EPA
United States
Environmental Protection
Agency

Identifying Susceptible Populations Using Exposure and Toxicokinetics

John Wambaugh
National Center for Computational Toxicology
Office of Research and Development
U.S. Environmental Protection Agency

Genetics and Environmental Mutagenesis Society
35th Annual Fall Meeting

November 7, 2017

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

orcid.org/0000-0002-4024-534X

EPA Office of Research and Development

United States Environmental Protection Agency

- The Office of Research and Development (ORD) is the scientific research arm of EPA
  - 558 peer-reviewed journal articles in 2016
- Research is conducted by ORD's three national laboratories, four national centers, and two offices
  - Includes **National Center for Computational Toxicology** and **National Exposure Research Laboratory**
- 14 facilities across the country and in Washington, D.C.
- Six research programs
  - Includes **Chemical Safety for Sustainability**
- Research conducted by a combination of Federal scientists; contract researchers; and postdoctoral, graduate student, and post-baccalaureate trainees

ORD Facility in
Research Triangle Park, NC

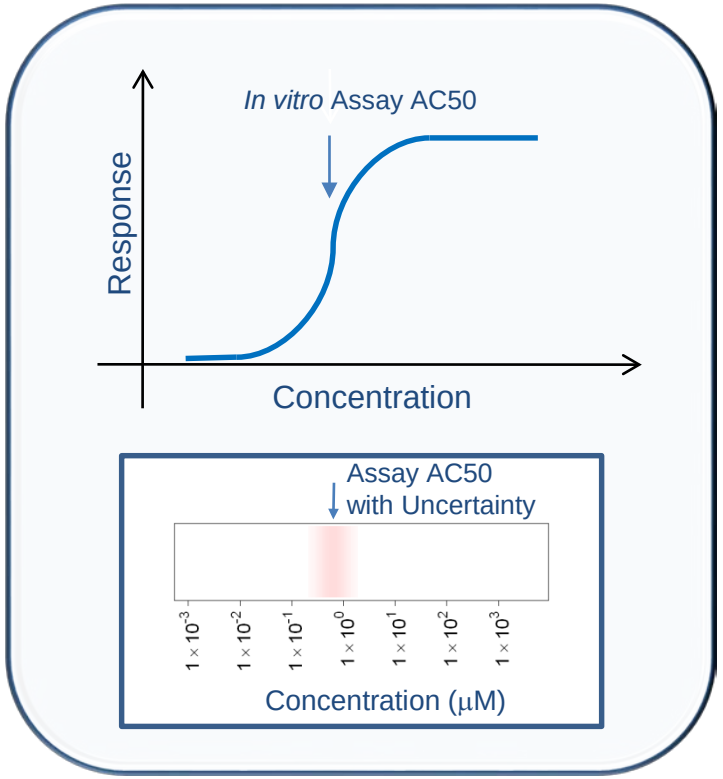
Chemical Regulation in the United States

- Park *et al.* (2012): At least 3221 chemicals in pooled human blood samples, many appear to be exogenous
- A tapestry of laws covers the chemicals people are exposed to in the United States (Breyer, 2009)
- Different testing requirements exist for food additives, pharmaceuticals, and pesticide active ingredients (NRC, 2007)
- Most other chemicals, ranging from industrial waste to dyes to packing materials are covered by the recently updated Toxic Substances Control Act (TSCA)
  - Thousands of chemicals on the market were either "grandfathered" in or were allowed without experimental assessment of hazard, toxicokinetics, or exposure
  - Thousands of new chemical use submissions are made to the EPA every year
  - **Methods are being developed to prioritize these existing and new chemicals for testing**

November 29, 2014

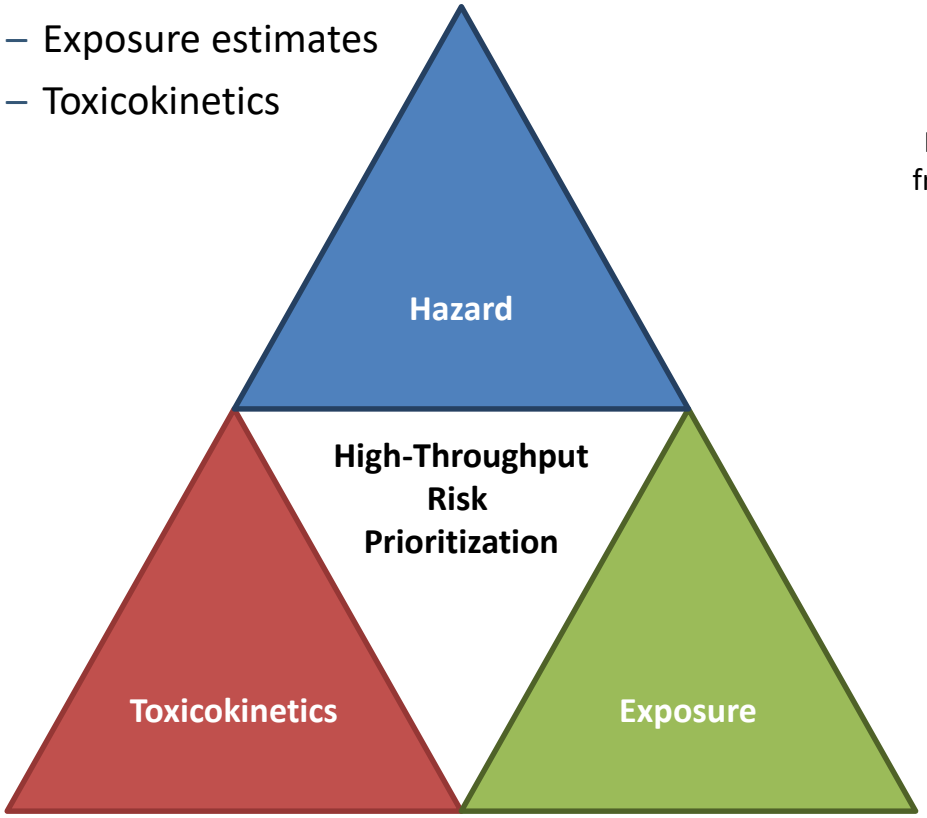
High-Throughput Bioactivity

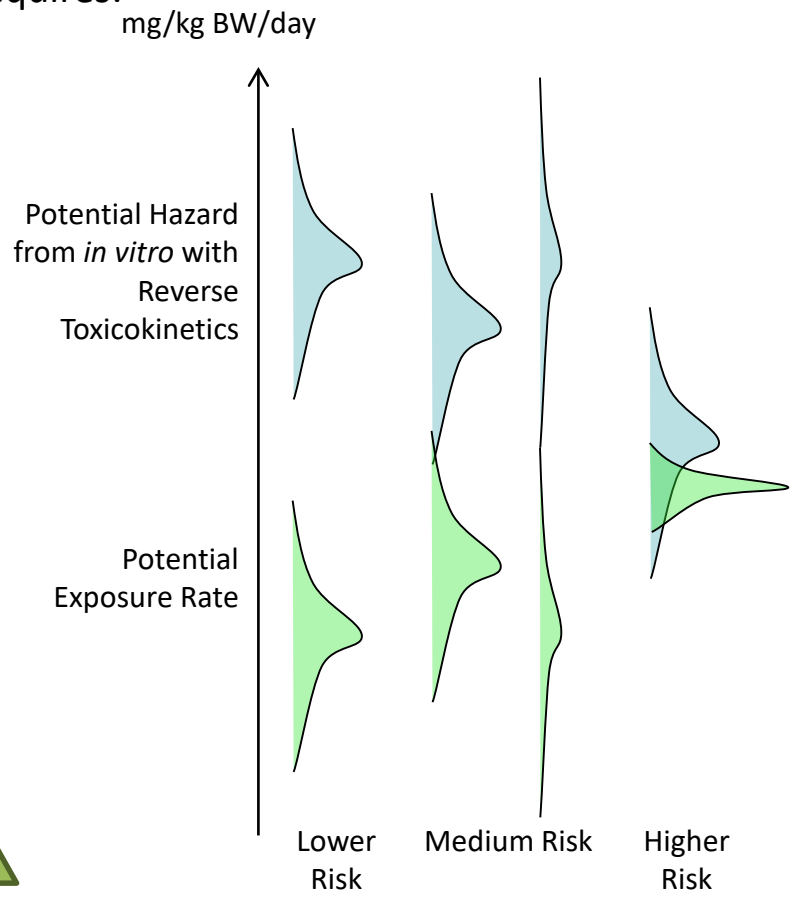
- We might estimate points of departure *in vitro* using high throughput screening (HTS)
- **Tox21**: Examining >8,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)
- **ToxCast**: For a subset (>2000) of Tox21 chemicals ran >1100 additional assays (Kavlock *et al.*, 2012)
- Most assays conducted in dose-response format (identify 50% activity concentration – AC50 – and efficacy if data described by a Hill function, Filer *et al.*, 2016)
- All data is public: http://comptox.epa.gov/
- However, we are not exposed to one chemical at a time



High-Throughput Risk-based Prioritization

- Risk is the product of chemical hazard and exposure (NRC, 1983)
- High throughput risk prioritization based upon HTS requires:
  - *in vitro-in vivo* extrapolation (IVIVE)
  - Exposure estimates
  - Toxicokinetics





CDC NHANES

Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) provides an important tool for monitoring public health

Large, ongoing CDC survey of US population: demographic, body measures, medical exam, biomonitoring (health and exposure), …

Designed to be representative of US population according to census data

Data sets publicly available (http://www.cdc.gov/nchs/nhanes.htm)

Includes measurements of:

- Body weight
- Height
- Chemical analysis of blood and urine

Kapraun et al. (2017) EHP

- Targeted analytical chemistry used to quantitate concentration of specific chemicals in urine
  - Samples must be divided up for each chemical tested
  - NHANES cohort divided up to allow enough sample for testing all chemicals

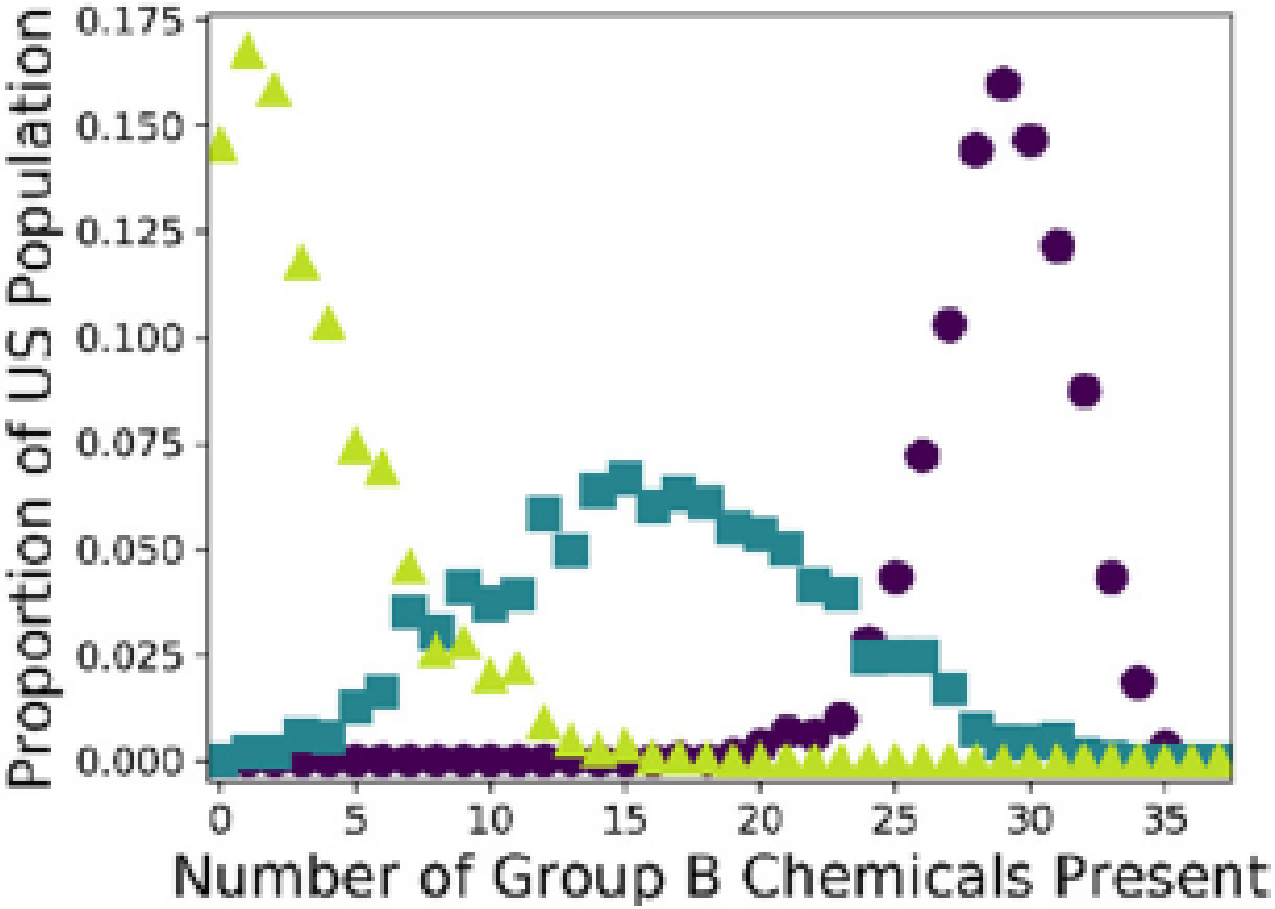
Table 4. Summary information for each of the National Health and Nutrition Examination Survey (NHANES) 2009–2010 subsamples.

