EPA
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Environmental Protection
Agency

Exposure-Based Screening and Priority-Setting

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Presentation to the
National Science and Technology Council (NSTC)
Committee on Environment, Natural Resources, and Sustainability (CENRS)
Toxics and Risk Subcommittee

February 16, 2017

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Introduction

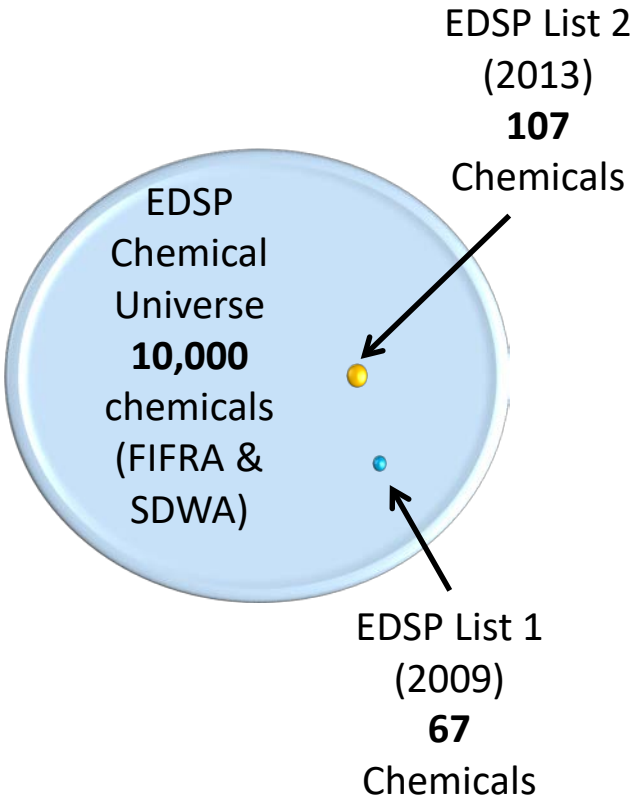
- The timely characterization of the human and ecological risk posed by thousands of existing and emerging commercial chemicals is a critical challenge facing EPA in its mission to protect public health and the environment
- Tools developed by EPA Exposure Forecasting "ExpoCast" project (co-leads Kristin Isaacs and John Wambaugh) inform chemical priority setting

November 29, 2014

Scale of the Problem

- Park *et al*. (2012): At least 3221 chemicals in humans, many appear to be exogenous

Endocrine Disruptor Screening Program (EDSP) Chemical List	Number of Compounds
Conventional Active Ingredients	838
Antimicrobial Active Ingredients	324
Biological Pesticide Active Ingredients	287
Non Food Use Inert Ingredients	2,211
Food Use Inert Ingredients	1,536
Fragrances used as Inert Ingredients	1,529
Safe Drinking Water Act Chemicals	3,616
TOTAL	**10,341**



So far 67 chemicals have completed testing and an additional 107 are being tested

December, 2014 Panel: "Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening" DOCKET NUMBER: EPA–HQ–OPP–2014–0614

EPA United States Environmental Protection Agency

New NAS Report

Using 21st Century Science to Improve Risk-Related Evaluations

The National Academies of

SCIENCES · ENGINEERING · MEDICINE

THE NATIONAL ACADEMIES PRESS

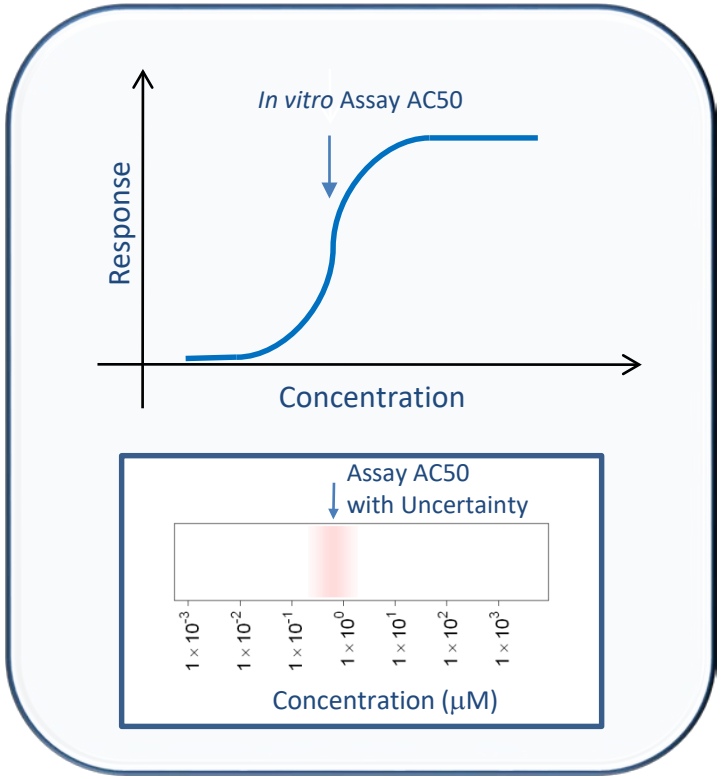
Washington, DC

www.nap.edu

"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 (see Chapter 1), and in high-throughput computational exposure assessment (Wambaugh et al. 2013, 2014) have enabled first-tier risk-based rankings of chemicals on the basis of margins of exposure"

