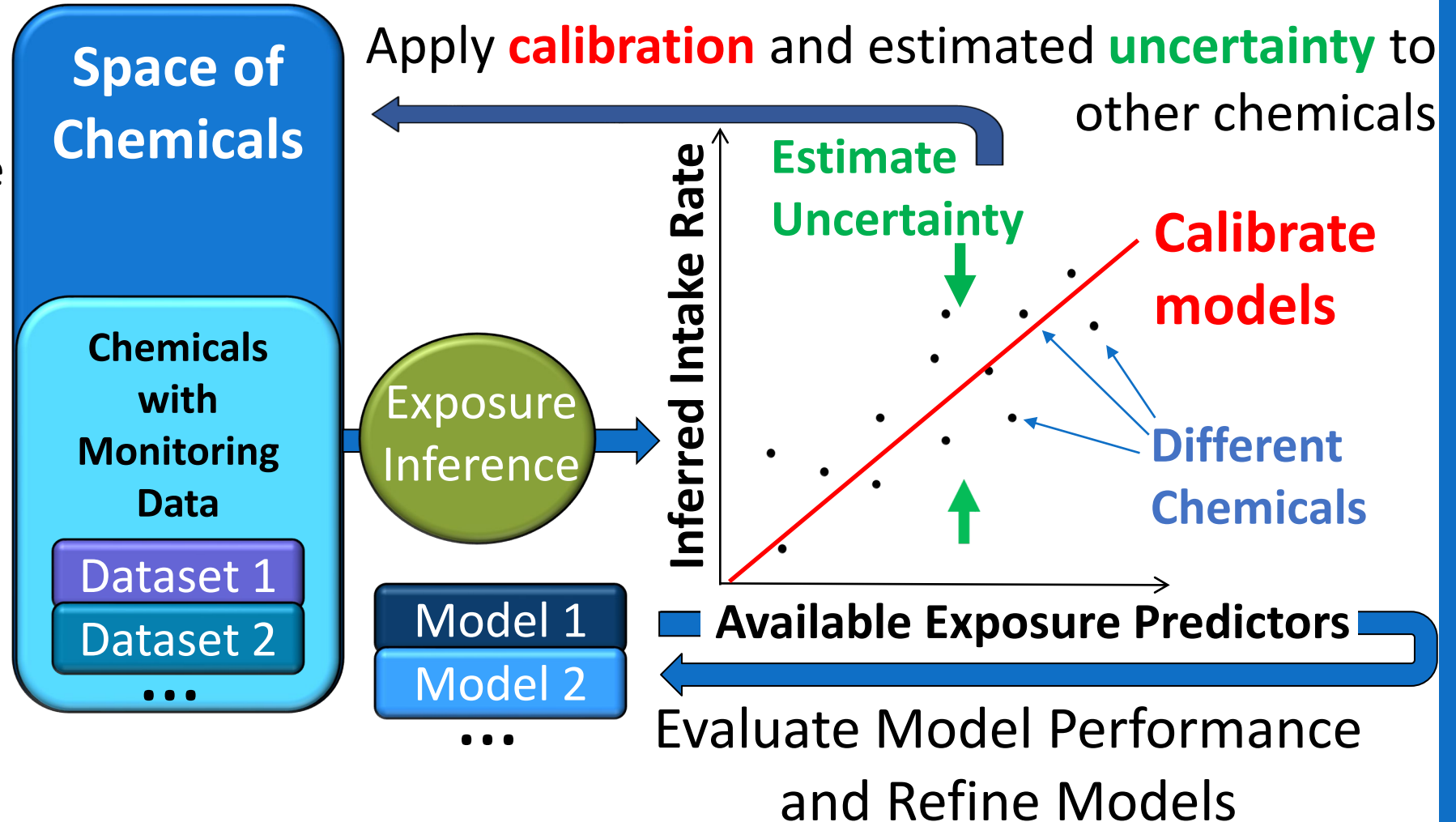
(Wambaugh et al., 2013, 2014; Ring et al., 2018)



Evaluating Exposure Models with the SEEM Framework

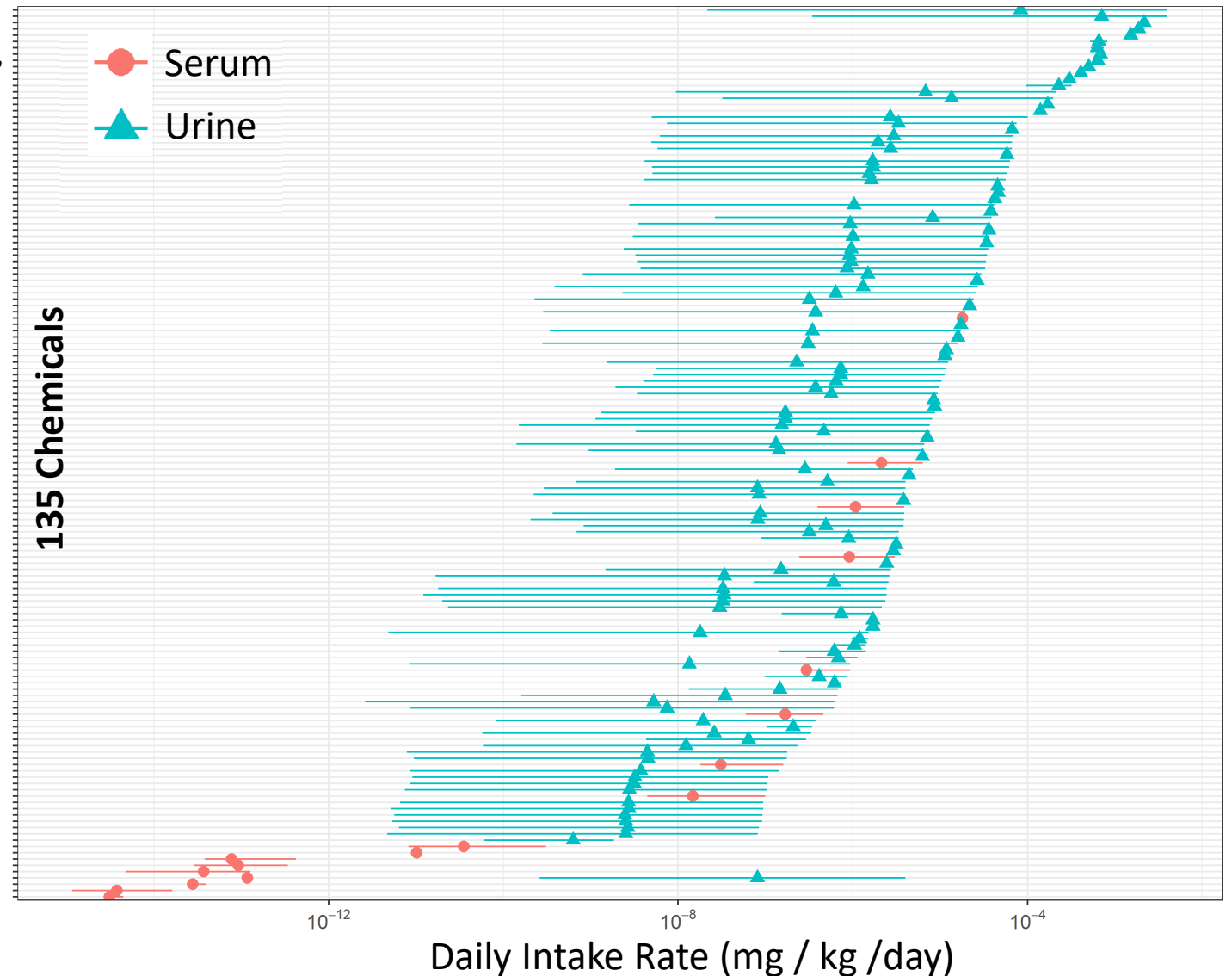
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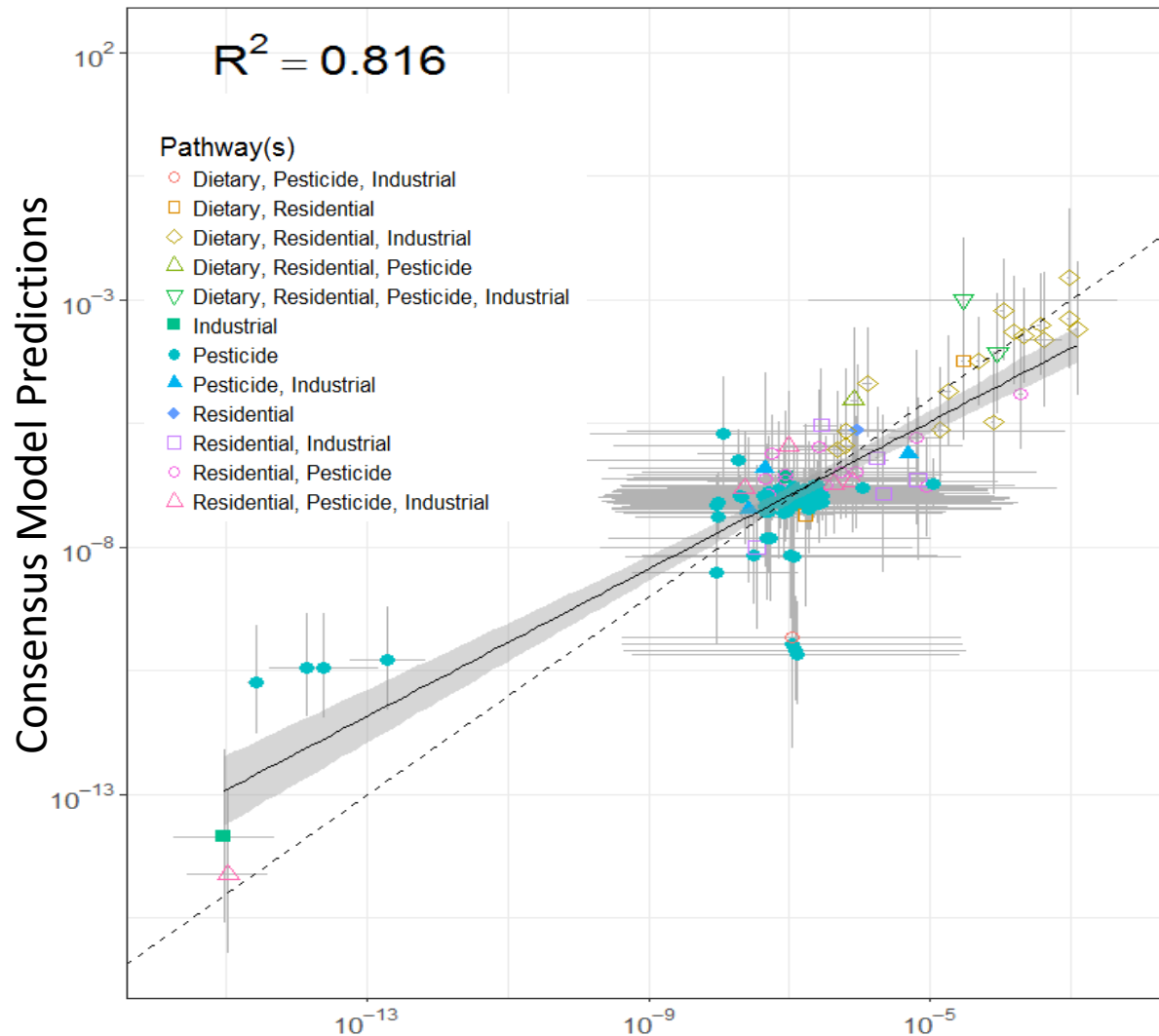
Inferred Exposure Rates from CDC NHANES