Category	Subsample A	Subsample B	Subsample C
Number of subjects	2,741	2,736	2,132
Number of chemicals	29	37	40
Maximum weight	476,883.0	426,061.1	413,068.1
Minimum weight	14,002.7	13,975.1	12,659.3
Sum of weights	258,281,689.4	272,911,664.0	226,021,580.6
Records needed	18,445.1	19,528.5	17,854.1

- We will focus on “Sub-sample B” PAHs, Phenols, Pesticides, and Phthalates

Co-Occurrence of Chemicals in Individuals

The number of chemicals (out of 37) “present” in individuals depends upon where you set the limit

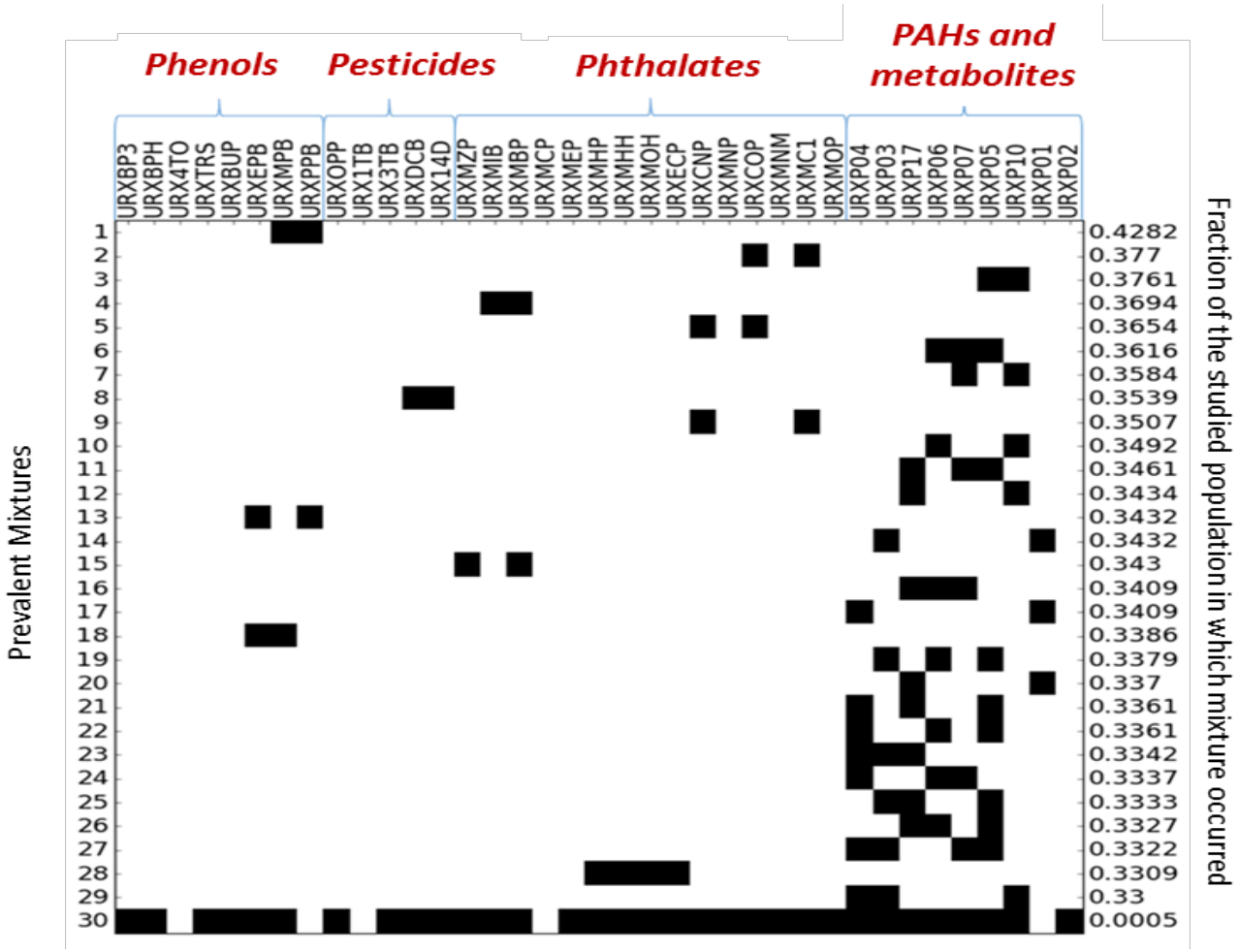


Ideally we would use some sort of chemical toxicity informed point of departure but don’t have that for all chemicals

Kapraun et al. (2017)

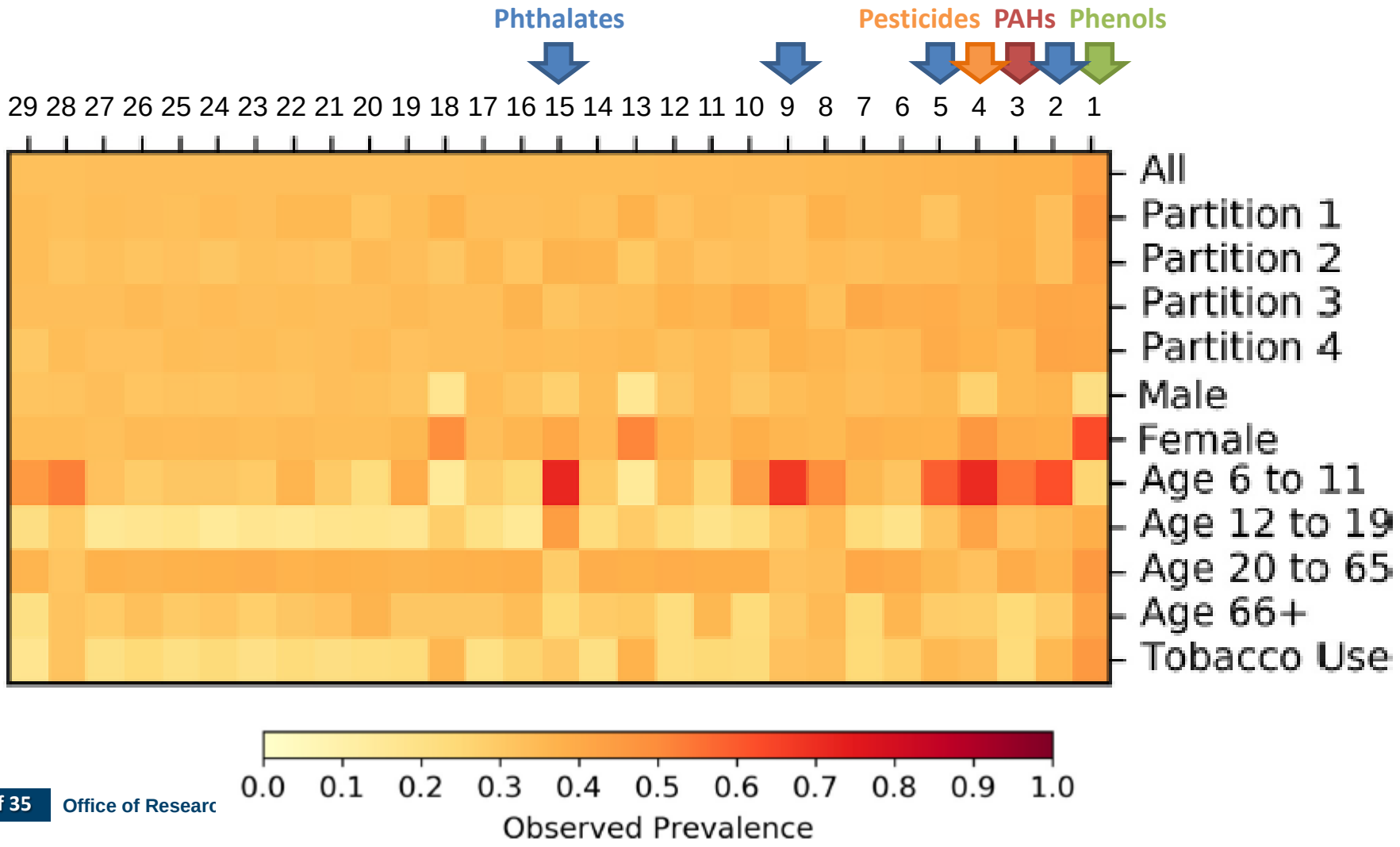
Identifying Prevalent Mixtures

- We are using data-mining methods (frequent itemset mining or FIM) to identify combinations of items (chemicals) that co-occur together within samples from same individual
- Used total population median concentration as threshold for “presence”
- Identified a few dozen mixtures present in >30% of U.S. population



Kapraun et al. (2017)

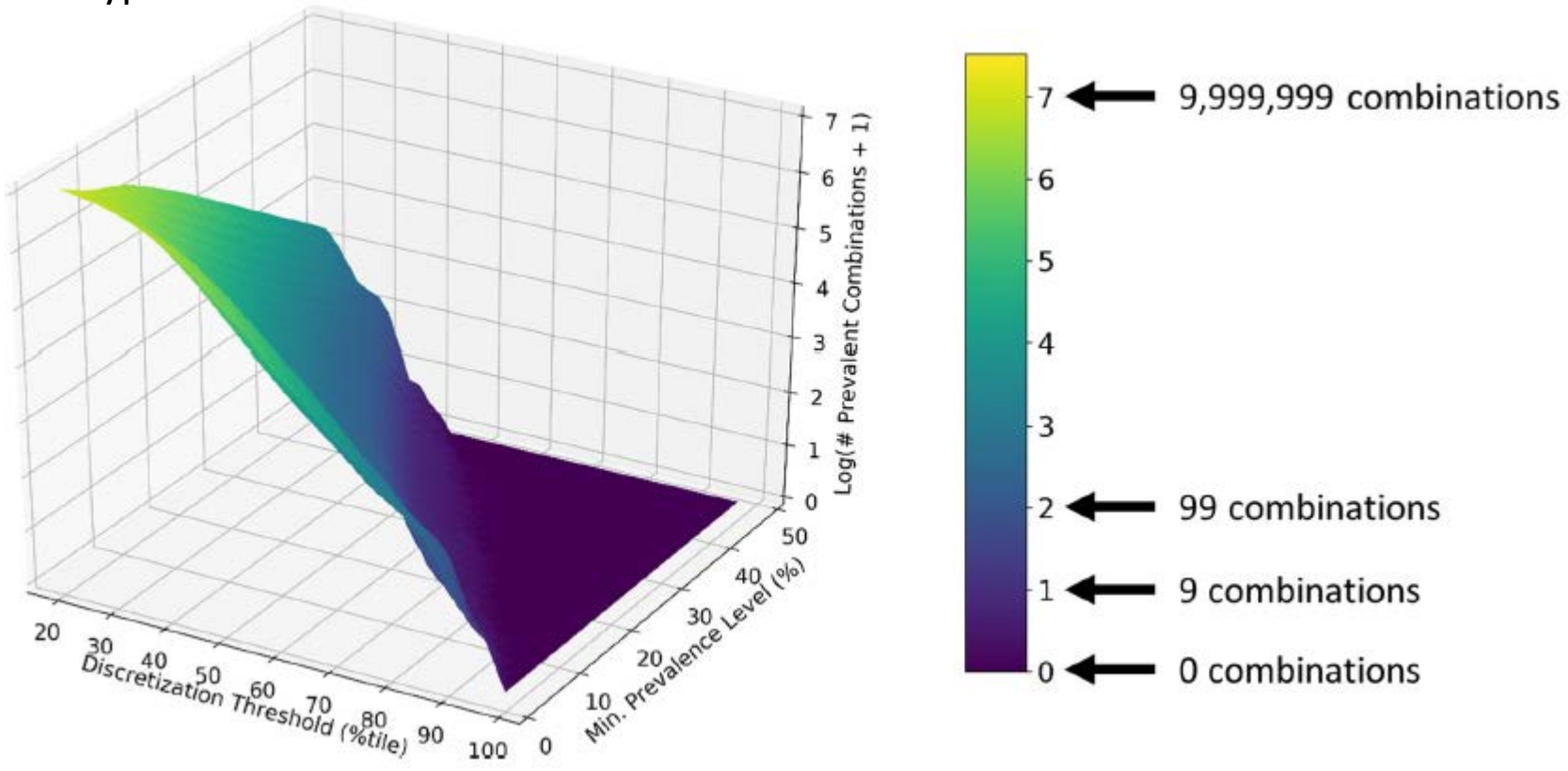
Demographic-Specific Prevalence of Combinations