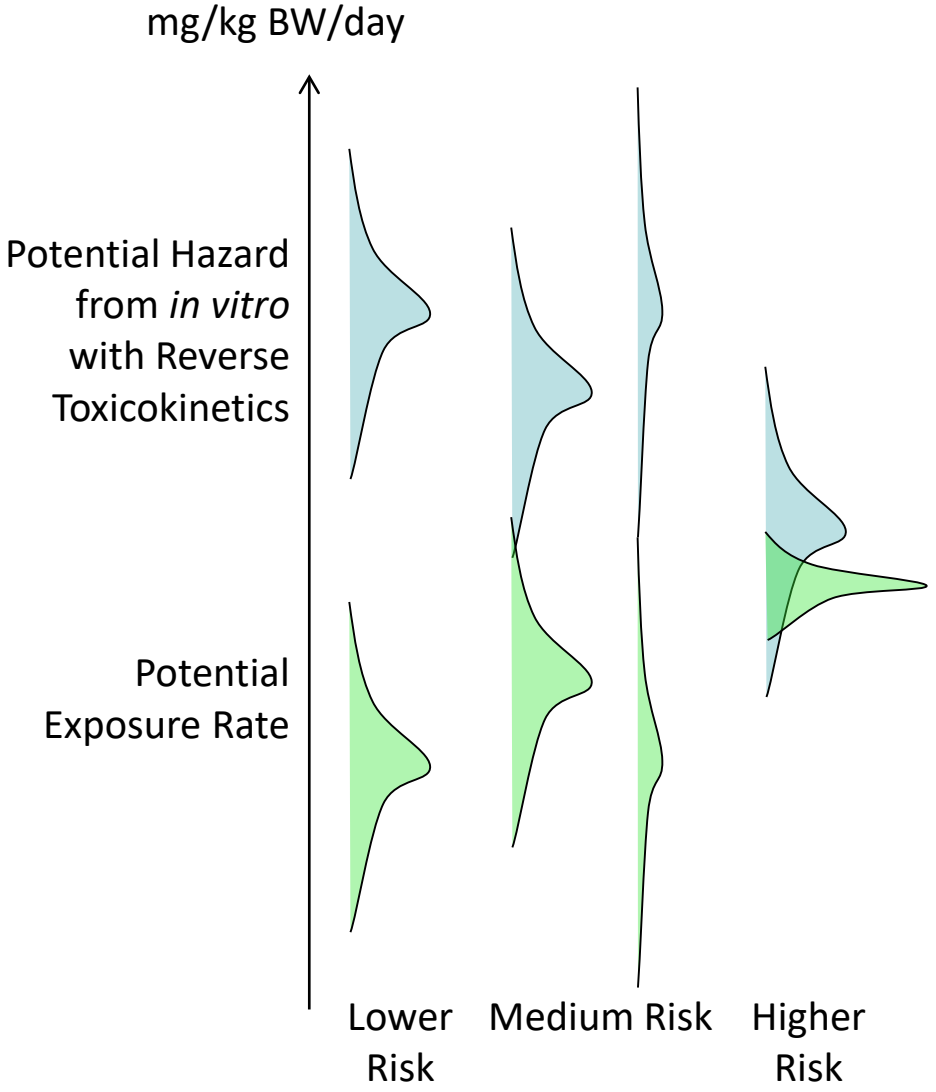
High-Throughput Bioactivity Screening

- **Tox21**: Examining >10,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)
- **ToxCast** : For a subset (>1000) of Tox21 chemicals ran >500 additional assays (Judson *et al.*, 2010)
- 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://actor.epa.gov/