- Monitoring data provides our “reference” exposures for SEEM
- We infer exposure from CDC NHANES biomarker data
- We propagate uncertainty in inferences
 - Considering multiple parent chemicals for a given analyte
 - Limit of detection issues
- These exposure inferences are made available on CCD



work with Miyuki Breen and Zach Stanfield

SEEM3: Pathway-Based Consensus Modeling



- SEEM3 consensus model provides estimates of human median intake rate (mg/kg/day) for nearly 500,000 chemicals via the CompTox Chemicals Dashboard (<http://comptox.epa.gov/dashboard>)
- SEEM3 first predicts relevant exposure pathways from chemical structure – model predictions are then weighted according to the models' abilities to explain NHANES data
- We rely on pathway determinations from CPDat
- We rely on NHANES biomonitoring data
 - 2014 FIFRA Scientific Advisory Panel identified need for broader sets of evaluation data

Intake Rate (mg/kg BW/day) Inferred from NHANES Serum and Urine

Computational Toxicology and Exposure

- Given a structure we can estimate bioactivity, distribution, and exposure
 - Quantitative structure-property relationships (QSPRs)
- If we have a sample, we can test it *in vitro*:
 - High throughput screening and toxicokinetics
 - HTTK QSPRs allow predictions from structure
- If we have monitoring data, can estimate daily intake
 - High throughput exposure tools provide forecasts from structure

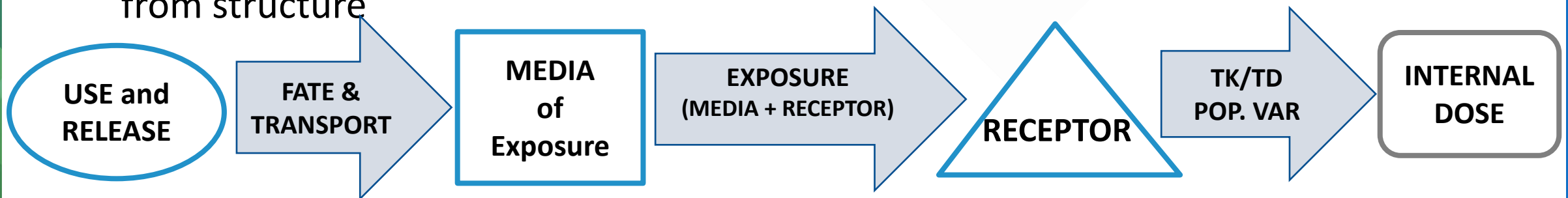
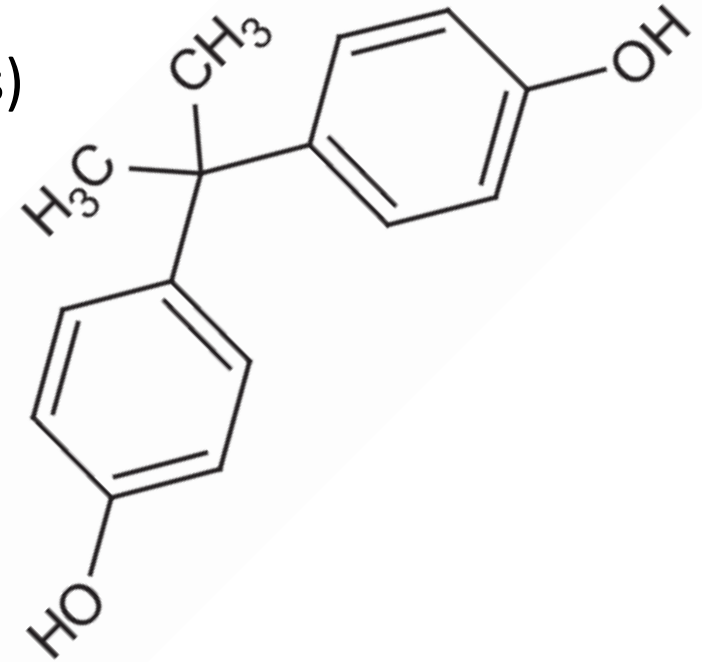
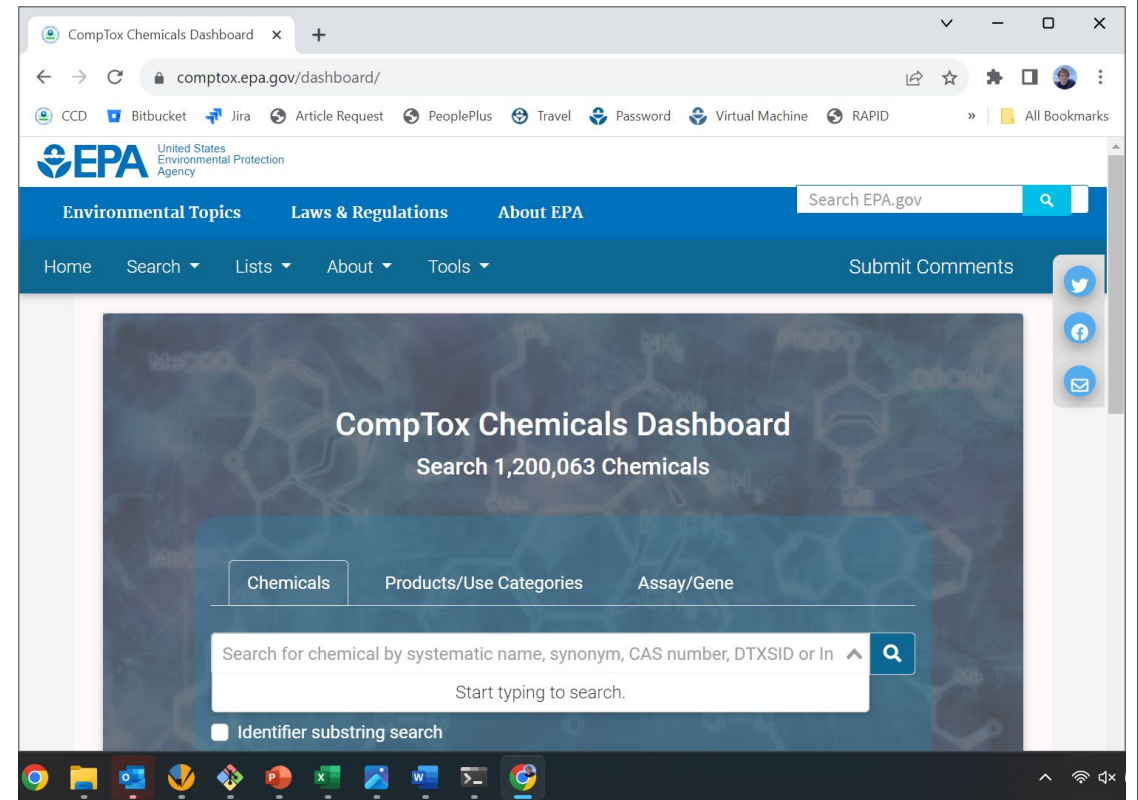