Kapraun et al. (2017)

A Testable Number of Combinations

While high throughput screening (HTS) allows thousands of tests, there are millions of hypothetical combinations



"Exposure based priority setting" (NAS, 2017) allows identification of most important mixtures to test

Kapraun et al. (2017)

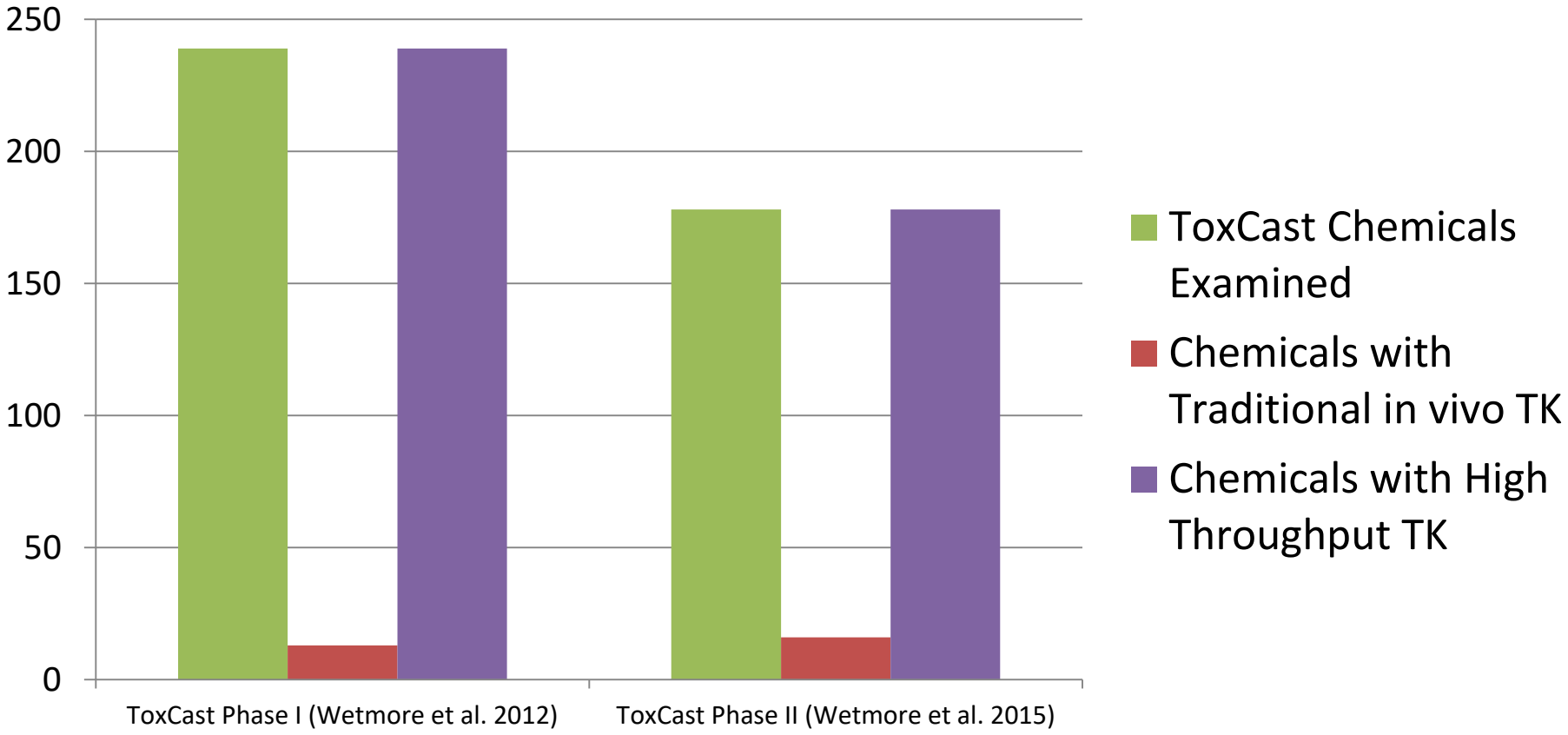
Combinations are not the same as Mixtures

- NHANES samples are one time "snapshots" of chemical concentration
- We don't actually know the **toxicokinetics** (absorption, distribution, metabolism, excretion) for most NHANES chemicals (Strope et al., 2017)
- **Chemical exposure patterns** not necessarily known – could be constant, sporadic, one time, or inherited from mother
  - Varies from chemical to chemical
- **Chemical clearance** (metabolism, excretion tells us the rate of change of chemical concentration
  - Different for different chemicals
- **Chemical distribution** tells us which tissues concentrate the chemical and the total body burden
  - Also varies between chemicals

What concentrations should be tested *in vitro*?

Addressing The Need for *In Vitro* Toxicokinetics

- Studies like Wetmore et al. (2012, 2015) generate TK data using *in vitro* methods



EPA United States Environmental Protection Agency

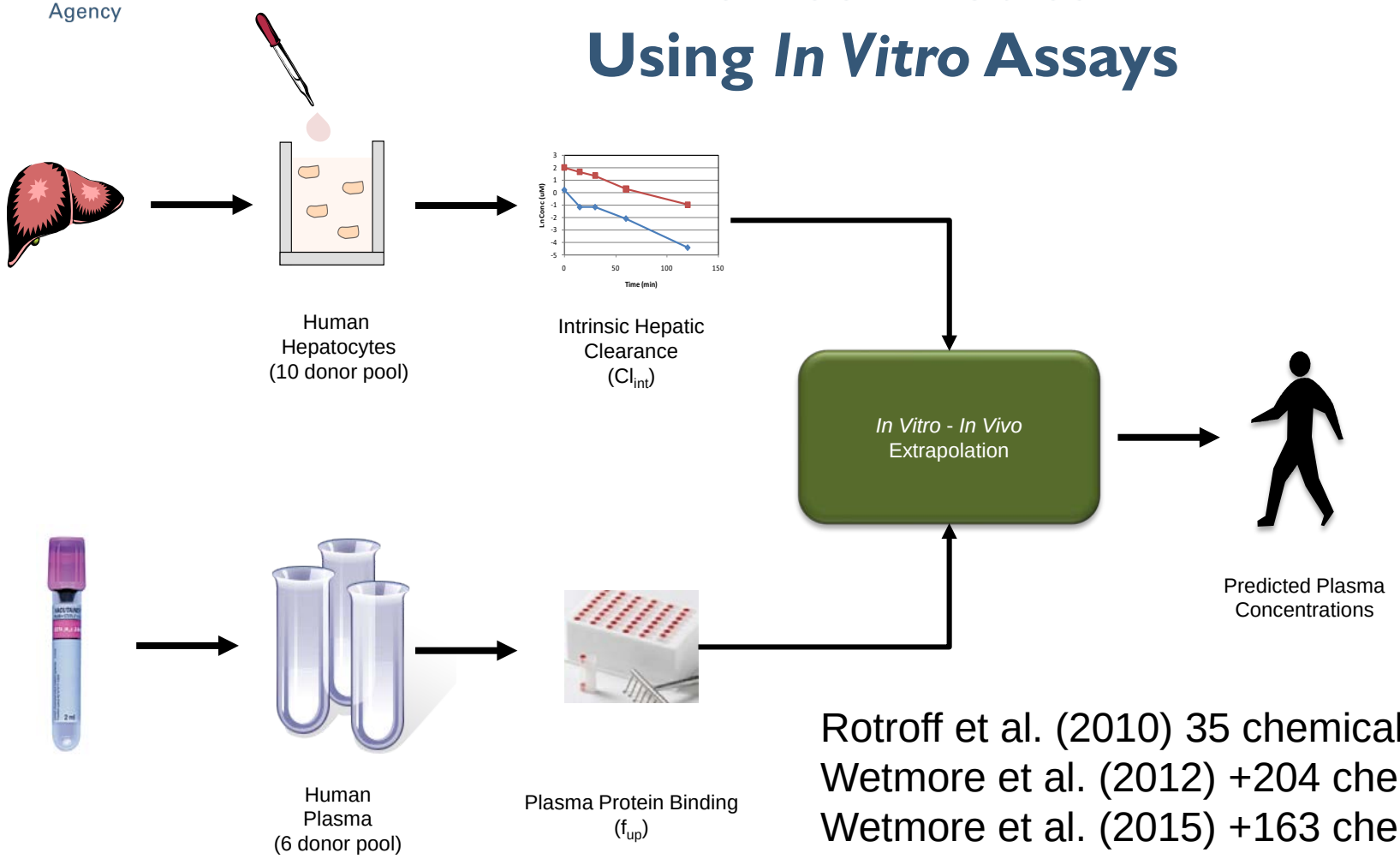
High Throughput Toxicokinetics (HTTK)

- Toxicokinetics (TK) provides a bridge between toxicity and exposure assessment by predicting tissue concentrations due to exposure
  - However traditional TK methods are resource intensive

- Relatively high throughput TK (HTTK) methods have been used by the pharmaceutical industry to determine range of efficacious doses and to prospectively evaluate success of planned clinical trials (Jamei, *et al.*, 2009; Wang, 2010)
  - A key application of HTTK has been “reverse dosimetry” (also called Reverse TK or RTK)
  - RTK can approximately convert *in vitro* HTS results to daily doses needed to produce similar levels in a human for comparison to exposure data (starting off with Rotroff, *et al*., 2010)

Characterizing Human *In Vivo* Toxicokinetics Using *In Vitro* Assays

United States Environmental Protection Agency



Rotroff et al. (2010) 35 chemicals
Wetmore et al. (2012) +204 chemicals
Wetmore et al. (2015) +163 chemicals

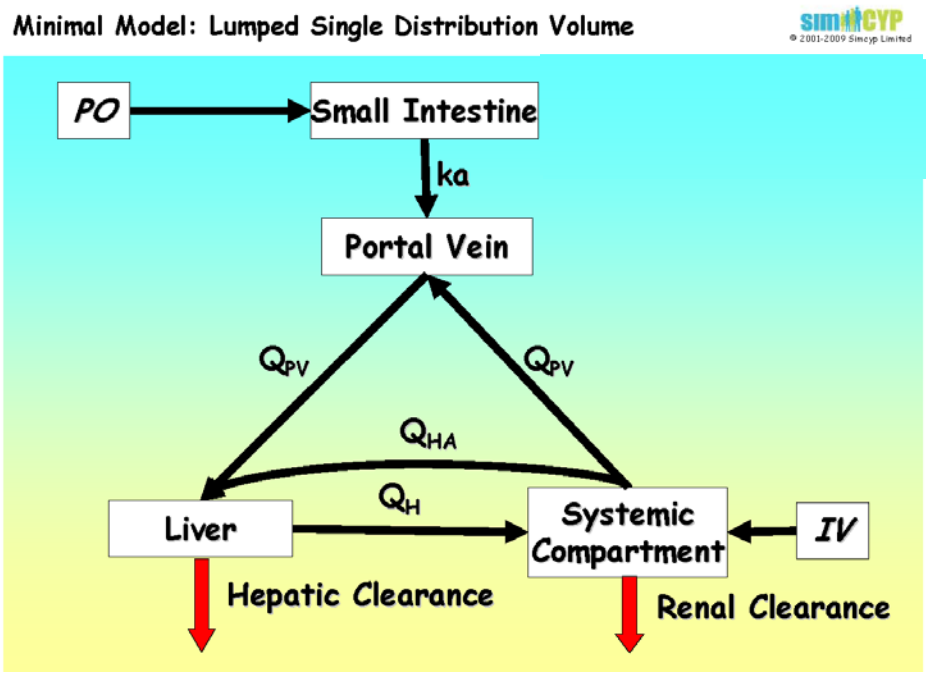
Figure from Barbara Wetmore

A Basic Model Allows HTTK

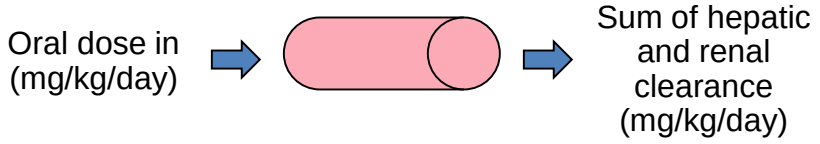
- *In vitro* plasma protein binding (fraction unbound in plasma – f_{up}) and intrinsic hepatic metabolic clearance (Cl_{int}) assays allow approximate hepatic and renal clearances to be calculated
- At steady state this allows conversion from concentration to administered dose
- 100% bioavailability assumed