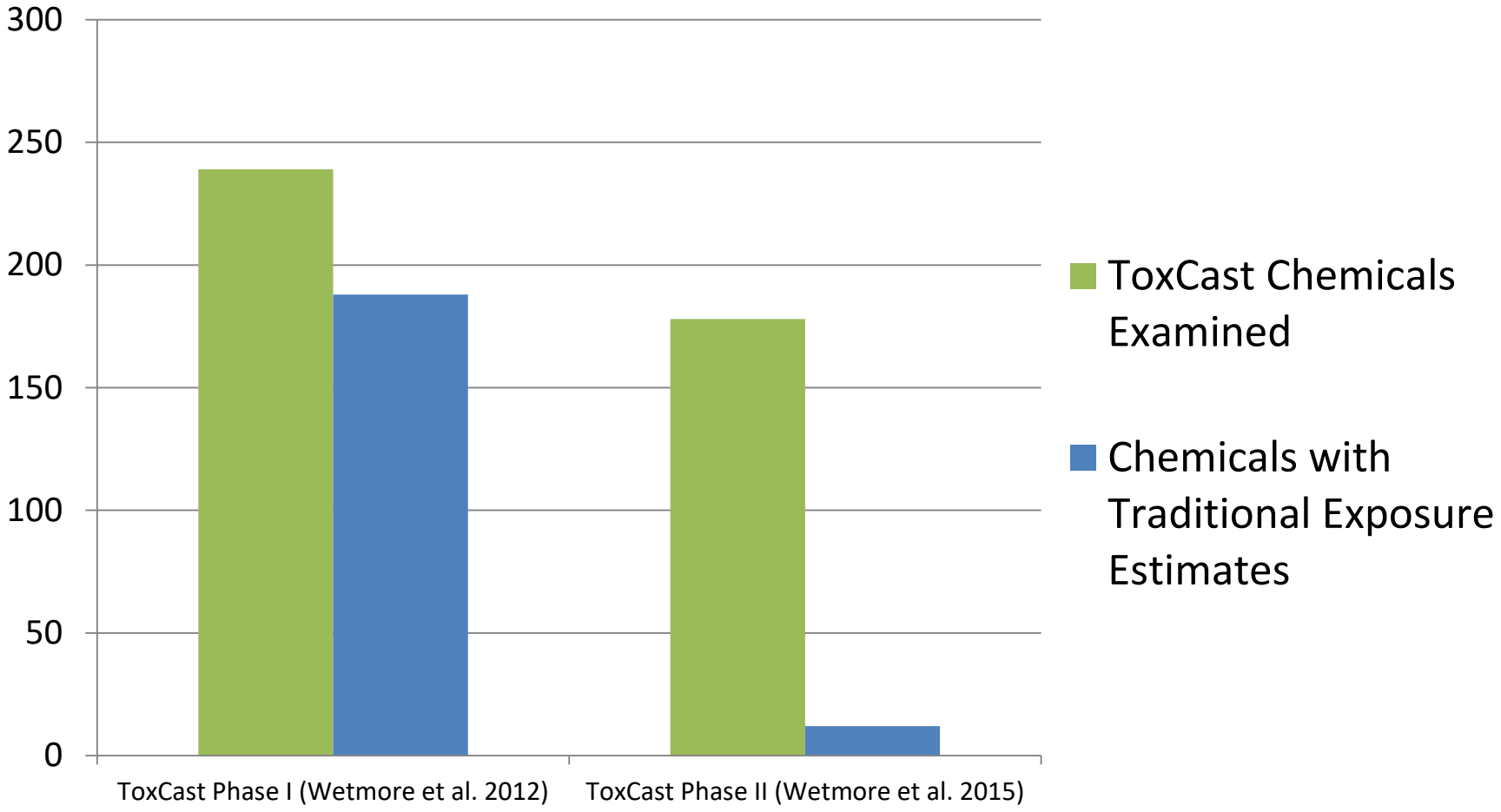


High Throughput Risk Prioritization

- **High throughput risk prioritization** needs:
  1. high throughput **hazard** characterization (e.g., ToxCast, Tox21)
  2. high throughput **exposure** forecasts
  3. high throughput **toxicokinetics** (*i.e.*, dosimetry)



Available Data for Exposure Estimations



- Egeghy et al. (2012) – Most chemicals lack exposure data
- We need high throughput exposure models

The Need for *In Vitro* Toxicokinetics

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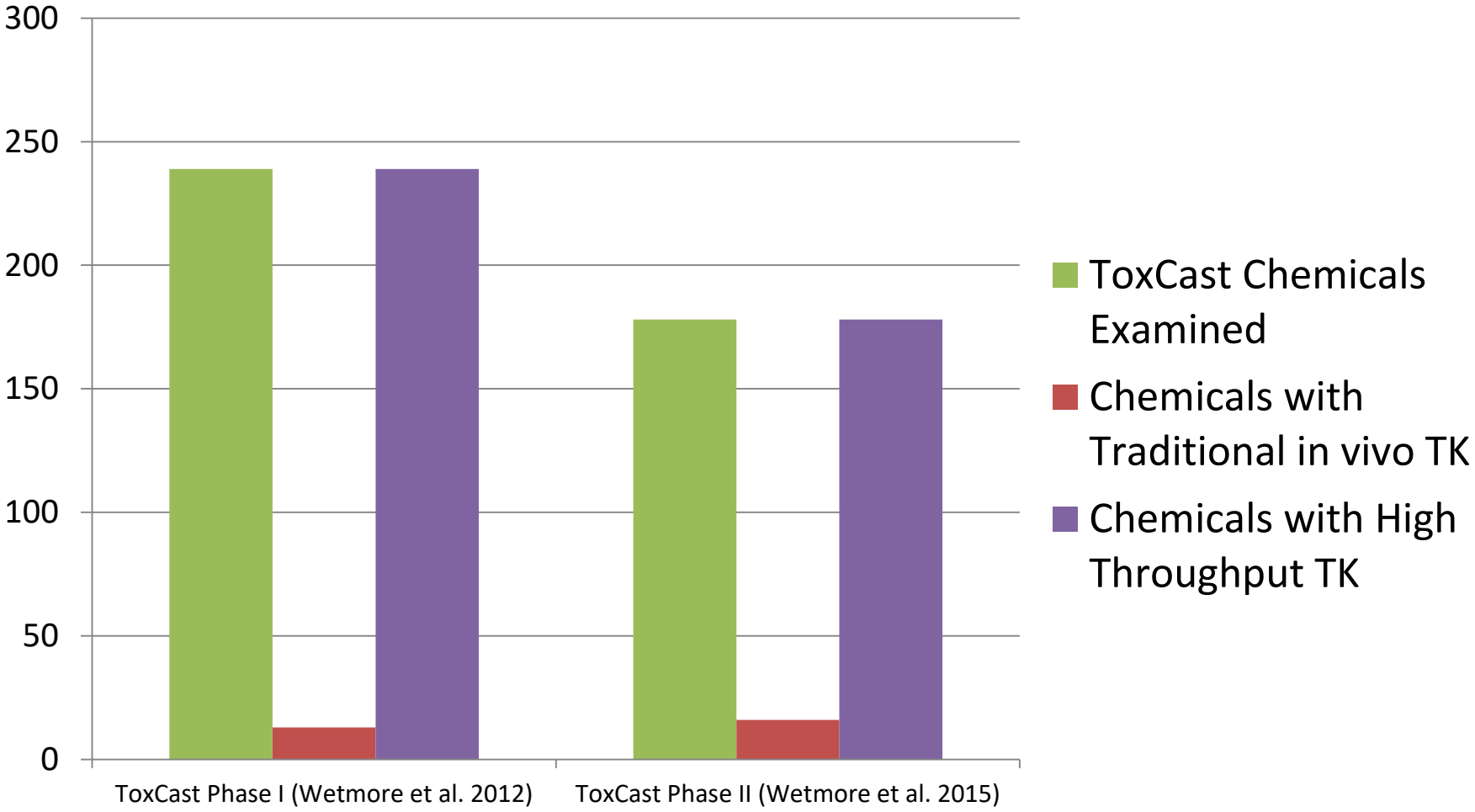
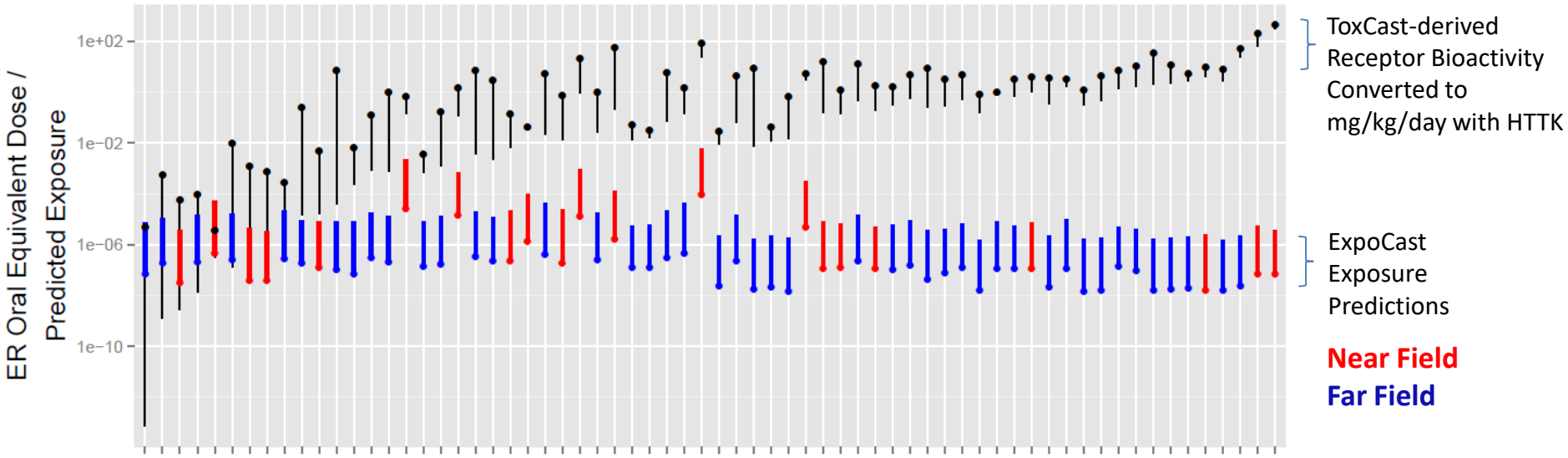