Figure from Caroline Ring

Standardized NAM Data and Tools

- **Hazard:** There are nearly 10,000 chemicals with *in vitro* bioactivity data
- **Exposure:** There are more than 400,000 chemicals with “exposure forecasts” (ExpoCast)
- **Dose-Response:** There are currently 7,569 chemicals with htkk data/predictions (including C_{ss} , V_d , t_{half}) available on the CompTox Chemicals Dashboard:

<https://comptox.epa.gov/dashboard>



Openly Available TK Information

- EPA's data and tools for HHTK are made available through R package "httk"
- The "httk" tool has been used to calculate key TK information that is available on the CompTox Chemicals Dashboard and elsewhere



CompTox Chemicals Dashboard v2.3.0

Fenazaquin
120928-09-8 | DTXSID4040476
Searched by Approved Name.

Chemical Details

Quality Control Notes

Intrinsic Properties

- Molecular Formula: $C_{20}H_{22}N_2O$
- Average Mass: 306.409 g/mol
- Monoisotopic Mass: 306.173213 g/mol

Structural Identifiers

Linked Substances

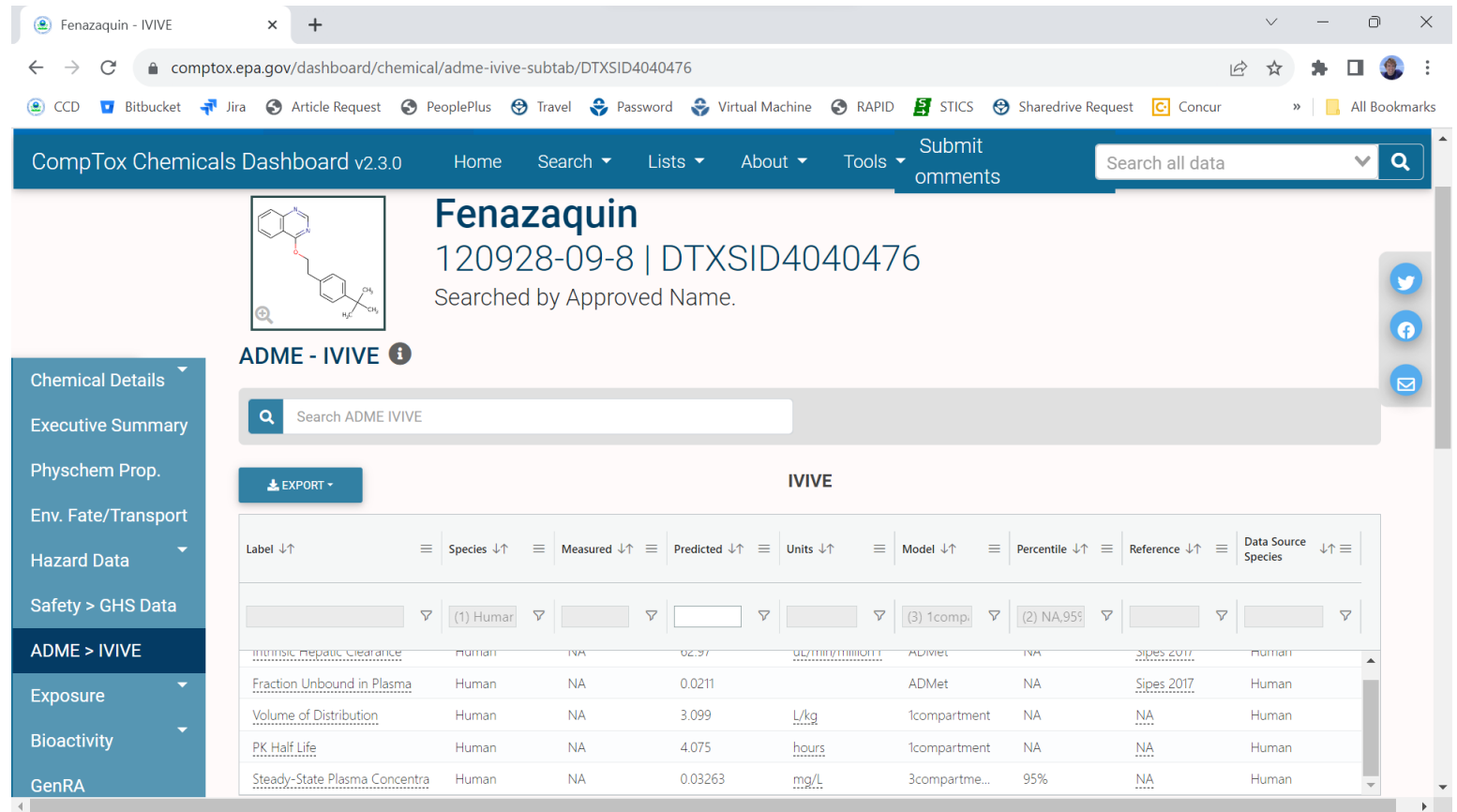
Presence in Lists

<https://comptox.epa.gov/dashboard>

The current HHTK data in CCD is HHTK v2.2.1. Please see the Data Sources table in the [Release Notes](#) for more information

Openly Available TK Information

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The screenshot displays the CompTox Chemicals Dashboard for Fenazaquin (DTXSID4040476). The page is titled "ADME - IVIVE" and includes a search bar for ADME IVIVE. Below the search bar, there is an "EXPORT" button and a table of IVIVE data. The table has columns for Label, Species, Measured, Predicted, Units, Model, Percentile, Reference, and Data Source Species. The data rows include:

Label	Species	Measured	Predicted	Units	Model	Percentile	Reference	Data Source Species
Intrinsic hepatic clearance	Human	NA	0.297	0.2/min/million	ADMet	NA	Sipes 2017	Human
Fraction Unbound in Plasma	Human	NA	0.0211		ADMet	NA	Sipes 2017	Human
Volume of Distribution	Human	NA	3.099	L/kg	1compartment	NA	NA	Human
PK Half Life	Human	NA	4.075	hours	1compartment	NA	NA	Human
Steady-State Plasma Concentra	Human	NA	0.03263	mg/L	3compartme...	95%	NA	Human

<https://comptox.epa.gov/dashboard>

The current HTTK data in CCD is HTTK v2.2.1. Please see the Data Sources table in the [Release Notes](#) for more information

Openly Available TK Information

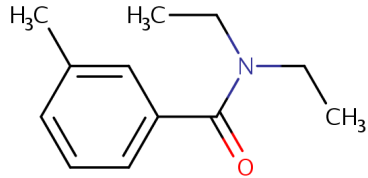
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	Label	Species	Measured	Predicted	Units	Model	Percentile	Reference	Data Source Species
V_d	<u>Volume of Distribution</u>	Human	NA	3.099	L/kg	1compartment	NA	NA	Human
t_{half}	<u>PK Half Life</u>	Human	NA	4.075	hours	1compartment	NA	NA	Human
C_{ss}	<u>Steady-State Plasma Concentra</u>	Human	NA	0.03263	mg/L	3compartme...	95%	NA	Human

<https://comptox.epa.gov/dashboard>

The current HTTK data in CCD is HTTK v2.2.1. Please see the Data Sources table in the [Release Notes](#) for more information

Calculation 1: Reverse Dosimetry, Steady-State Exposure



DEET (CASRN 134-62-3, DTXSID2021995):

$$C_{ss,95} = 1.37 \text{ mg/L per } 1 \text{ mg/kg/day}$$

In vitro
estimated
point of
departure
($POD_{in\ vitro}$)
(in μM units)



Perform
chemical-
specific unit
conversion
from μM to
 mg/L

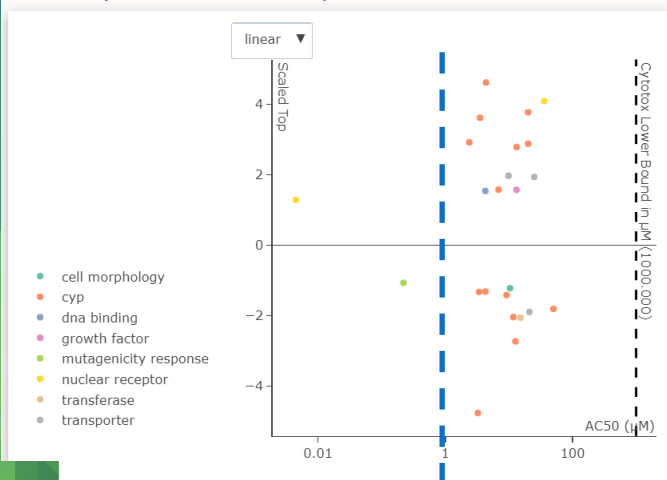


Divide by
chemical-
specific TK C_{ss}
(mg/L for 1
 mg/kg/day
intake rate)



Estimated
steady-state
intake rate
needed to
cause plasma
concentration
= $POD_{in\ vitro}$

Bioactivity - TOXCAST Summary



1 μM seems protective

$$1 \mu\text{M} = 1 \mu\text{mol} / \text{L} \\ \times \text{Molecular Weight} \\ (191.2 \text{ g/mol}) \\ \times 1 \text{ mg} / 1000 \mu\text{g} \\ = 0.191 \text{ mg} / \text{L}$$

$$0.191 \text{ mg} / \text{L} \\ / 1.37 \text{ mg} / \text{L} \\ \text{per } 1 \text{ mg/kg/day}$$

0.14 mg/kg/day
exposure will
produce
0.191 mg/L
plasma
concentration
at steady-state

Openly Available Exposure Information

<https://comptox.epa.gov/dashboard>

- For chemicals monitored by the Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) we have inferred daily intake rates (mg/kg/day) for the median U.S. population (Only ~100 chemicals)

- Product & Use Categories
- Chemical Weight Fraction
- Chemical Functional Use
- Toxics Release Inventory
- Biomonitoring Data**
- Exposure Predictions
- Production Volume

The screenshot shows the EPA Comptox Dashboard for the chemical DEET (DTXSID2021995). The page includes a chemical structure, a search bar, and a table of monitoring data. The table displays median, upper bound, and lower bound intake rates for various demographic groups.

DEET
134-62-3 | DTXSID2021995
Searched by DSSTox_Substance_Id.

National Health and Nutrition Examination Survey (NHANES) Inferences (mg/kg-bw/day)

Search Monitoring Data

Demographic ↓↑	Median ↓↑	Upper Bound (Median) ↓↑	Lower Bound (Median) ↓↑
Total	4.09e-7	8.67e-7	9.89e-8
Male	4.82e-7	9.49e-7	1.46e-7
Female	3.68e-7	8.33e-7	6.71e-8
6 - 11 years	5.93e-7	1.08e-6	2.22e-7
12 - 19 years	5.31e-7	1.03e-6	1.59e-7

Openly Available Exposure Information

<https://comptox.epa.gov/dashboard>

- The 95th percentile refers to the uncertainty about the median value, **it does not reflect variability**
- We typically use the upper 95th limit on the uncertainty to be conservative – but this is still only for the population median

DEET
134-62-3 | DTXSID2021995
Searched by DSSTox_Substance_Id.

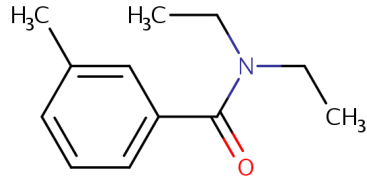
National Health and Nutrition Examination Survey (NHANES) Inferences (mg/kg-bw/day)

Monitoring Data

Demographic ↓↑	Median ↓↑	Upper Bound (Median) ↓↑	Lower Bound (Median) ↓↑
Total	4.09e-7	8.67e-7	9.89e-8
Male	4.82e-7	9.49e-7	1.46e-7
Female	2.69e-7	8.22e-7	6.71e-8

Inferences from Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) made using R package bayesMarker (Stanfield et al., 2022 & 2024)

Calculation 2: NHANES Bioactivity:Exposure Ratio



DEET (CASRN 134-62-3, DTXSID2021995):
NHANES Total Population Daily Intake Rate
Upper 95th: 8.67×10^{-7} mg/kg/day

Estimated
steady-state
intake rate
needed to
cause plasma
concentration
= $POD_{in\ vitro}$



Divide by
Inferred
Exposure for
Median U.S.
Population



Bioactivity
Exposure Ratio
(BER)

0.14 mg/kg/day
exposure will produce
0.191 mg/L
plasma concentration
at steady-state

0.14 mg/kg/day
/
0.000000867
mg/kg/day

Bioactive Dose is
162,000 times
higher than dose
inferred from CDC
biomonitoring
data



Low general
population
risk

Openly Available Exposure Information

<https://comptox.epa.gov/dashboard>

- Demographic-specific inferences from NHANES are available for certain demographic groups
- Again, only for median
- Again, we have only ~100 chemicals

DEET
134-62-3 | DTXSID2021995
Searched by DSSTox_Substance_Id.