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

GFR: Glomerular filtration rate (kidney)
Q_l: Liver blood flow



Jamei *et al*. (2009)



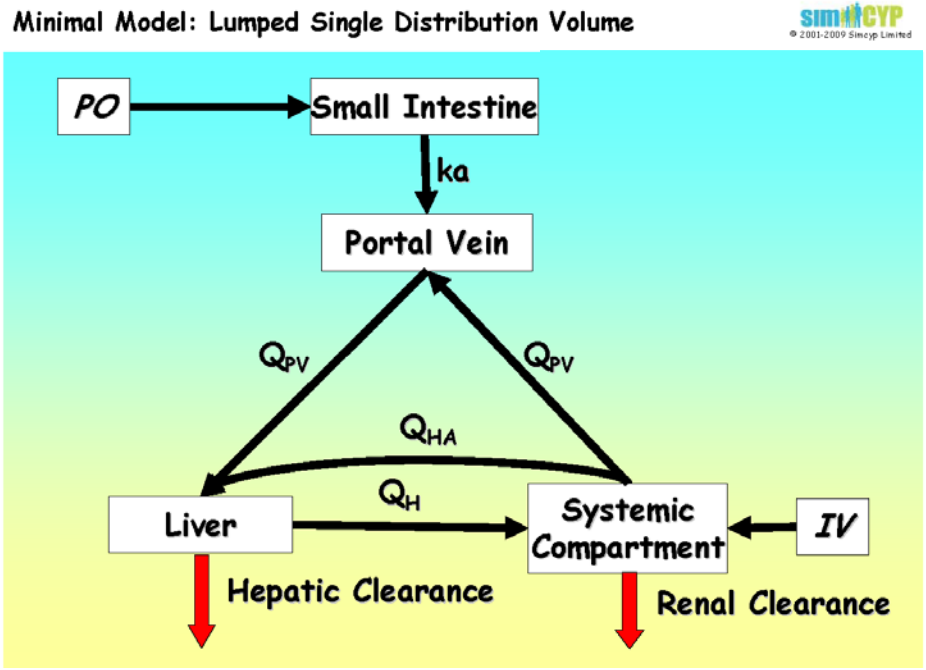
Variability in the Basic Steady-State TK Model

- Because we use a simple model for steady-state plasma concentration (C_{ss}) the amount of human physiology that can be varied is limited
- *In vitro* clearance (µL/min/10^6 hepatocytes) is scaled to a whole organ clearance using the density of hepatocytes per gram of liver and the volume of the liver (which varies between individuals)

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

(Passive) Renal Clearance

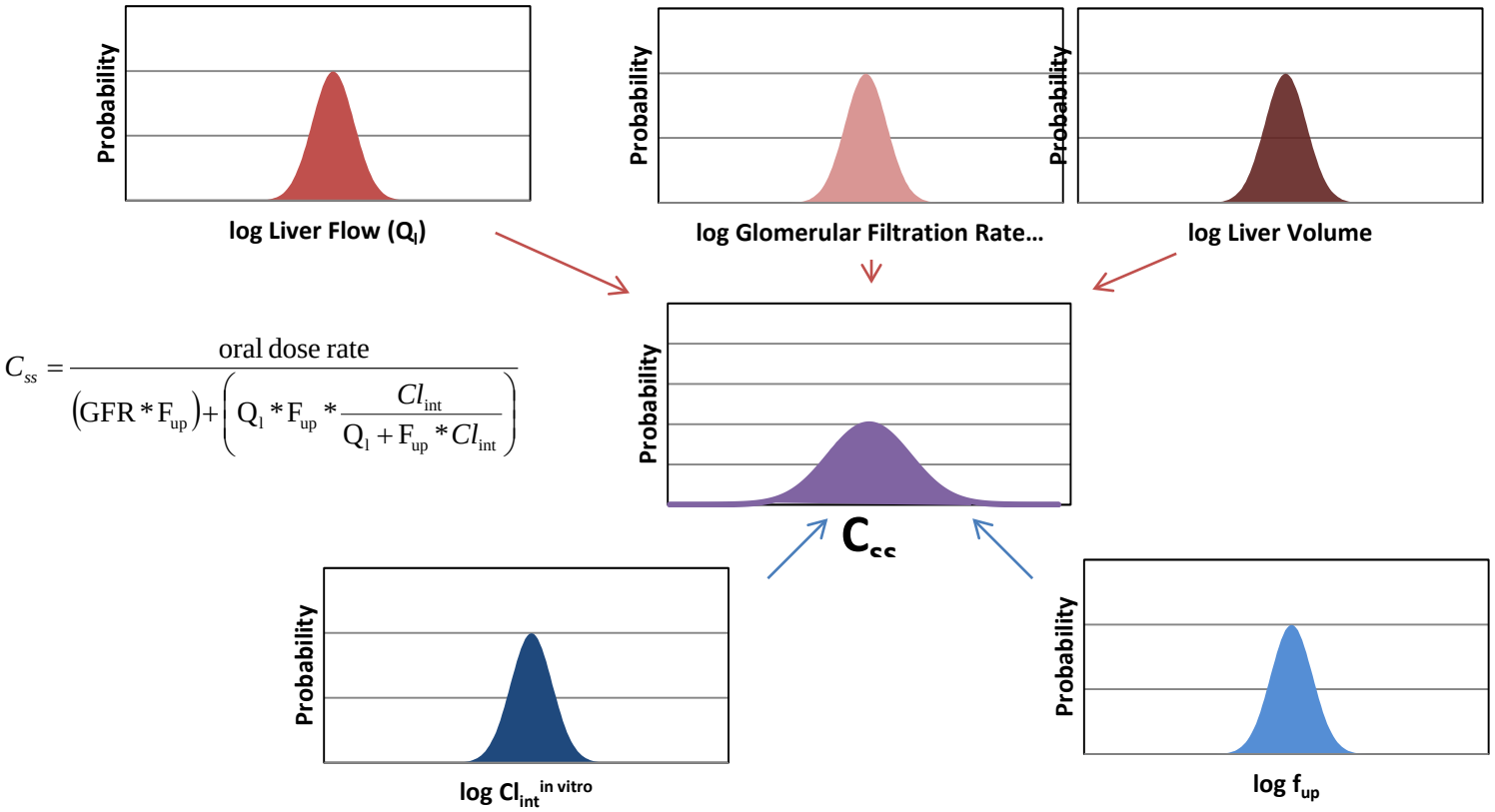
Hepatic Clearance (Metabolism)



Jamei *et al*. (2009)

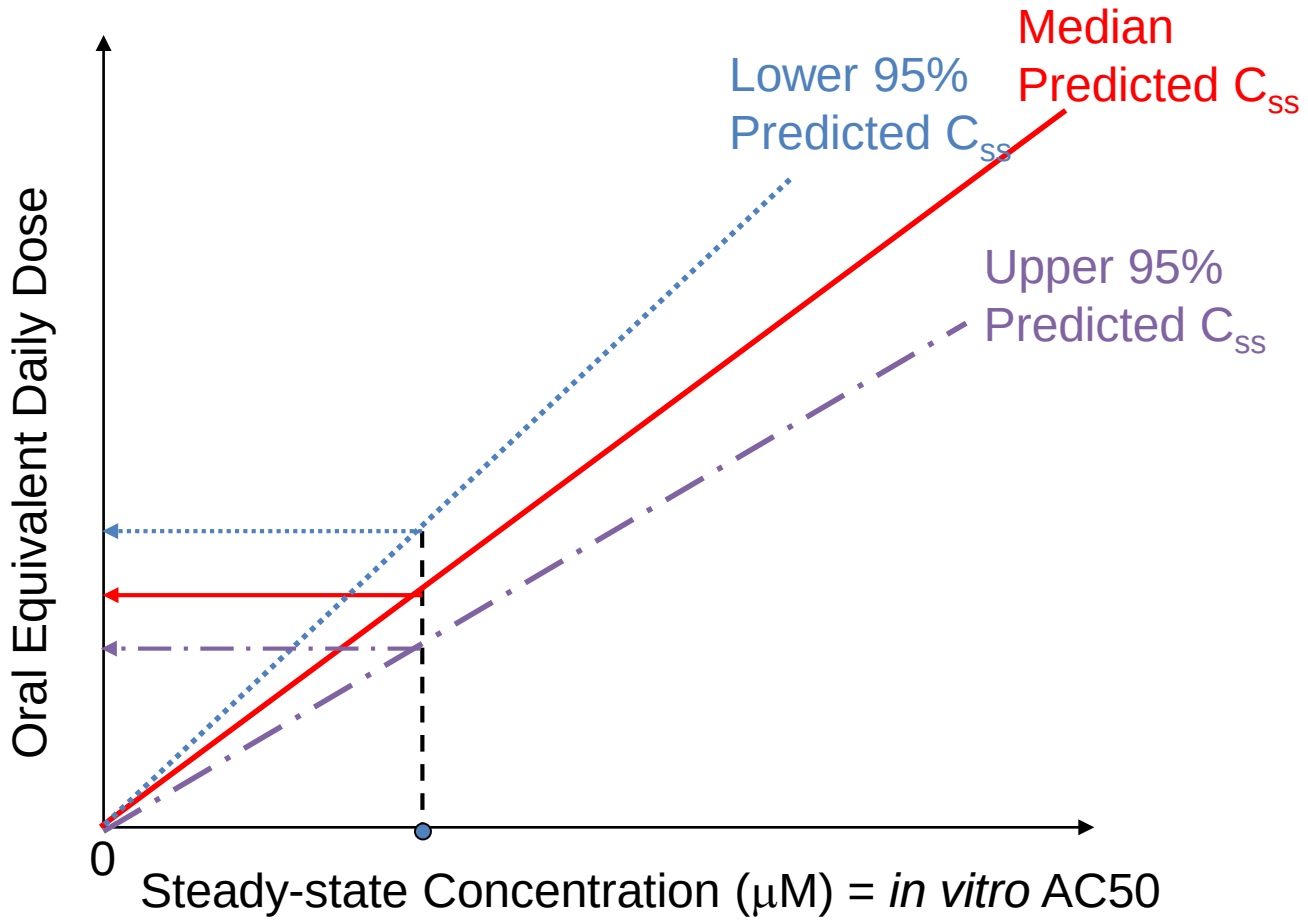
- Glomerular filtration rate (GFR) and blood flow to the liver (Q_l) both vary from individual to individual

Monte Carlo (MC) Approach to Variability



We use the upper 95th percentile Css for a 1 mg/kg bw/day exposure to convert from *in vitro* concentration to *in vivo* dose

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



- The higher the predicted C_{ss}, the lower the oral equivalent dose, so the upper 95% predicted C_{ss} from the MC has a lower oral equivalent dose

In Vitro - In Vivo Extrapolation (IVIVE)