- We need high throughput toxicokinetics (HTTK)
- Studies like Wetmore et al. (2012,2015), address the need for TK data using *in vitro* methods

High Throughput Risk Prioritization in Practice



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

- July and December EPA FIFRA Scientific Advisory Panels reviewed ExpoCast research as it applies to the Endocrine Disruptor Screening Program

Thinking About Exposure

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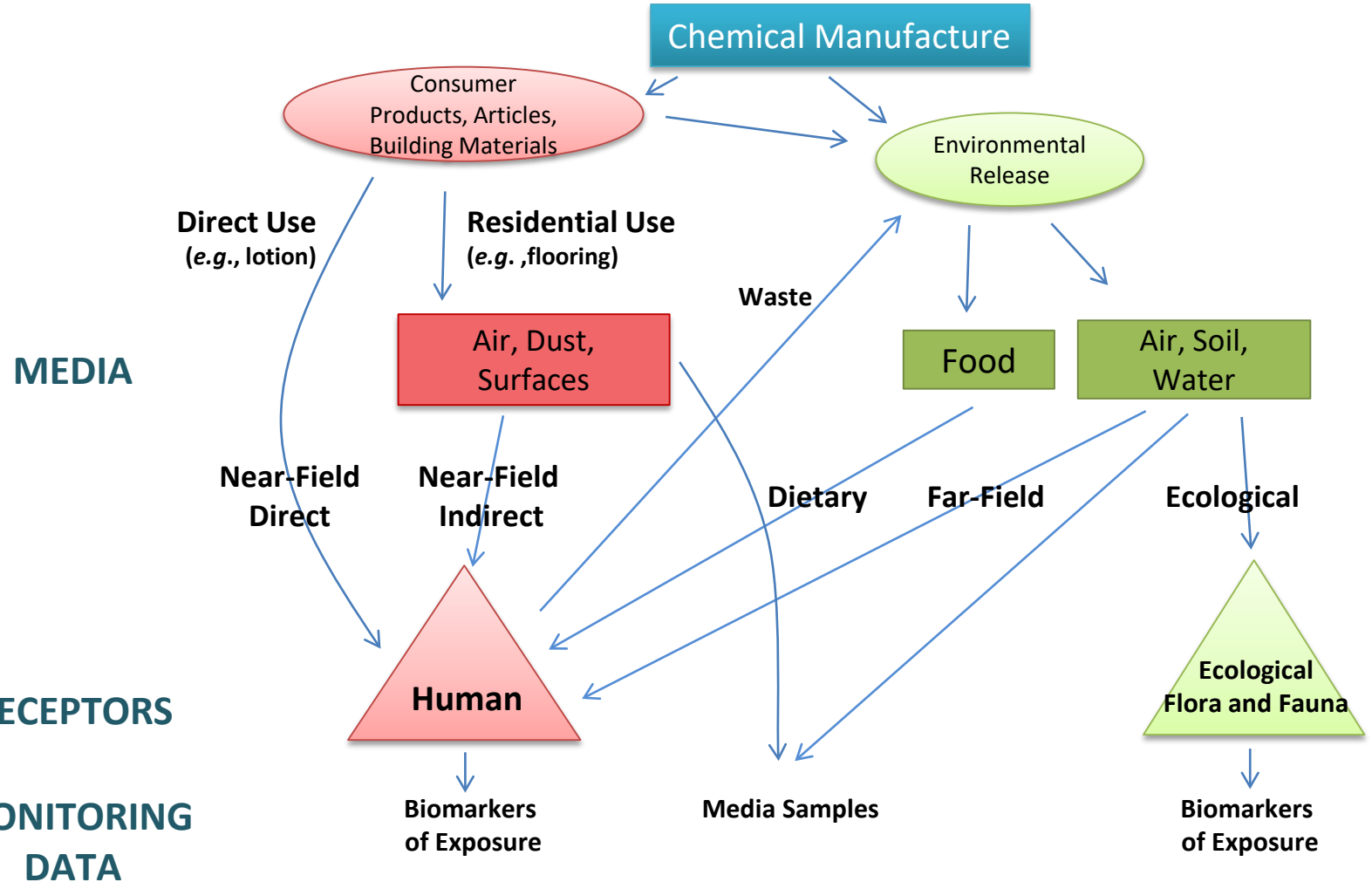