National Health and Nutrition Examination Survey (NHANES) Inferences (mg/kg-bw/day)

Search Monitoring Data

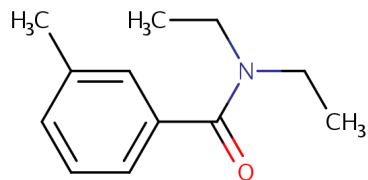
Monitoring Data

Demographic ↓↑	Median ↓↑	Upper Bound (Median) ↓↑	Lower Bound (Median) ↓↑
66 years and older	4.35e-7	8.64e-7	1.08e-7
ReproAgeFemale	3.82e-7	8.89e-7	7.74e-8
BMI <= 30	4.42e-7	9.18e-7	1.07e-7

Demographic ↓↑	Median ↓↑	Upper Bound (Median) ↓↑	Lower Bound (Median) ↓↑
66 years and older	4.35e-7	8.64e-7	1.08e-7
ReproAgeFemale	3.82e-7	8.89e-7	7.74e-8
BMI <= 30	4.42e-7	9.18e-7	1.07e-7

Examination Survey (NHANES) made using R package bayesMarker (Stanfield et al., 2022 & 2024)

Calculation 3: NHANES Demographic Bioactivity:Exposure Ratio



DEET (CASRN 134-62-3, DTXSID2021995):
NHANES Total Population Daily Intake Rate
Upper 95th: 8.89×10^{-7} mg/kg/day

Estimated steady-state intake rate needed to cause plasma concentration = $POD_{in\ vitro}$



Divide by Inferred Exposure for Median U.S. Population



Bioactivity Exposure Ratio (BER)

0.14 mg/kg/day exposure will produce 0.191 mg/L plasma concentration at steady-state

0.14 mg/kg/day / 0.000000889 mg/kg/day

Bioactive Dose is **157,000 times higher** than dose inferred from CDC biomonitoring data



Low risk for median woman of reproductive age

Openly Available Exposure Information

<https://comptox.epa.gov/dashboard>

- For most chemicals we do not have intake rates from NHANES
- However, systematic empirical evaluation of models (SEEM) gives estimated intake rates for hundreds of thousands of chemicals

Product & Use Categories
Chemical Weight Fraction
Chemical Functional Use
Toxics Release Inventory
Biomonitoring Data
Exposure Predictions
Production Volume

DEET
134-62-3 | DTXSID2021995
Searched by DSSTox_Substance_Id.

Exposure - Exposure Predictions (mg/kg-bw/day)

Search Demographics Predictions Data

Demographics Predictions Data

Demographic	Predictor	Median	Upper 95%ile	Units
Age 6-11	(2) SEEM2_Heuristic,SEEM3_Cd	1.29e-7	4.37e-5	mg/kg/day
Age 12-19	SEEM2_Heuristic	1.54e-7	8.91e-5	mg/kg/day
Age 20-65	SEEM2_Heuristic	1.19e-7	4.86e-5	mg/kg/day
Age 66+	SEEM2_Heuristic	1.25e-7	5.25e-5	mg/kg/day

Openly Available Exposure Information

<https://comptox.epa.gov/dashboard>

- SEEM forecasts are much more uncertain than NHANES measurements
- For sake of conservatism, we use upper 95th limit on estimated median intake
- **This does not reflect population variability**

DEET
134-62-3 | DTXSID2021995
Searched by DSSTox_Substance_Id.

Exposure - Exposure Predictions (mg/kg-bw/day)

Search Demographics Predictions Data

Demographics Predictions Data

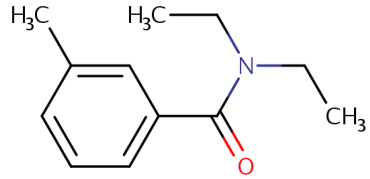
Demographic ↓↑	Predictor ↓↑	Median ↓↑	Upper 95%ile ↓↑	Units ↓↑
	(2) SEEM2 Heuristic, SEEM3 Cd			
Age 6-11	SEEM2 Heuristic	1.29e-7	4.37e-5	mg/kg/day
Age 12-19	SEEM2 Heuristic	1.54e-7	8.91e-5	mg/kg/day
Age 20-65	SEEM2 Heuristic	1.19e-7	4.86e-5	mg/kg/day
Age 66+	SEEM2 Heuristic	1.25e-7	5.25e-5	mg/kg/day

Demographics Predictions Data

Demographic ↓↑	Predictor ↓↑	Median ↓↑	Upper 95%ile ↓↑	Units ↓↑
Repro. Age Females	SEEM2 Heuristic	3.11e-7	7.36e-5	mg/kg/day
Total	SEEM3 Consensus	1.52e-6	1.11e-4	mg/kg/day

SEEM3 Consensus Model described in Ring et al. (2019)

Calculation 4: SEEM Bioactivity:Exposure Ratio



DEET (CASRN 134-62-3, DTXSID2021995):
SEEM Total Population Daily Intake Rate
Upper 95th: 1.11×10^{-4} mg/kg/day

Estimated
steady-state
intake rate
needed to
cause plasma
concentration
= $POD_{in\ vitro}$



Divide by
Inferred
Exposure for
Median U.S.
Demographic
Group



Bioactivity
Exposure Ratio
(BER)

0.14 mg/kg/day
exposure will produce
0.191 mg/L
plasma concentration
at steady-state

0.14 mg/kg/day
/
0.000111
mg/kg/day

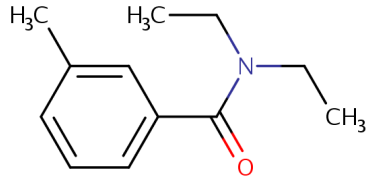
Bioactive Dose is
1,260 times
higher than
intake rate
forecast by SEEM



Low general
population
risk

**Note that this BER is less than for NHANES
because of greater uncertainty**

Calculation 5: Forward Dosimetry, Single Exposure



DEET (CASRN 134-62-3, DTXSID2021995):

$V_d = 1.7 \text{ L/kg}$, $t_{\text{half}} = 6.5 \text{ h}$

Single exposure
(mg/kg)



If oral dose multiply by fraction bioavailable (F_{bio})



Divide by volume of distribution (V_d) (units of L/kg)



Peak concentration (C_{max})

95% of dose gone after three half-lives ($3 \times t_{\text{half}}$)

15% in sun lotion,
use 60 g lotion,
Body weight 70 kg:
 $60 * 1000 * 0.15 / 70 =$
129 mg/kg

100%
absorption in
lieu of data to
contrary

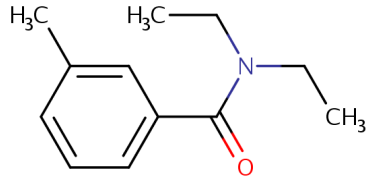
129 mg/kg /
1.737 L/kg

74 mg/L

$3 \times 6.5 =$
19.5 h

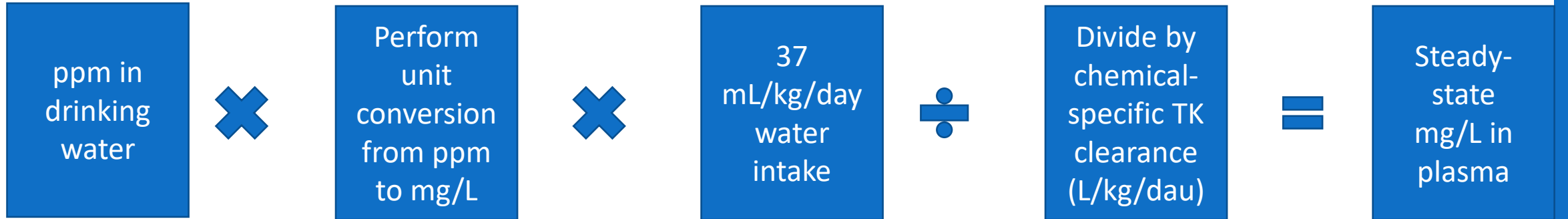
Note that if we use a mathematical simulation tool (such as R package `httk`) we could do more elaborate, tissue specific predictions (`httk::solve_pbtk`) as well as simulate other exposure routes (inhalation – `httk::solve_gas_pbtk()`, gestational – `httk::solve_fetal_pbtk()`)

Calculation 6: Forward Dosimetry, Steady-State Exposure



DEET (CASRN 134-62-3, DTXSID2021995):

$$C_{ss,95} = 1.37 \text{ mg/L per } 1 \text{ mg/kg/day} = 1.37 \text{ kg-day/L}$$