Definition:
IVIVE is the 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

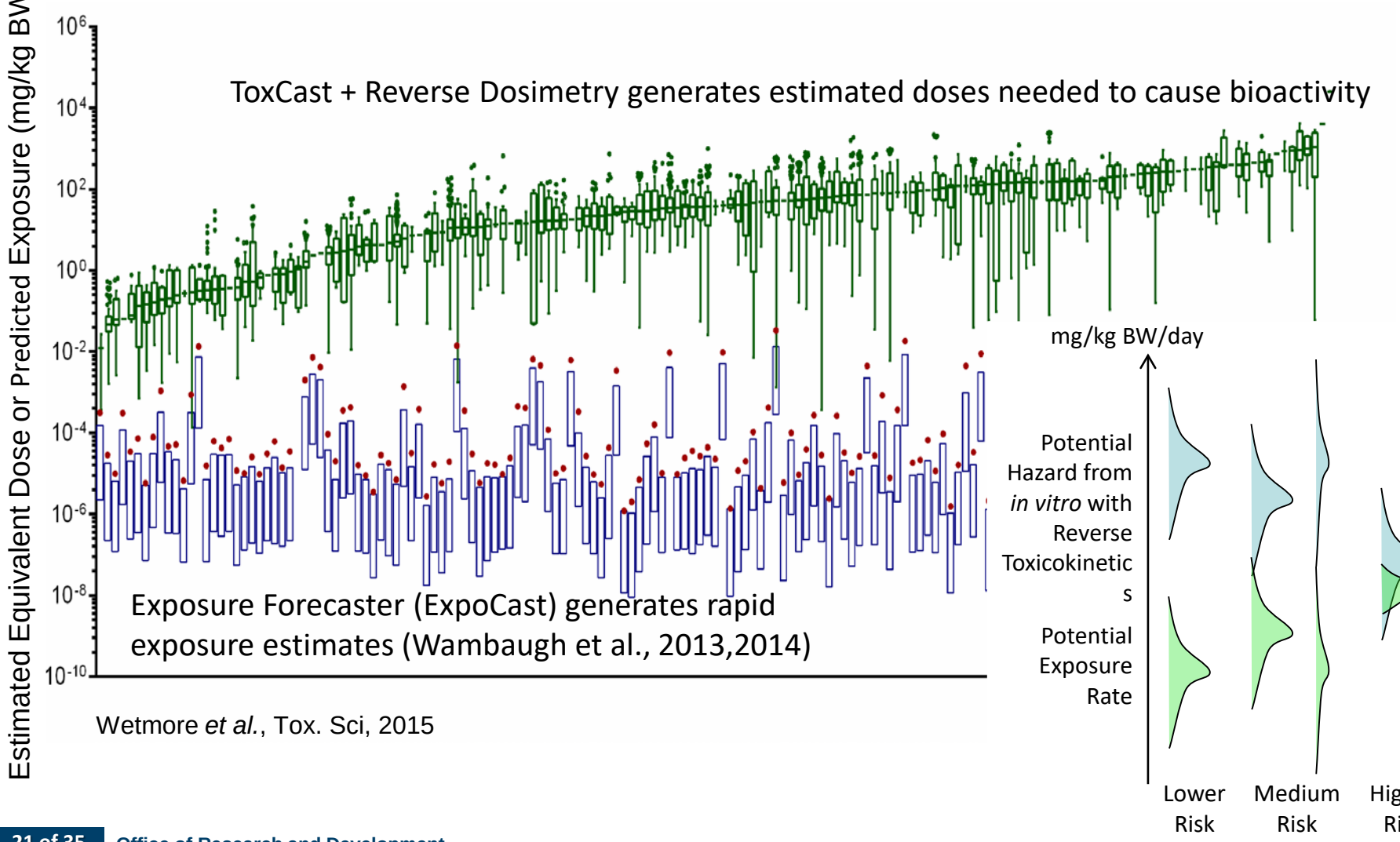
- IVIVE-PD/TD (Pharmacodynamics/Toxicodynamics):
  - Effect of molecules/chemicals at biological target *in vivo*
  - Assay design/selection important
  - Perturbation as adverse/therapeutic effect, reversible/ irreversible

- Both contribute to predict *in vivo* effects

Slide from Barbara Wetmore

Incorporating Dosimetry-Adjusted ToxCast Bioactivity Data with Exposure

United States Environmental Protection Agency

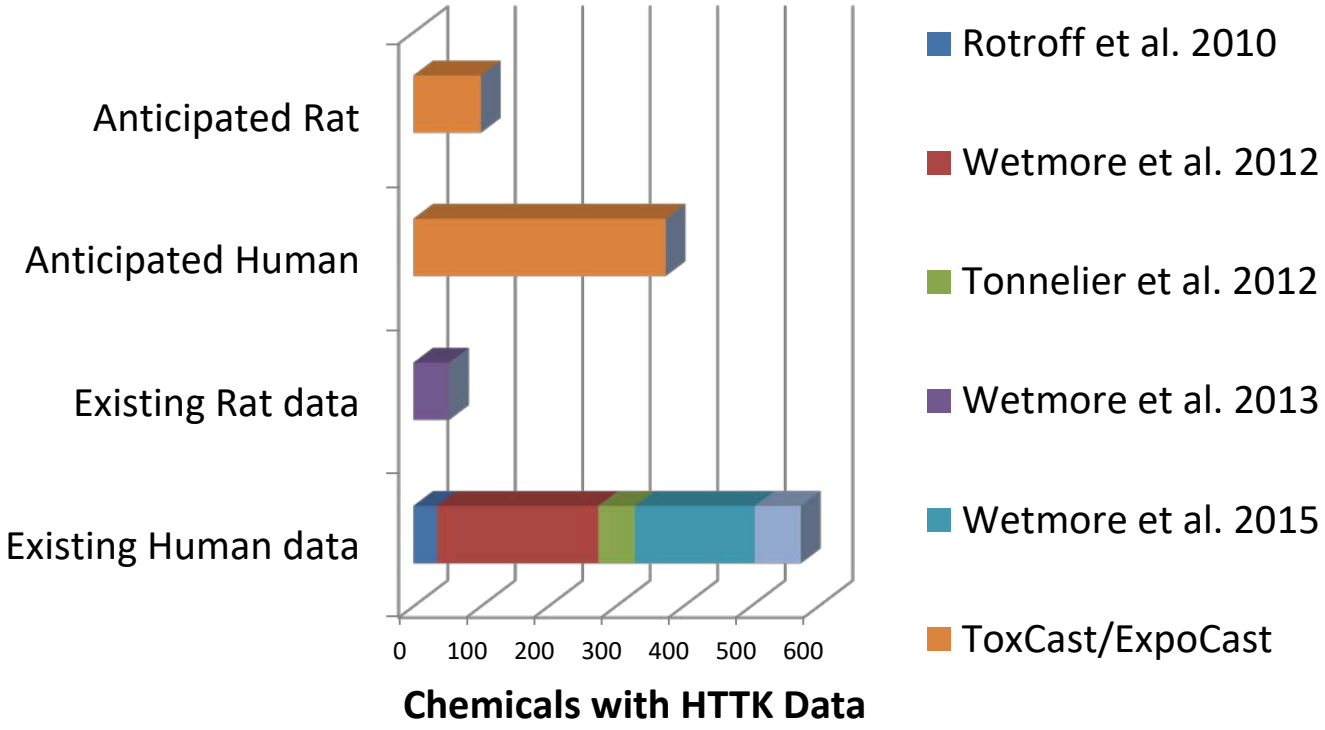


Wetmore *et al.*, Tox. Sci, 2015

Chemicals with HTTK Data

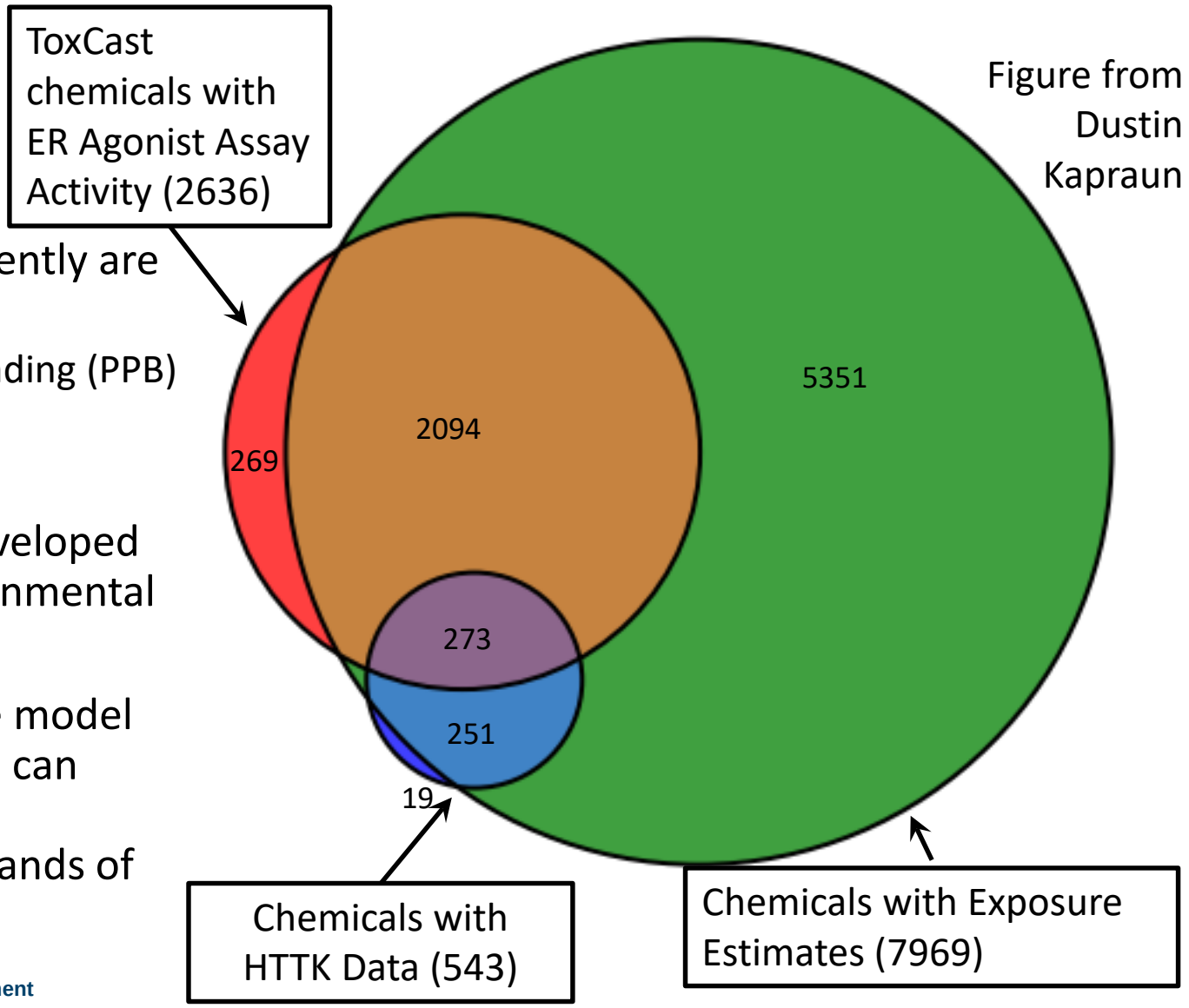
Measurement of *in vitro* clearance and binding both require chemical-specific analytical chemistry methods – these can be difficult to develop

Methods are appropriate for chemicals that are soluble, non-volatile only



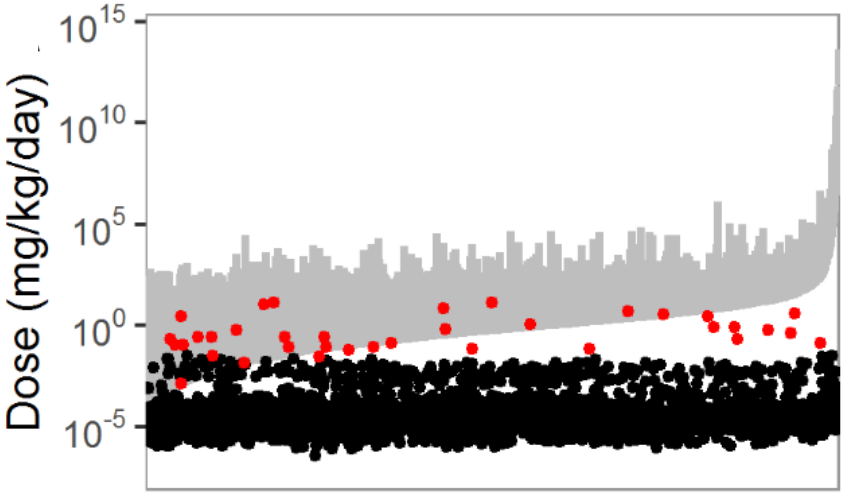
Predicting Critical TK Parameters

- Two parameters currently are key to HTTK model:
  - Plasma protein binding (PPB)
  - Hepatic clearance (metabolism)
- Ingle *et al.* (2016) developed PPB model for environmental chemicals
- If a hepatic clearance model can be developed we can provide tentative TK predictions for thousands of more chemicals