Figure from Kristin Isaacs

Exposure Pathways

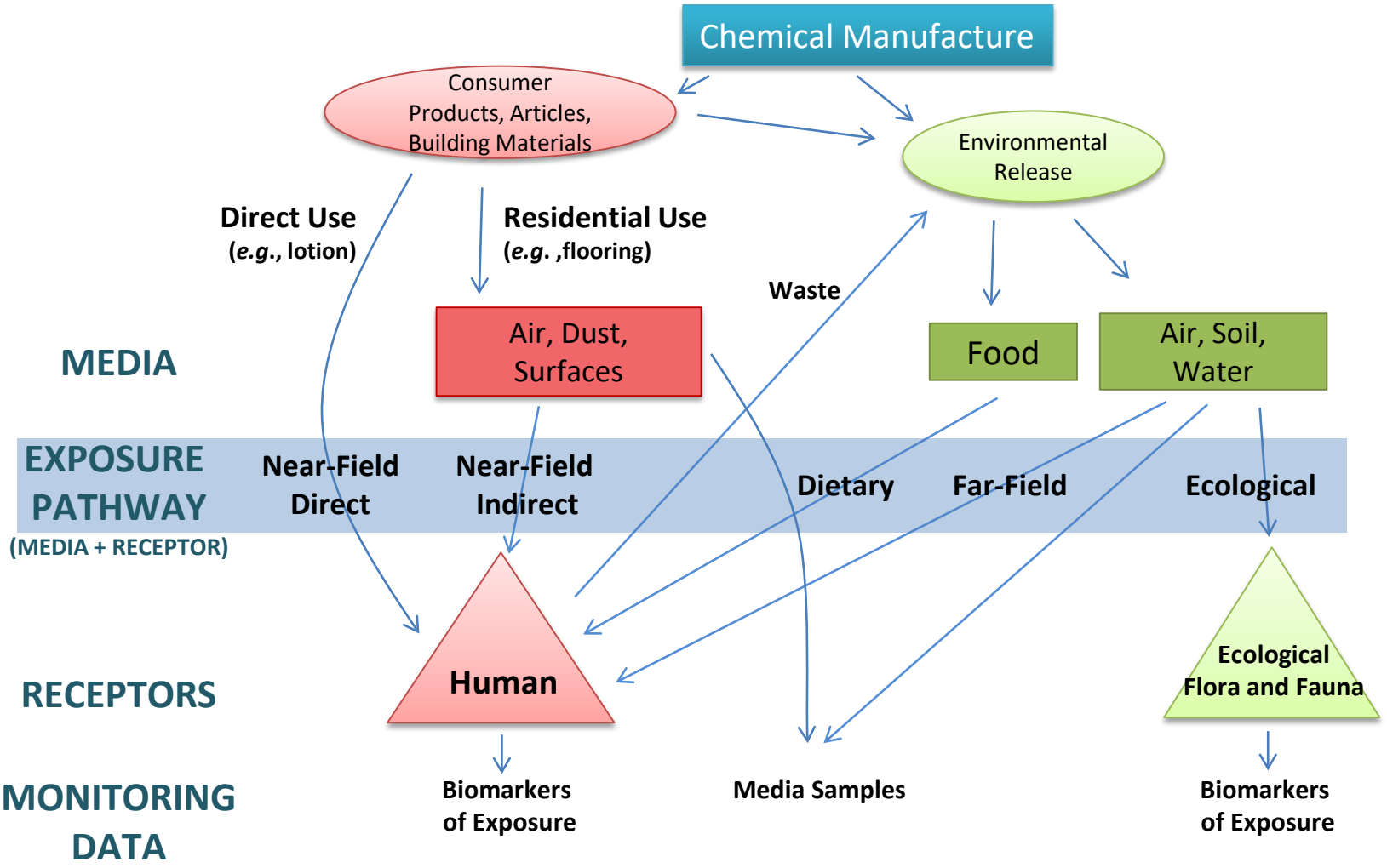
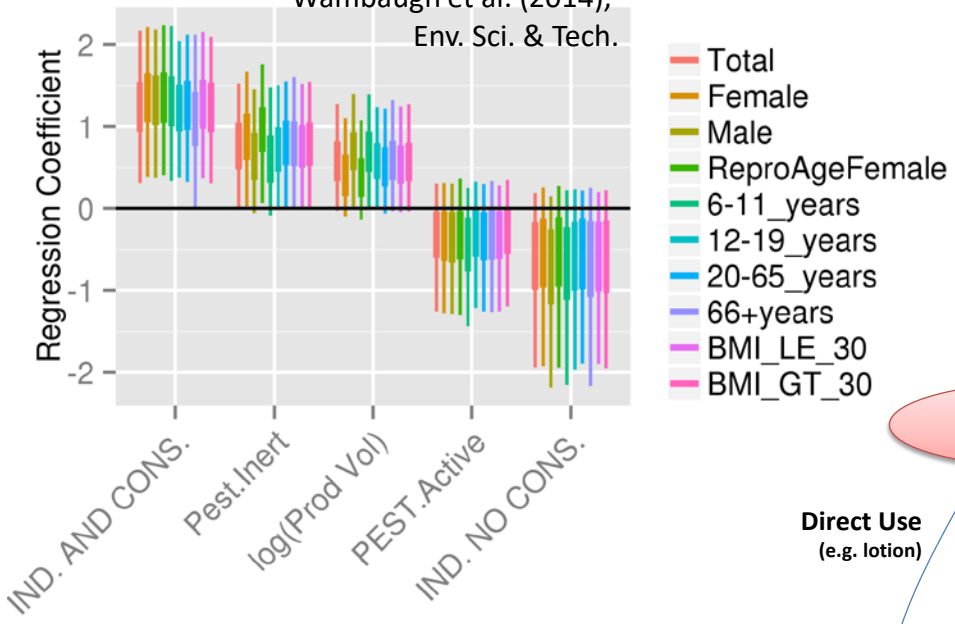