Scenario, chemical spill causes 1 ppm in drinking water:

$$\begin{aligned} & 1 \text{ ppm} = 1 \mu\text{g} / \text{g} \\ & / 1 \text{ mL/g (water density)} \\ & \times 1 \text{ mg} / 1000 \mu\text{g} \\ & = 0.001 \text{ mg} / \text{mL} \end{aligned}$$

$$\begin{aligned} & 0.001 \text{ mg} / \text{mL} \\ & \times 37 \text{ mL/kg/day} \\ & = 0.037 \text{ mg/kg/day} \end{aligned}$$

$$\begin{aligned} & 0.037 \text{ mg/kg/day} \\ & / \text{CL (L/kg/day)} = \\ & 0.037 * 1.37 \end{aligned}$$

Convenient TK fact:
Clearance (Cl) = $1/C_{ss}$

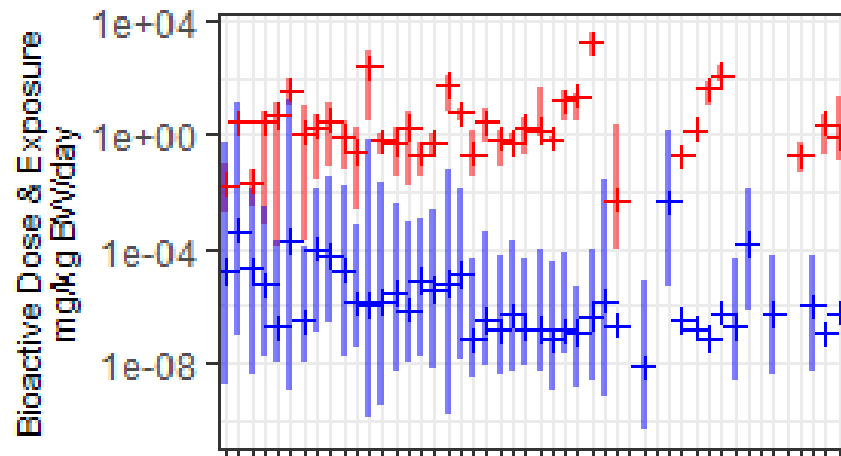
0.051 mg/L

37 mL/kg/day water intake is from the EPA Exposure Factors Handbook Table 3-1 for all ages, 95th Percentile

Online Tutorial on Bioactivity : Exposure Ratios

https://cran.r-project.org/web/packages/httk/vignettes/V2_IntrotoIVIVE.html

- An online R tutorial walks you through the steps needed to calculate Bioactivity:Exposure Ratios. It covers:
 - Loading *in vitro* screening data into R
 - HTTK-based IVIVE
 - Applying relevant QSPRs
 - Comparing with Exposure predictions
 - Making a BER Plot



Introduction to *In Vitro-In Vivo* Extrapolation (IVIVE) with R Package httk

John Wambaugh and Elaina Kenyon

November, 2022

Please send questions to wambaugh.john@epa.gov

Introduction

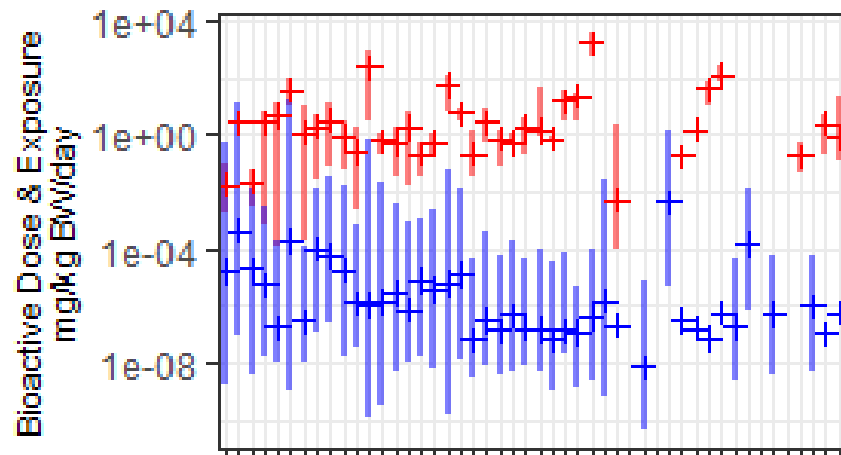
Chemical risk assessment depends on knowledge of inherent chemical hazard, the dose-response relationship, and the extent of exposure that occurs ([NASEM 2017](#)). High throughput screening (HTS) for *in vitro* bioactivity allows characterization of potential hazard for thousands of chemicals for which no other testing has occurred ([Judson et al., 2009](#)).

Toxicokinetics (TK) describes the Absorption, Distribution, Metabolism, and Excretion (ADME) of a chemical by the body. TK relates external exposures to internal tissue

Online Tutorial on Bioactivity : Exposure Ratios

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Prepare R to run the vignette

R package **knitr** generates html and PDF documents from this RMarkdown file, Each bit of code that follows is known as a “chunk”. We start by telling **knitr** how we want our chunks to look.

```
knitr::opts_chunk$set(collapse = TRUE, comment = '#>')
options(rmarkdown.html_vignette.check_title = FALSE)
```

Clear the memory

It is a bad idea to let variables and other information from previous R sessions float around, so we first remove everything in the R memory.

```
rm(list=ls())
```

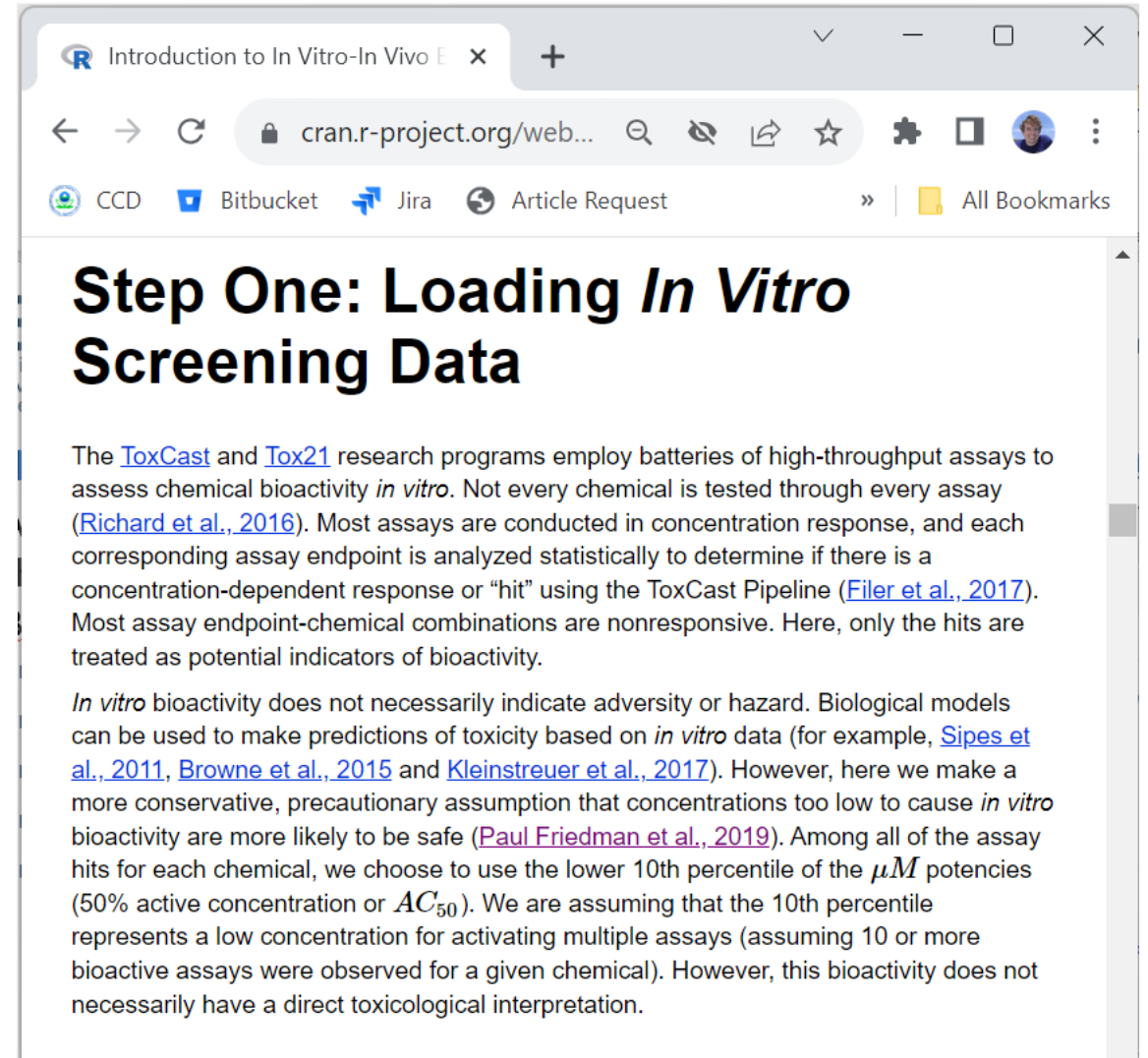
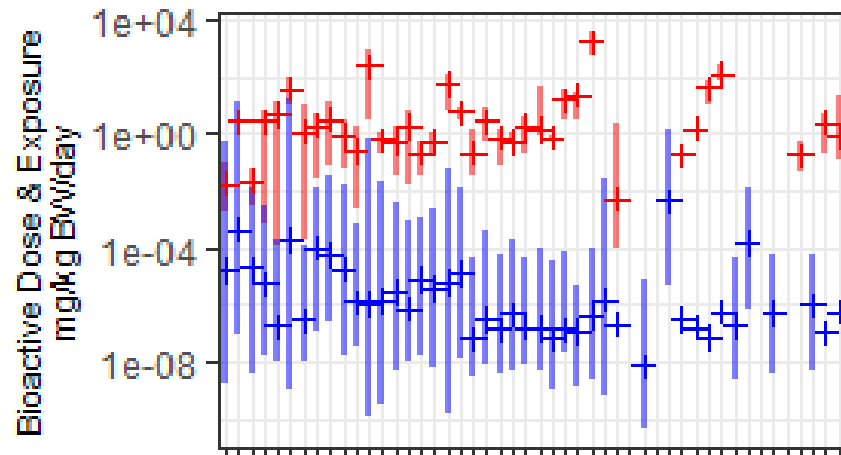
HTTK Version