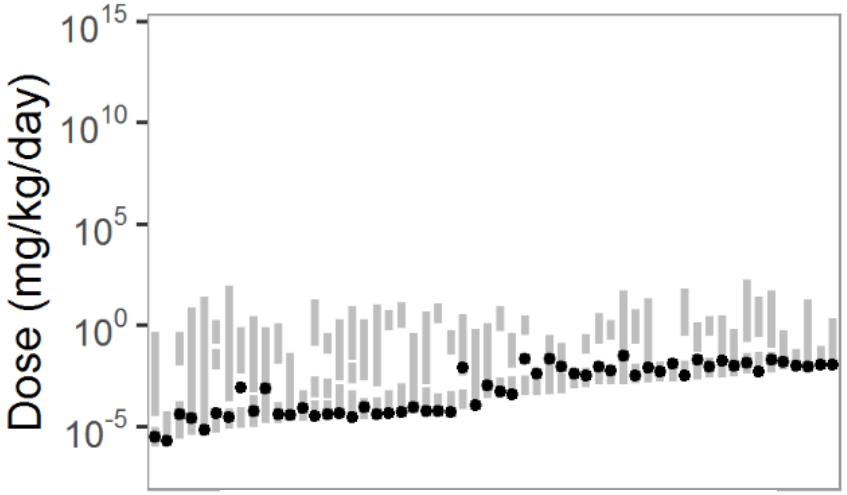


Using Predicted HTTK for Risk Prioritization

Sipes et al. used Simulations Plus ADMET Predictor to make *in silico* predictions of metabolism and protein binding:



Doses ranges for all 3925 Tox21 compounds eliciting a 'possible'-to-'likely' human *in vivo* interaction alongside estimated daily exposure

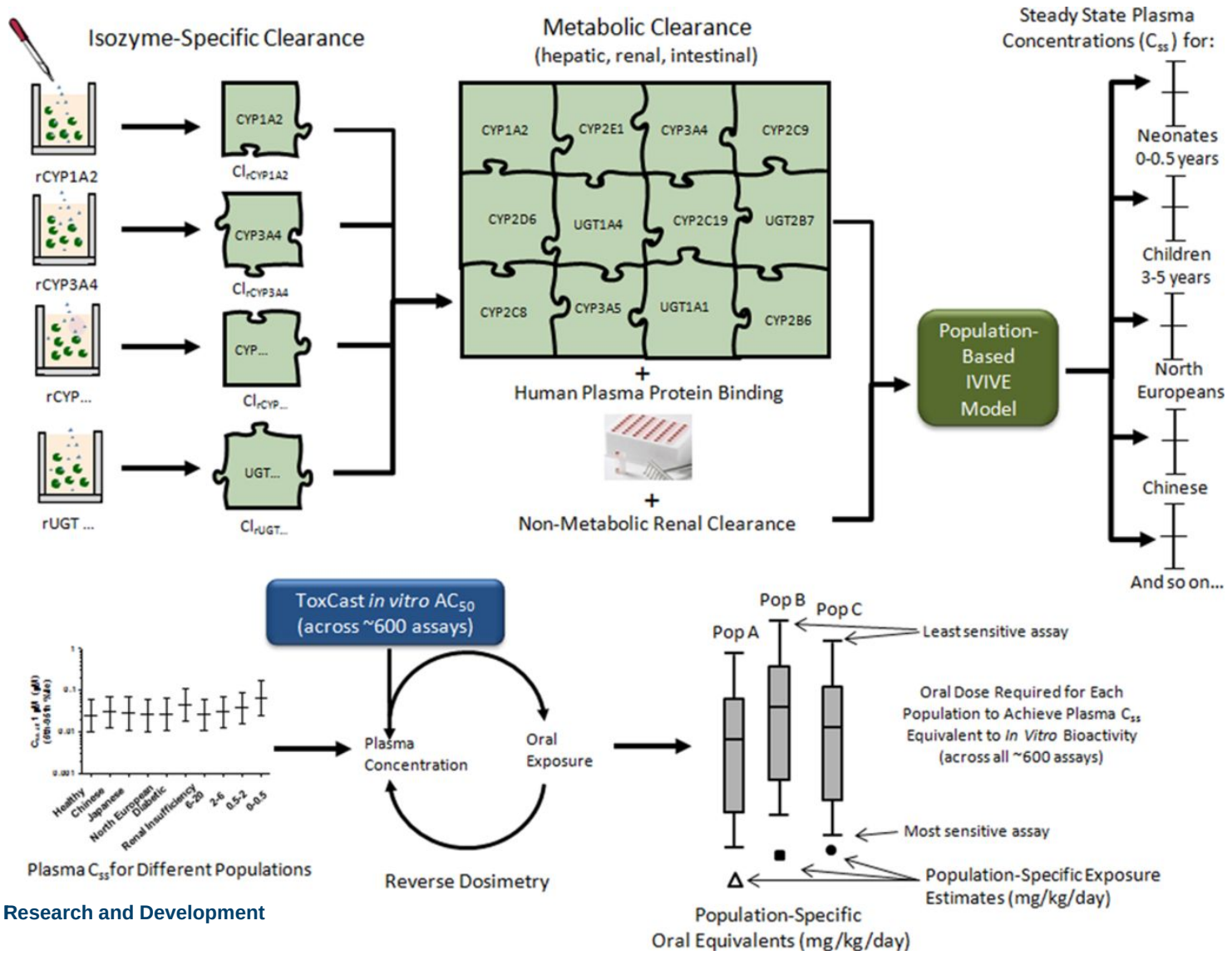


56 compounds with potential *in vivo* biological interaction at or above estimated environmental exposures

Sipes et al., (2017)

Population Variability in Metabolism

Single assay per enzyme per chemical – *lower throughput*!



Wetmore *et al*. (2014)

McNally et al. (2014) Linear Regressions for Population Simulation

Toxicology 315 (2014) 70–85

Contents lists available at ScienceDirect

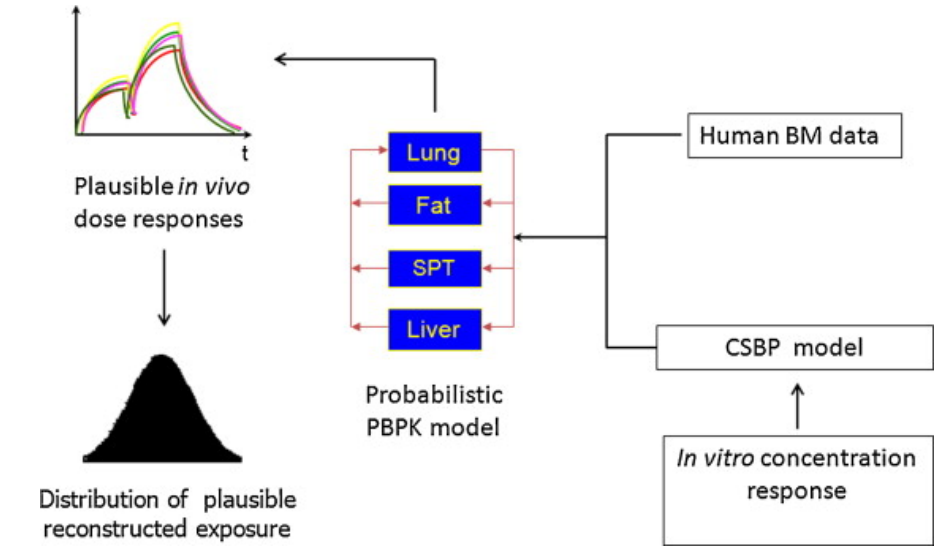
Toxicology

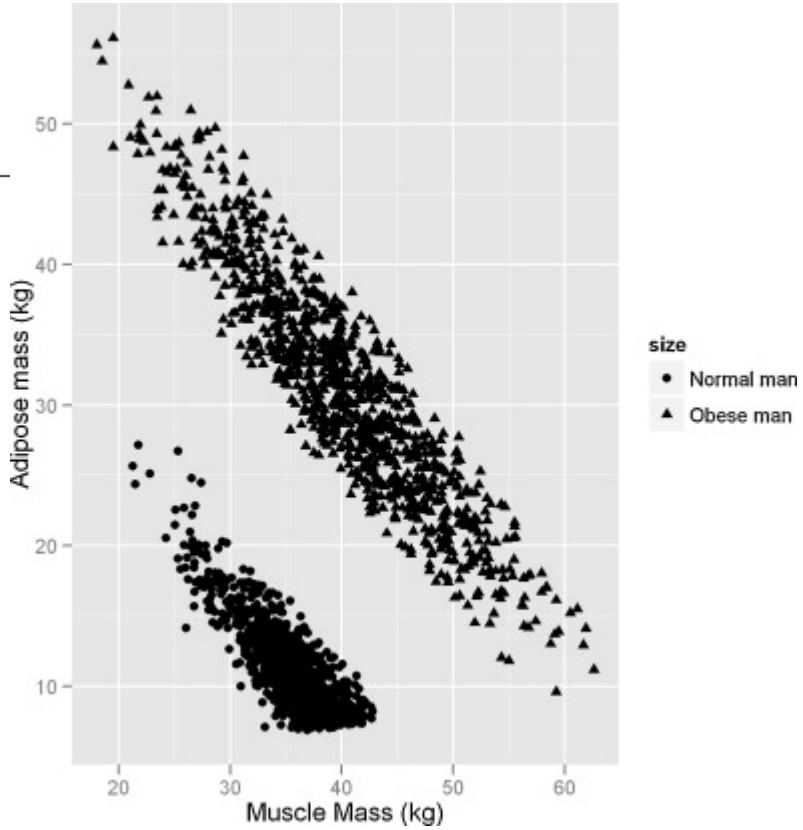
journal homepage: www.elsevier.com/locate/toxicol

PopGen: A virtual human population generator

Kevin McNally[a], Richard Cotton[b], Alex Hogg[a], George Loizou[a,*]

[a] Health & Safety Laboratory, Buxton, Derbyshire, UK
[b] TDL Ltd, Buxton, Derbyshire, UK





Modern U.S. Population Simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters

Sample quantities from

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

Ring *et al*. (2017)

Modern U.S. Population Simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters

Sample quantities from

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

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

Ring *et al*. (2017)

Modern U.S. Population Simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters

Sample quantities from

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

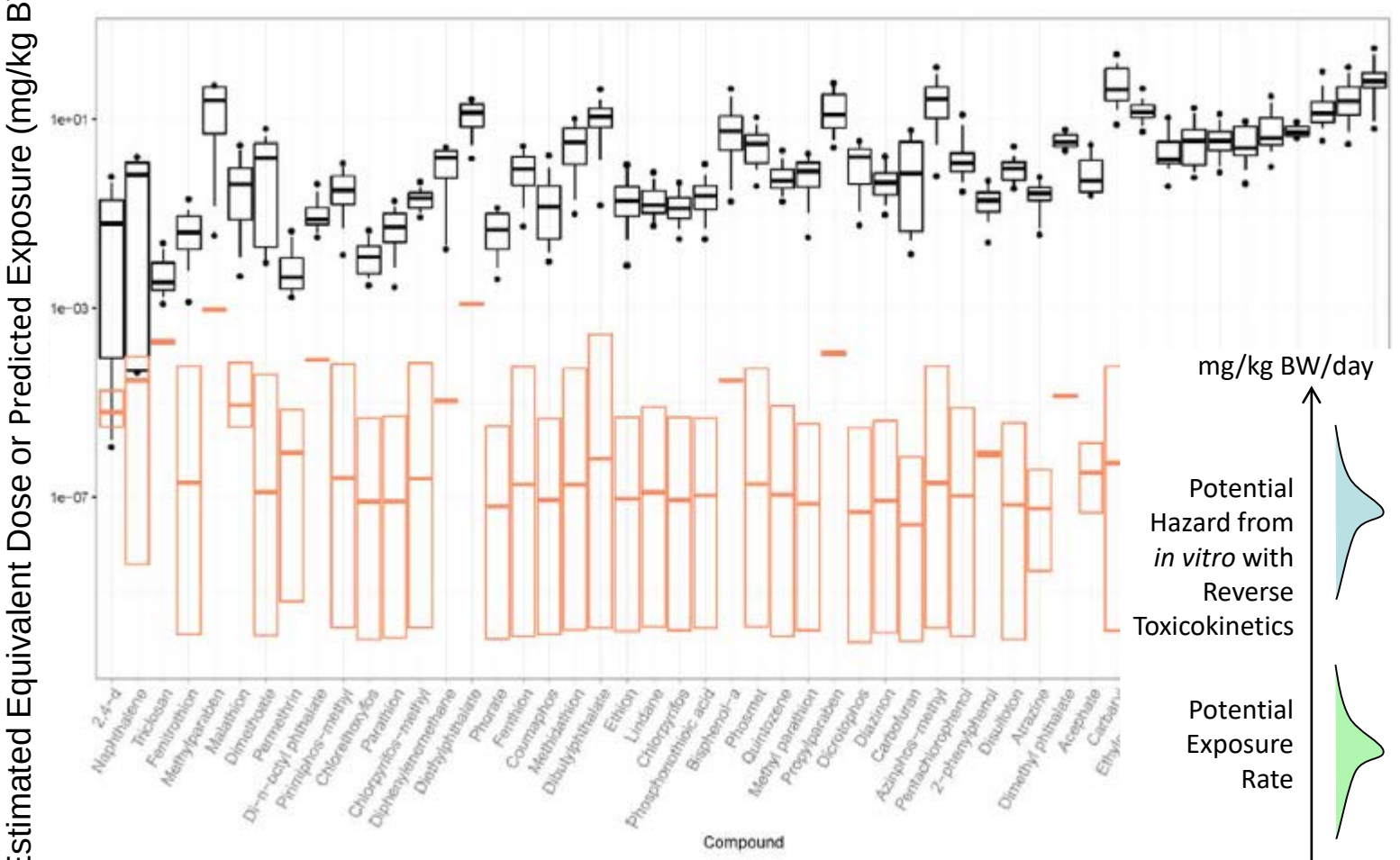
Use equations from literature
(McNally *et al.*, 2014)
(+ residual marginal variability)