Figure from Kristin Isaacs

Chemical Use Identifies Relevant Pathways

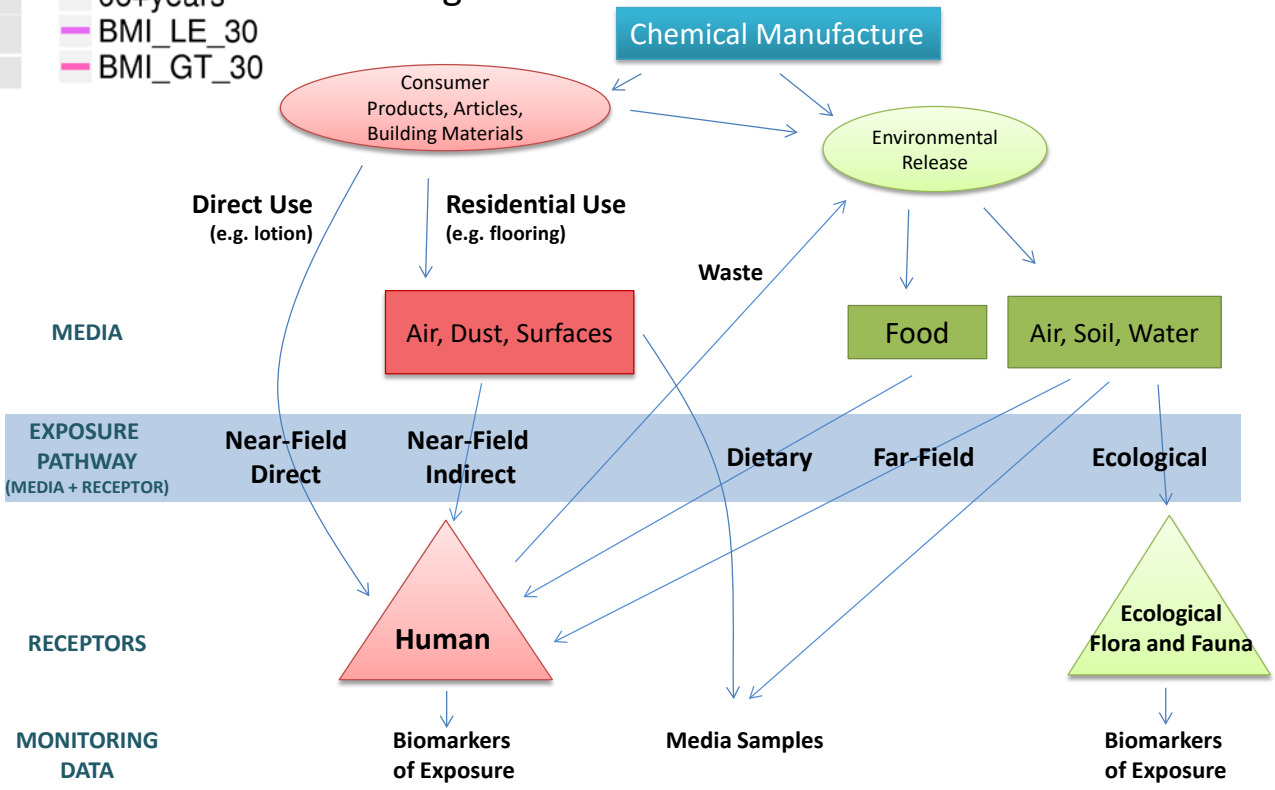
United States Environmental Protection Agency

Wambaugh et al. (2014), Env. Sci. & Tech.



…and some pathways have much higher average exposures!

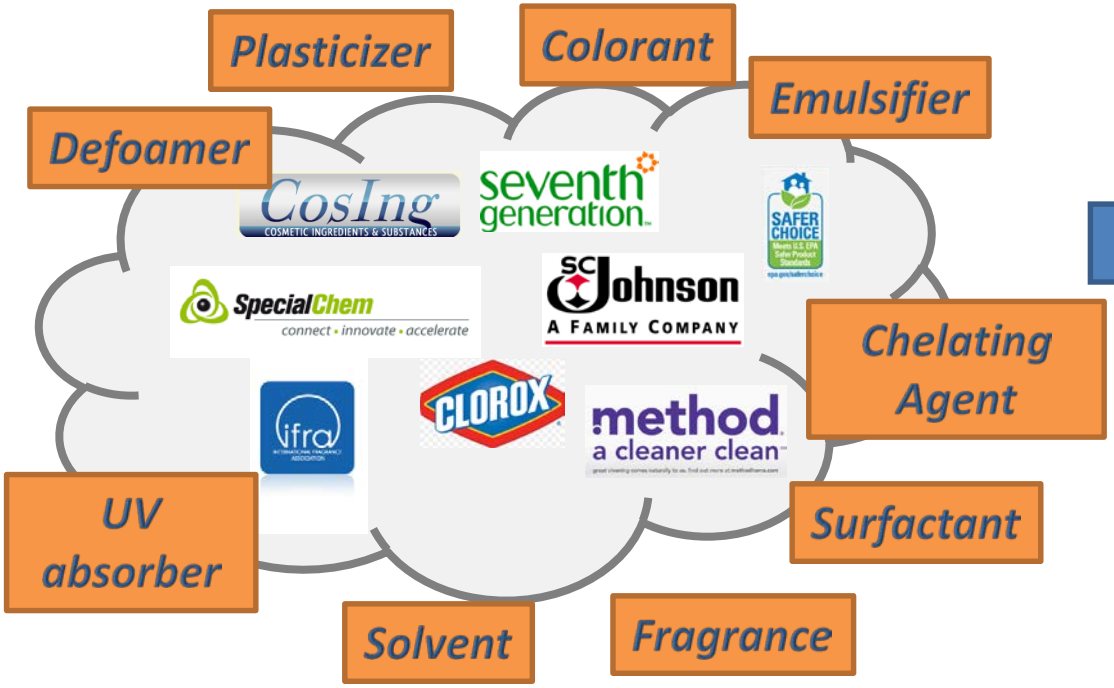
In particular, NHANES biomonitoring indicates exposures to consumer product chemicals are highest



SHEDS-HT (High Throughput Stochastic Human Exposure Dose Simulation Model) simulates human exposure in the indoor environment
(Isaacs et al. (2014), Env. Sci. & Tech.)

Databases and Models for Predicting Function of Chemicals

>2000 chemicals with Material Safety Data Sheets (MSDS) in CPCPdb (Goldsmith *et al.* (2014), Food Chem. Tox.)