This vignette was created using **httk** v2.2.2 in 2022. Although we attempt to maintain backward compatibility, if you encounter issues with the latest release of **httk** and cannot easily address the changes, historical versions of **httk** are available from: https://cran.r-project.org/web/packages/httk/vignettes/V2_IntrotoIVIVE.html

Online Tutorial on Bioactivity : Exposure Ratios

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Step One: Loading *In Vitro* Screening Data

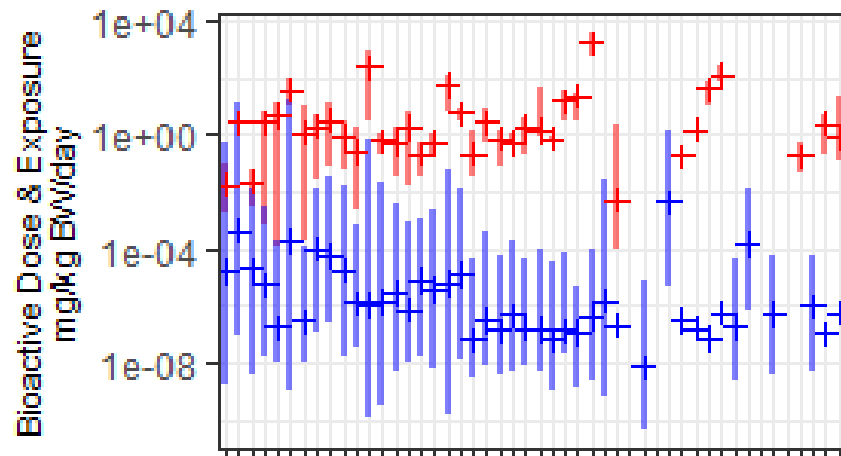
The [ToxCast](#) and [Tox21](#) research programs employ batteries of high-throughput assays to assess chemical bioactivity *in vitro*. Not every chemical is tested through every assay ([Richard et al., 2016](#)). Most assays are conducted in concentration response, and each corresponding assay endpoint is analyzed statistically to determine if there is a concentration-dependent response or “hit” using the ToxCast Pipeline ([Filer et al., 2017](#)). Most assay endpoint-chemical combinations are nonresponsive. Here, only the hits are treated as potential indicators of bioactivity.

In vitro bioactivity does not necessarily indicate adversity or hazard. Biological models can be used to make predictions of toxicity based on *in vitro* data (for example, [Sipes et al., 2011](#), [Browne et al., 2015](#) and [Kleinstreuer et al., 2017](#)). However, here we make a more conservative, precautionary assumption that concentrations too low to cause *in vitro* bioactivity are more likely to be safe ([Paul Friedman et al., 2019](#)). Among all of the assay hits for each chemical, we choose to use the lower 10th percentile of the μM potencies (50% active concentration or AC_{50}). We are assuming that the 10th percentile represents a low concentration for activating multiple assays (assuming 10 or more bioactive assays were observed for a given chemical). However, this bioactivity does not necessarily have a direct toxicological interpretation.

Online Tutorial on Bioactivity : Exposure Ratios

https://cran.r-project.org/web/packages/httk/vignettes/V2_IntrotoIVIVE.html

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Critical Step: Select your chemicals of interest

We might have chemicals we are interested in for one reason or another – you could type any chemical ID's you want into the following `my.chems` vector, or even load it from a file. Here we'll pick 50 chemicals at random from among the ToxCast chemicals:

```
set.seed(1234)
my.chems <- sample(mc5$dsstox_substance_id,50)
example.toxcast <- as.data.frame(mc5[mc5$dsstox_substance_id %in% my.chems,])
```

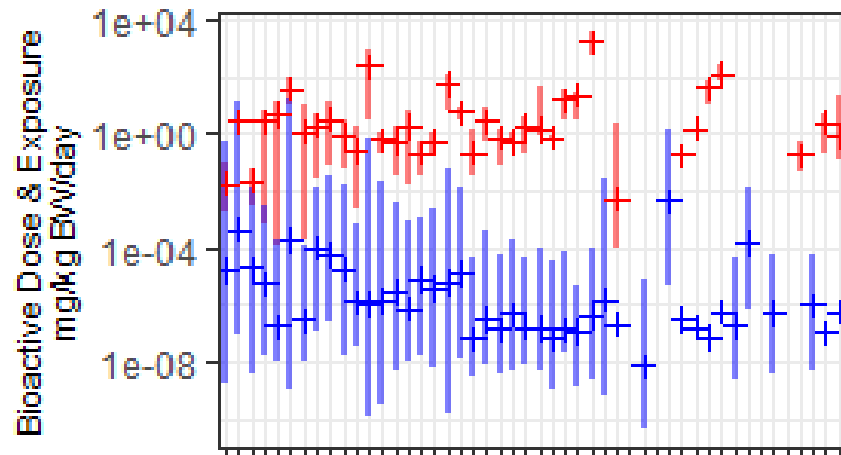
Unfortunately for this vignette there are too many ToxCast data to fit into a 5mb R package. So we will subset to just the selected chemicals and distribute only those data. In addition, out of 78 columns in the data, we will keep only eight. Download the full data following the instructions above.

```
example.toxcast <- example.toxcast[, c("chnm",
  "dsstox_substance_id",
  "spid",
  "hitc",
  "l1")]
```