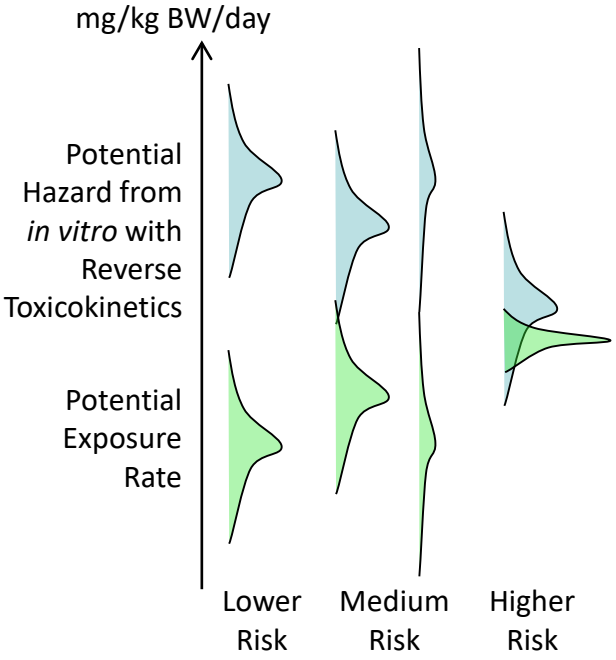
Predict physiological quantities

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

Ring *et al*. (2017)

Risk-Based Ranking for Total NHANES Population

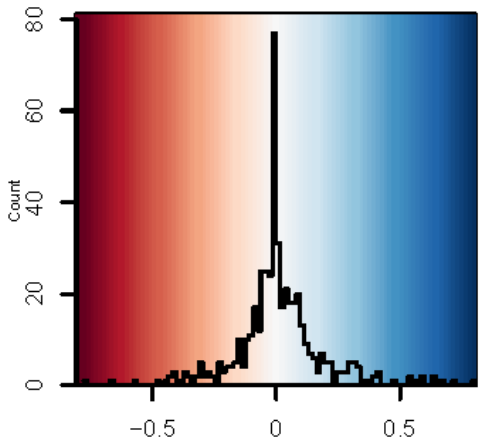




Ring *et al*. (2017)

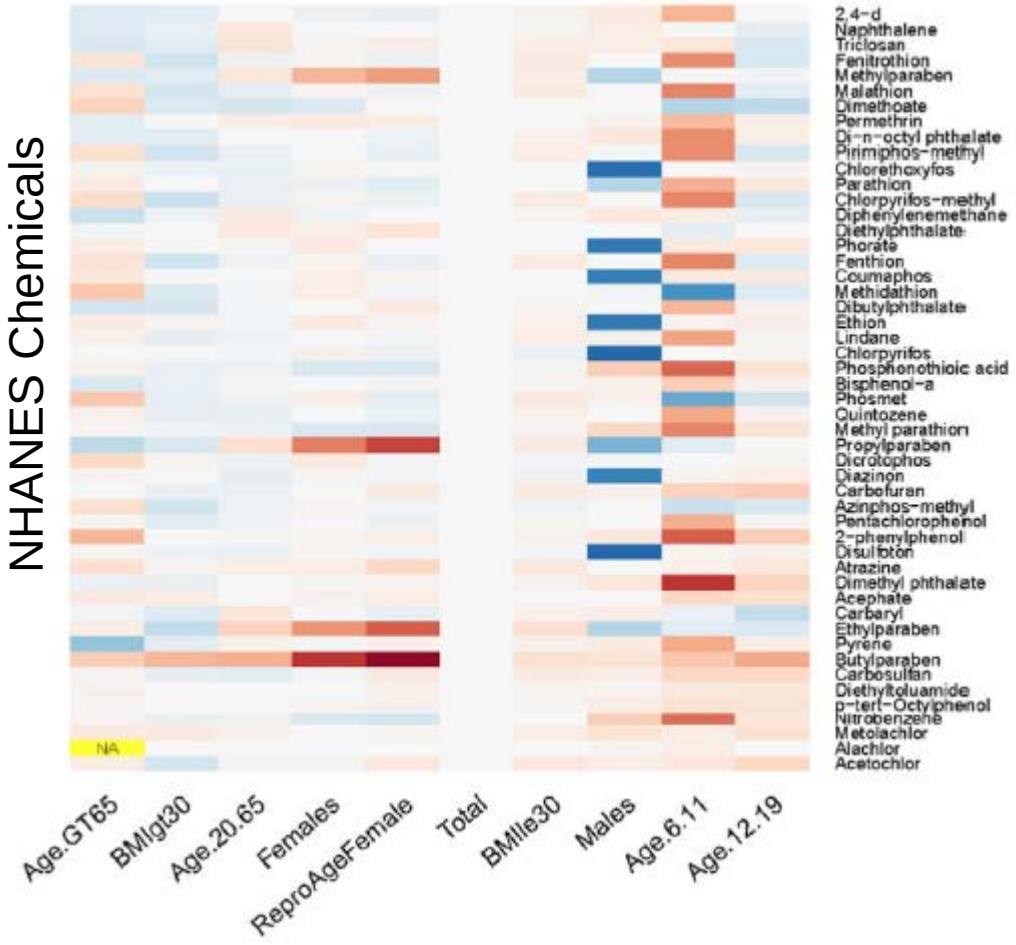
Life-stage and Demographic Variation in Exposure

- Wambaugh *et al.* (2014) made steady-state inferences of exposure rate (mg/kg/day) from NHANES data for various demographic groups



Change in Exposure Relative to Total Population

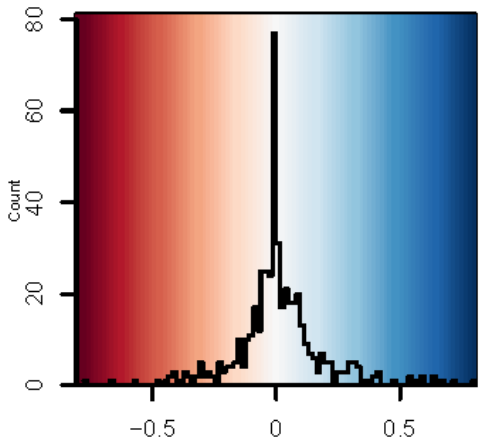
Change in Exposure (mg/kg bodyweight/day)



Ring *et al.* (2017)

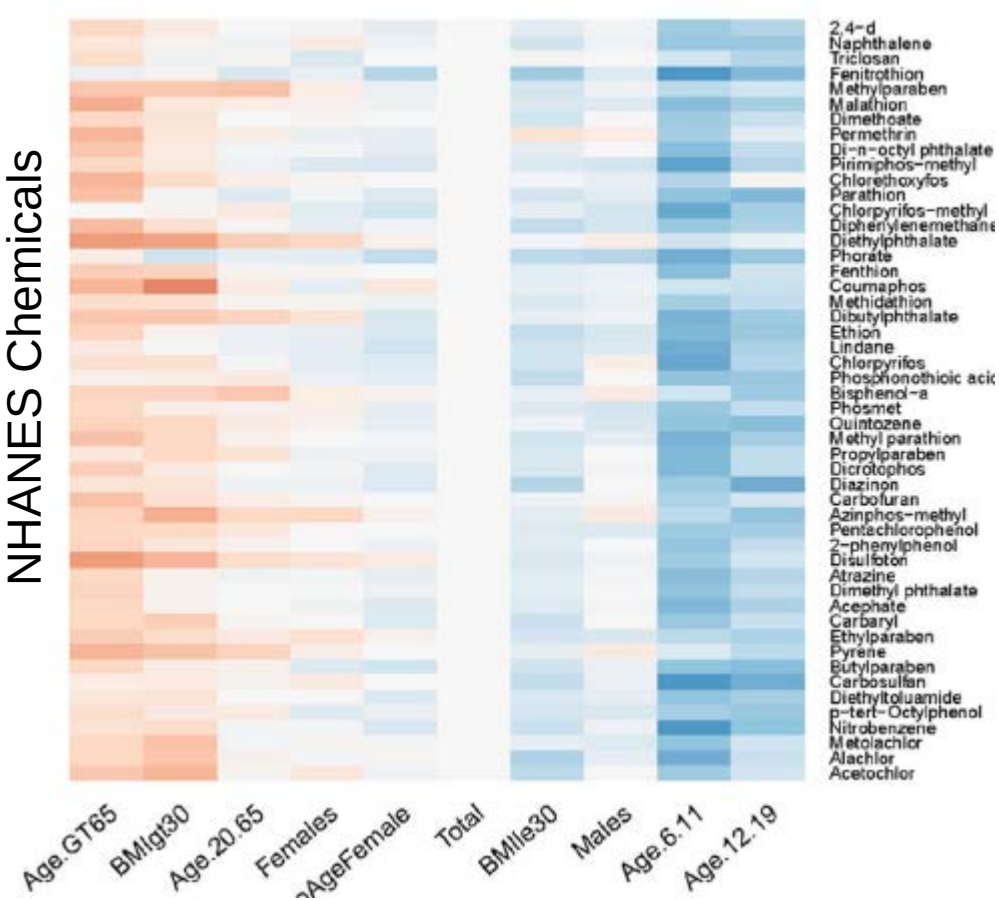
Life-stage and Demographic Variation in Exposure

- Ring *et al.* (2017) made demographic-specific predictions of change in plasma concentrations for a 1 mg/kg bw/day exposure



Change in Plasma Concentration Relative to Total Population

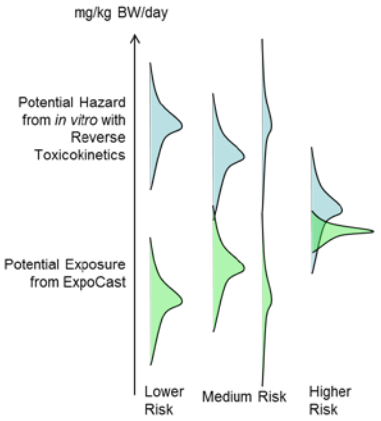
Change in Toxicokinetics (µM/unit exposure)

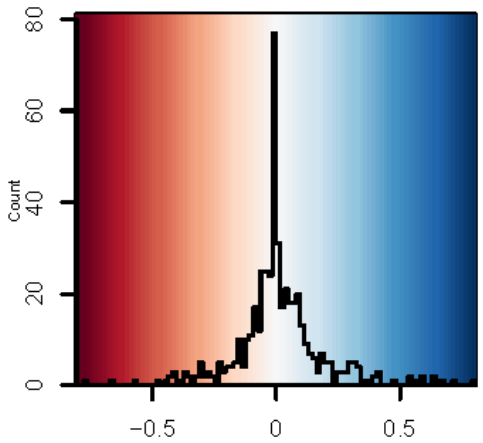


Ring *et al.* (2017)