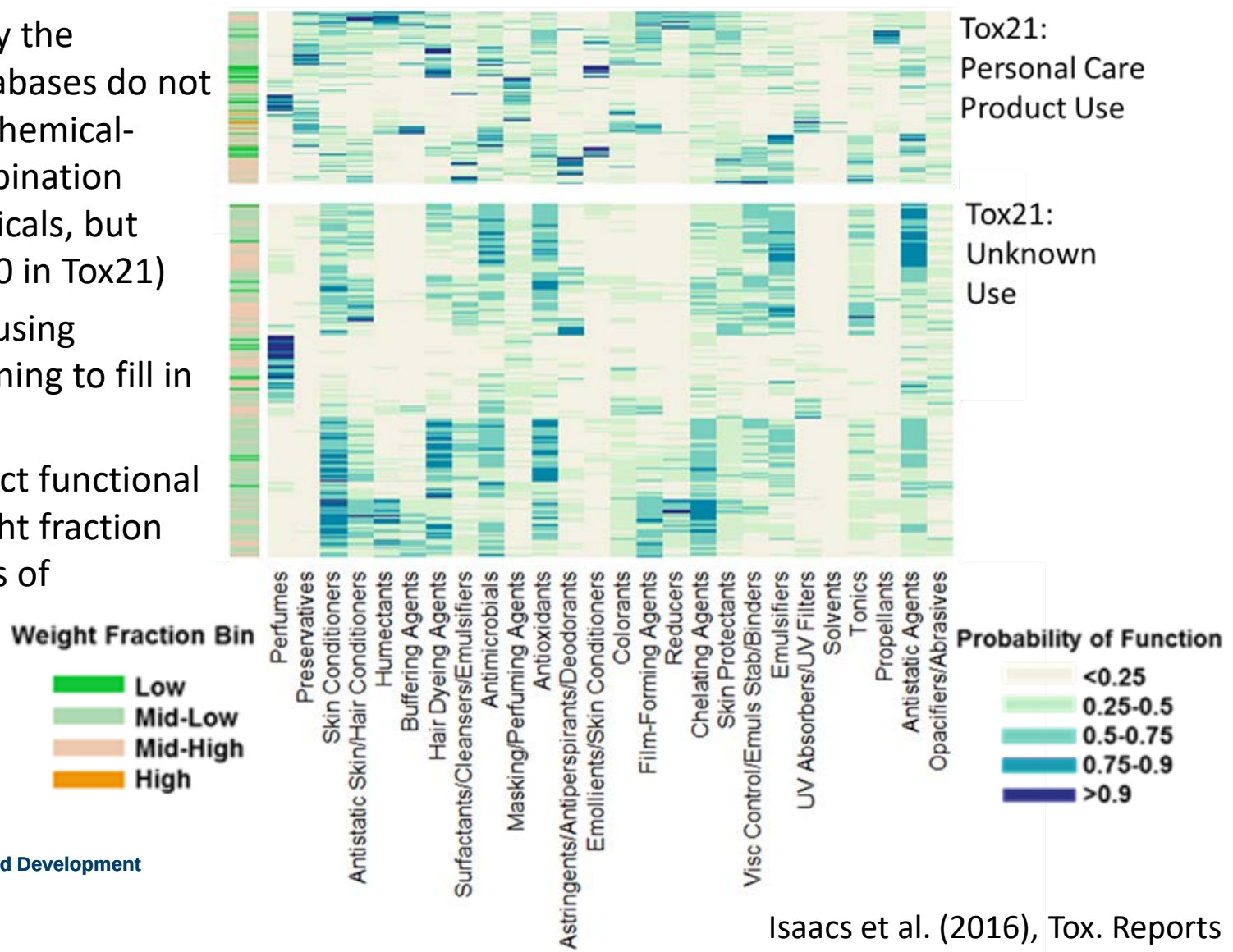
Functional Use (FUse) Dataset
14,000+ Chemicals
200+ Functions

Allows for Modeling of Function in Terms of Chemical Properties or Structures

Isaacs et al. (2016), Tox. Reports

Predicting Whether Chemicals Are in Consumer Products

- Unfortunately the available databases do not cover every chemical-product combination (~2000 chemicals, but already >8000 in Tox21)
- We are now using machine learning to fill in the rest
- We can predict functional use and weight fraction for thousands of chemicals



Isaacs et al. (2016), Tox. Reports

Obtaining New Data

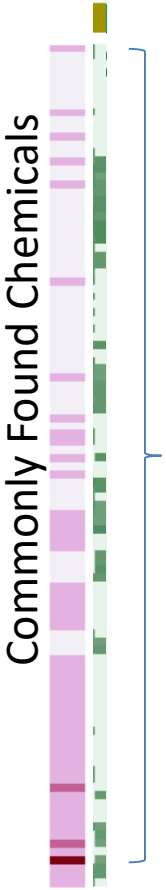
- Emphasis on suspect screening and non-targeted analysis mass spectrometry
- Ongoing ExpoCast contract consumer product scanning and blood sample monitoring
- EPA has developed significant in house capabilities
  - Published on analysis of house dust from American homes – can identify many of the most prevalent chemicals but only 2% overall, *Rager et al. (2016)*

"I'm searching for my keys."

- EPA is coordinating a comparison of non-targeted screening workflows used by leading academic and government groups using known chemical mixtures (ToxCast) and standardized environmental/biological samples (led by Jon Sobus and Elin Ulrich)

ExpoCast Consumer Product Scan

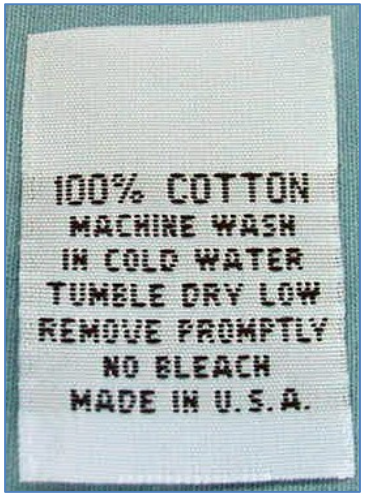
United States Environmental Protection Agency