Online Tutorial on Bioactivity : Exposure Ratios

https://cran.r-project.org/web/packages/httk/vignettes/V2_IntrotoIVIVE.html

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The screenshot shows a web browser window with the URL [cran.r-project.org/web/...](https://cran.r-project.org/web/packages/httk/vignettes/V2_IntrotoIVIVE.html). The page content includes R code for loading ToxCast data and a table titled "Summarized ToxCast Data".

```
knitr::kable(toxcast.table[1:10,c("Compound","Q10.AC50","Css","EquivDose")],  
             caption = "Summarized ToxCast Data",  
             floating.environment="sidewaystable")
```

Compound	Q10.AC50	Css	EquivDose
Methyl perfluoro(3-(1-ethenyloxypropan-2-yloxy)propanoate)	-0.435	NA	NA
Nonafluoropentanamide	1.110	NA	NA
Bisphenol A	-0.196	7.735	8.23e-02
Tris(2-ethylhexyl) trimellitate	-0.532	2.017	1.46e-01
1-Octen-3-ol	1.220	NA	NA
Thalidomide	-0.761	7.366	2.35e-02
Tributyl phosphate	0.605	1143.000	3.52e-03
Monocrotophos	-3.490	3.465	9.34e-05
N-Butyl-p-toluenesulfonamide	-0.762	11.060	1.56e-02
Anilazine	0.508	37.670	8.55e-02

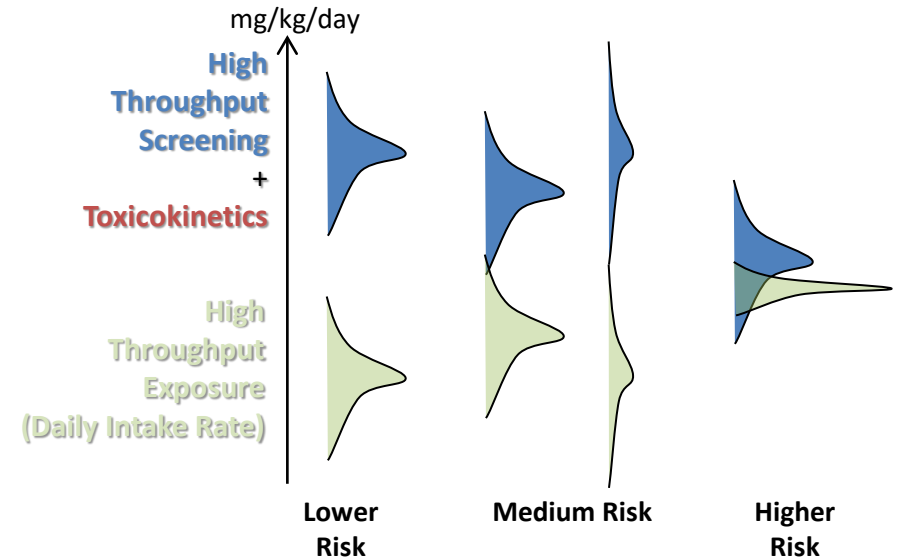
Step Five: Compare with

Conclusions

Please send any questions to:

wambaugh.john@epa.gov

- High throughput toxicokinetics (HTTK) are available to convert from bioactive *in vitro* concentrations to putative dose rates that might cause those concentrations (**new approach method-based points of departure or POD_{NAM}**)
- We calculate the **Bioactivity:Exposure Ratio (BER)** by comparing POD_{NAM} to a daily intake rate
- For a small subset of chemicals daily intake rates are monitored by the U.S. CDC
- The **systematic empirical evaluation of models (SEEM)** tool provides estimated intake rates for most chemicals
The 95% interval on SEEM intake rates reflects uncertainty on median population value and does not reflect population variability



References

- Bell, Shannon M., et al. "In vitro to in vivo extrapolation for high throughput prioritization and decision making." *Toxicology in vitro* 47 (2018): 213-227.
- Bell, Shannon, et al. "An integrated chemical environment with tools for chemical safety testing." *Toxicology in Vitro* 67 (2020): 104916.
- Breen, Miyuki, et al. "High-throughput PBTK models for in vitro to in vivo extrapolation." *Expert opinion on drug metabolism & toxicology* 17.8 (2021): 903-921.
- Coecke, Sandra, et al. "Toxicokinetics as a key to the integrated toxicity risk assessment based primarily on non-animal approaches." *Toxicology in vitro* 27.5 (2013): 1570-1577.
- Dorne, Jean-Lou, et al. "Reconnecting exposure, toxicokinetics and toxicity in food safety: OpenFoodTox and TKplate for human health, animal health and ecological risk assessment." *54. Congress of the European Societies of Toxicology (EUROTOX 2018)*. Vol. 295. No. Suppl. 1. 2018.
- Kapraun, Dustin F., et al. "Evaluation of a rapid, generic human gestational dose model." *Reproductive Toxicology* 113 (2022): 172-188.
- Khalidi, Hiba, et al. "SimRFlow: An R-based workflow for automated high-throughput PBPK simulation with the Simcyp® simulator." *Frontiers in Pharmacology* 13 (2022).
- Linakis, Matthew W., et al. "Development and evaluation of a high throughput inhalation model for organic chemicals." *Journal of exposure science & environmental epidemiology* 30.5 (2020): 866-877.
- National Research Council. "Risk assessment in the federal government: managing the process." (1983).
- Paul Friedman, Katie, et al. "Utility of in vitro bioactivity as a lower bound estimate of in vivo adverse effect levels and in risk-based prioritization." *Toxicological Sciences* 173.1 (2020): 202-225.
- Pearce, Robert G., et al. "Httk: R package for high-throughput toxicokinetics." *Journal of Statistical Software* 79.4 (2017a): 1.
- Pearce, Robert G., et al. "Evaluation and calibration of high-throughput predictions of chemical distribution to tissues." *Journal of pharmacokinetics and pharmacodynamics* 44 (2017b): 549-565.
- Ring, Caroline L., et al. "Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability." *Environment International* 106 (2017): 105-118.
- Sayre, Risa R., John F. Wambaugh, and Christopher M. Grulke. "Database of pharmacokinetic time-series data and parameters for 144 environmental chemicals." *Scientific data* 7.1 (2020): 122.
- Sobels, F. H. (1982) "The parallelogram; an indirect approach for the assessment of genetic risks from chemical mutagens." In: *Progress in Mutation Research*, Vol. 3 (K. C. Bora, G. R. Douglas, and E. R. Nestman, Eds.), Elsevier, Amsterdam, pp. 233-327.
- Stanfield, Zachary, et al. "Bayesian inference of chemical exposures from NHANES urine biomonitoring data." *Journal of Exposure Science & Environmental Epidemiology* 32.6 (2022): 833-846.
- Stanfield, Zachary, et al. "Characterizing Chemical Exposure Trends from NHANES Urinary Biomonitoring Data." *Environmental Health Perspectives* 132.1 (2024): 017009.
- Tan, Yu-Mei, Kai H. Liao, and Harvey J. Clewell. "Reverse dosimetry: interpreting trihalomethanes biomonitoring data using physiologically based pharmacokinetic modeling." *Journal of exposure science & environmental epidemiology* 17.7 (2007): 591-603.
- Wambaugh, John F., et al. "Evaluating in vitro-in vivo extrapolation of toxicokinetics." *Toxicological Sciences* 163.1 (2018): 152-169.
- Wang, Ying-Hong. "Confidence assessment of the Simcyp time-based approach and a static mathematical model in predicting clinical drug-drug interactions for mechanism-based CYP3A inhibitors." *Drug Metabolism and Disposition* 38.7 (2010): 1094-1104.
- Wetmore, Barbara A., et al. "Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment." *Toxicological Sciences* 125.1 (2012): 157-174.
- Wetmore, Barbara A. "Quantitative in vitro-to-in vivo extrapolation in a high-throughput environment." *Toxicology* 332 (2015a): 94-101.
- Wetmore, Barbara A., et al. "Incorporating high-throughput exposure predictions with dosimetry-adjusted in vitro bioactivity to inform chemical toxicity testing." *Toxicological Sciences* 148.1 (2015b): 121-136.
- Williams, Antony J., et al. "The CompTox Chemistry Dashboard: a community data resource for environmental chemistry." *Journal of cheminformatics* 9 (2017): 1-27.

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