Life-stage and Demographic Specific Predictions

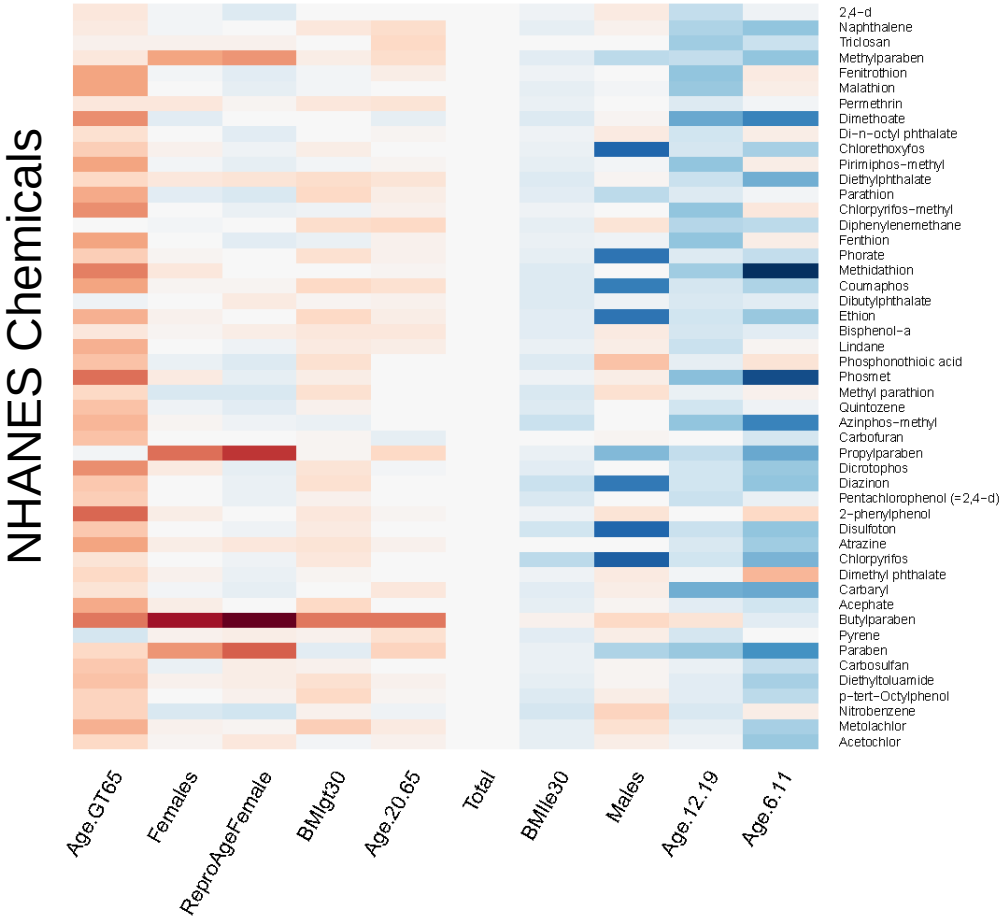
- Can calculate margin between bioactivity and exposure for specific populations





Change in Risk Relative to Total Population

Change in Activity:Exposure Ratio



Ring *et al*. (2017)

All Models and Data Open Source and Public

Download R:

https://www.r-project.org/

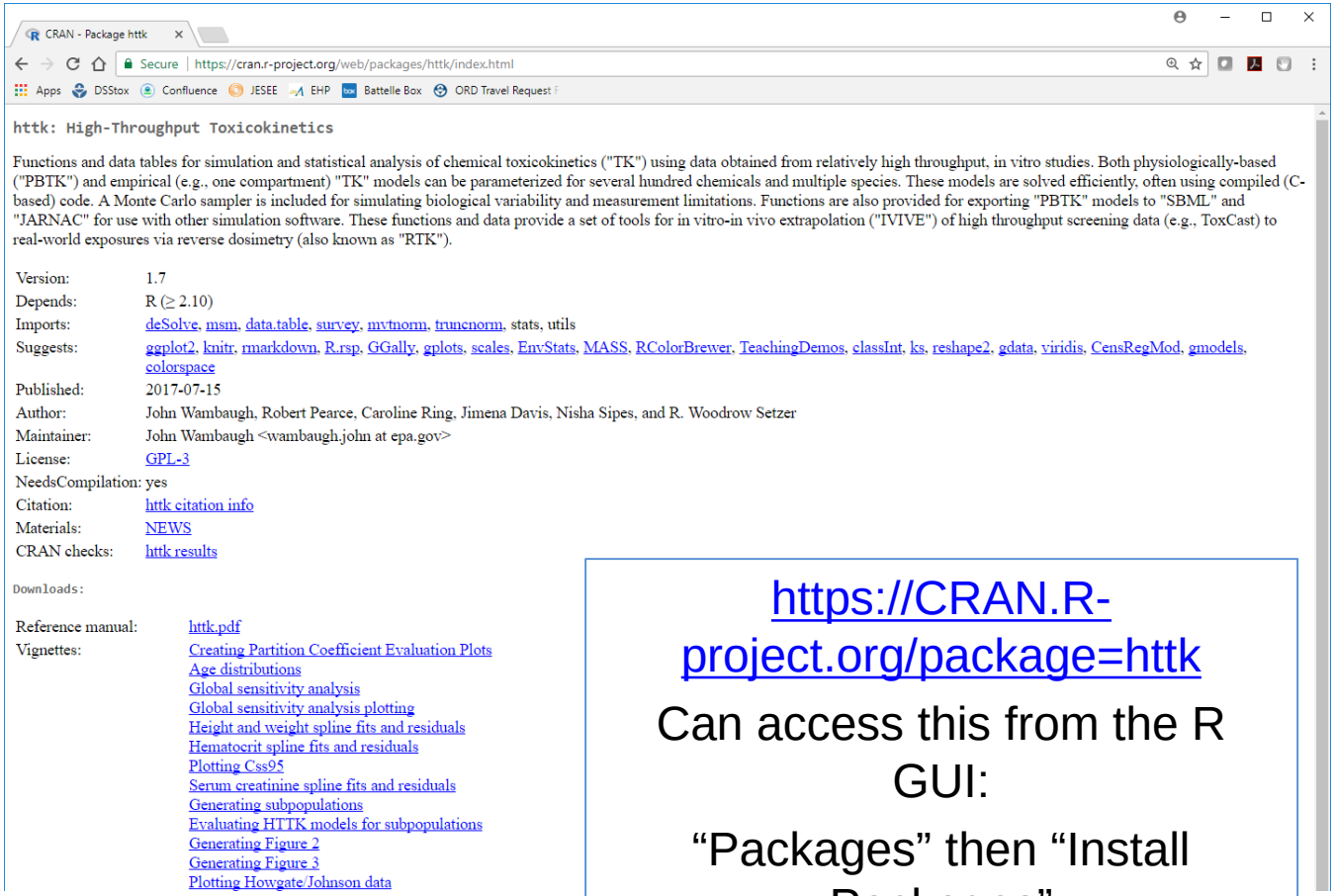
within R, type:

```
install.packages("httk")
```

Then

```
library("httk")
```

- "httk" R Package for IVIVE and **PBTK**
- 553 chemicals to date
- 100's of additional chemicals being studied
- Pearce *et al.* (2017a) provides documentation and examples
- Built-in vignettes provide further examples of how to use many functions



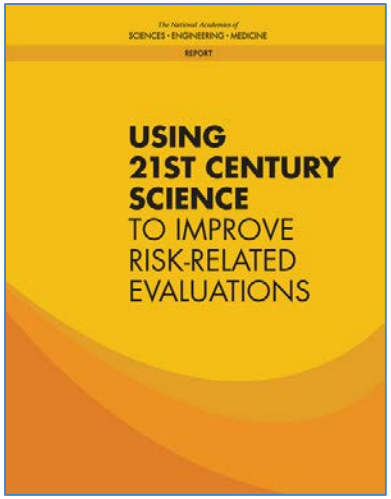
https://CRAN.R-project.org/package=httk

Can access this from the R GUI:

"Packages" then "Install Packages"

Conclusions

- We would like to know more about the risk posed by thousands of chemicals in the environment – which ones should we start with?
  - High throughput screening (HTS) provides a path forward for identifying potential hazard
- Using big data analytics we can identify priority combinations of chemicals
- Using *in vitro* methods developed for pharmaceuticals, we can relatively efficiently predict TK for large numbers of chemicals, but we are limited by analytical chemistry



National Academy of Sciences, January, 2017:
"Translation of high-throughput data into risk-based rankings is an important application of exposure data for chemical priority-setting. Recent advances in high-throughput toxicity assessment, notably the ToxCast and Tox21 programs… and in high-throughput computational exposure assessment… have enabled first-tier risk-based rankings of chemicals on the basis of margins of exposure…"

EPA United States Environmental Protection Agency

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

NCCT
Chris Grulke
Greg Honda*
Richard Judson
Andrew McEachran*
Robert Pearce*
Ann Richard
Risa Sayre*
Woody Setzer
Rusty Thomas
John Wambaugh
Antony Williams

NRMRL
Yirui Liang*
Xiaoyu Liu

NHEERL
Linda Adams
Christopher Ecklund
Marina Evans
Mike Hughes
Jane Ellen Simmons

NERL
Craig Barber
Namdi Brandon*
Peter Egeghy
Hongtai Huang*
Brandall Ingle*
Kristin Isaacs
Seth Newton
Katherine Phillips
Paul Price
Jeanette Reyes*
Jon Sobus
John Streicher*
Mark Strynar
Mike Tornero-Velez
Elin Ulrich
Dan Vallero
Barbara Wetmore

*Trainees

Lead CSS Matrix Interfaces:
John Kenneke (NERL)
John Cowden (NCCT)

Collaborators

Arnot Research and Consulting
Jon Arnot
Battelle Memorial Institute
Anne Louise Sumner
Anne Gregg
Chemical Computing Group
Rocky Goldsmith
National Institute for Environmental Health Sciences (NIEHS) National Toxicology Program
Mike Devito
Steve Ferguson
Nisha Sipes
Research Triangle Institute
Timothy Fennell
ScitoVation
Harvey Clewell
Kamle Mansouri
Chantel Nicolas
Silent Spring Institute
Robin Dodson
Southwest Research Institute
Alice Yau
Kristin Favela
Summit Toxicology
Lesa Aylward
Tox Strategies
Caroline Ring
University of California, Davis
Deborah Bennett
Hyeong-Moo Shin
University of Michigan
Olivier Jolliet
University of North Carolina, Chapel Hill
Alex Tropsha

References

- Breyer, Stephen. Breaking the vicious circle: Toward effective risk regulation. Harvard University Press, 2009
- Filer, Dayne L.. "The ToxCast analysis pipeline: An R package for processing and modeling chemical screening data." US Environmental Protection Agency: http://www. epa. gov/ncct/toxcast/files/MySQL% 20Database/Pipeline_Overview. pdf (2014)
- Ingle, Brandall L., et al. "Informing the Human Plasma Protein Binding of Environmental Chemicals by Machine Learning in the Pharmaceutical Space: Applicability Domain and Limits of Predictability." Journal of Chemical Information and Modeling 56.11 (2016): 2243-2252.
- Jamei, et al. "The Simcyp® population-based ADME simulator." Expert opinion on drug metabolism & toxicology 2009;5:211-223
- Kapraun, Dustin et al., "A Method for Identifying Prevalent Chemical Combinations in the US Population," Environmental Health Perspectives, 2017
- Kavlock, Robert, et al. "Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management." Chemical research in toxicology 25.7 (2012): 1287-1302.
- McNally, et al., "PopGen: a virtual human population generator." Toxicology 2014
- National Research Council. (1983). Risk Assessment in the Federal Government: Managing the Process Working Papers. National Academies Press.
- National Research Council. Toxicity testing in the 21st century: a vision and a strategy. National Academies Press, 2007.
- Park, Youngja, H., et al. "High-performance metabolic profiling of plasma from seven mammalian species for simultaneous environmental chemical surveillance and bioeffect monitoring." Toxicology 295:47-55 (2012)
- Pearce, Robert, et al. "httk: R Package for High-Throughput Toxicokinetics." Journal of Statistical Software, 2017
- Ring, Caroline, et al., "Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability", Environment International, 2017
- Schmidt, Charles W. "TOX 21: new dimensions of toxicity testing." Environmental health perspectives 117.8 (2009): A348.
- Sipes, Nisha, et al. (2017) "An Intuitive Approach for Predicting Potential Human Health Risk with the Tox21 10k Library", Environmental Science and Technology
- Strope, Cory L., et al. (2018) "High-throughput in-silico prediction of ionization equilibria for pharmacokinetic modeling." Science of The Total Environment
- Tonnelier, A., Coecke, S., & Zaldívar, J. M. (2012). Screening of chemicals for human bioaccumulative potential with a physiologically based toxicokinetic model. Archives of toxicology, 86(3), 393-403.
- Wambaugh, John F., et al. "High-throughput models for exposure-based chemical prioritization in the ExpoCast project." Environmental science & technology 47.15 (2013): 8479-848.
- Wambaugh, John F., et al. "High Throughput Heuristics for Prioritizing Human Exposure to Environmental Chemicals." Environmental science & technology (2014).
- Wetmore, Barbara A., et al. "Integration of dosimetry, exposure and high-throughput screening data in chemical toxicity assessment." Toxicological Sciences (2012): kfr254.
- Wetmore, Barbara A., et al., "Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing. Toxicological sciences 2014;142:210-224
- 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 (2015): 121-136.