Scanned 5 examples each of 20 class of consumer products

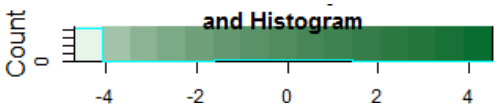
Found >3500 chemicals in total across the 100 products

The chemicals found in a cotton shirt



GC-MS with DCM Extraction

- Common Chemical (n>19)
- ToxCast
- Flame Retardant
- Potent ER



Log_{10} (µg/g)

- Air freshener
- Baby soap
- Carpet
- Carpet padding
- Cereals
- Cotton clothing
- Deodorant
- Fabric upholstery
- Glass cleaners
- Hand soap
- Indoor house paint
- Lipstick
- Plastic children's toys
- Shampoo
- Shaving cream
- Shower curtain
- Skin lotion
- Sunscreen
- Toothpaste
- Vinyl upholstery

Phillips et al. (in preparation)

ExpoCast Consumer Product Scan

United States Environmental Protection Agency

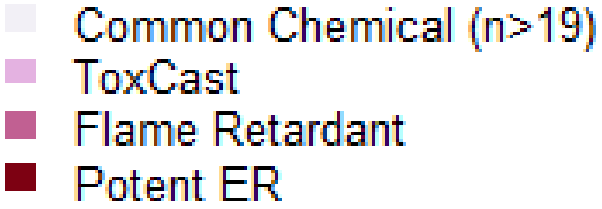
Scanned 5 examples each of 20 class of consumer products

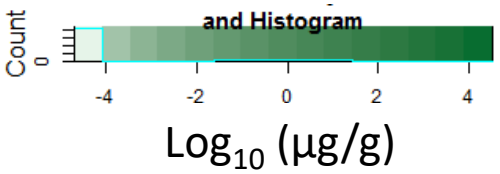
Found >3500 chemicals in total across the 100 products

Dark green is a high concentration

Light green is not detected

GC-MS with DCM Extraction





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ExpoCast Consumer Product Scan

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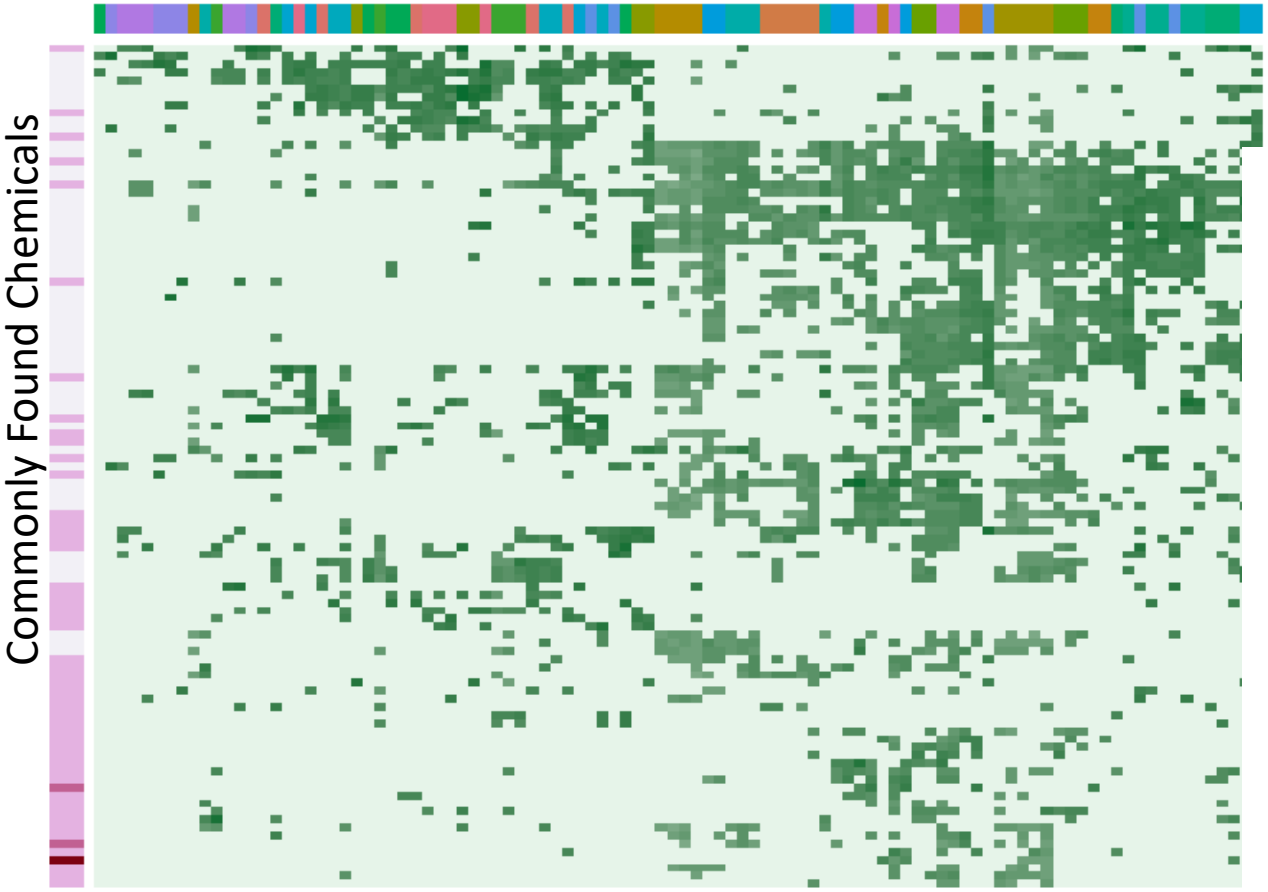

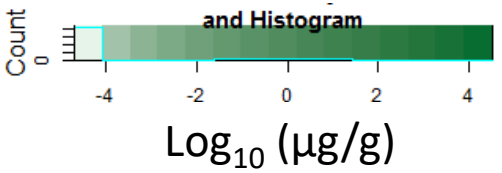

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GCXGC-MS with DCM Extraction

- Common Chemical (n>19)
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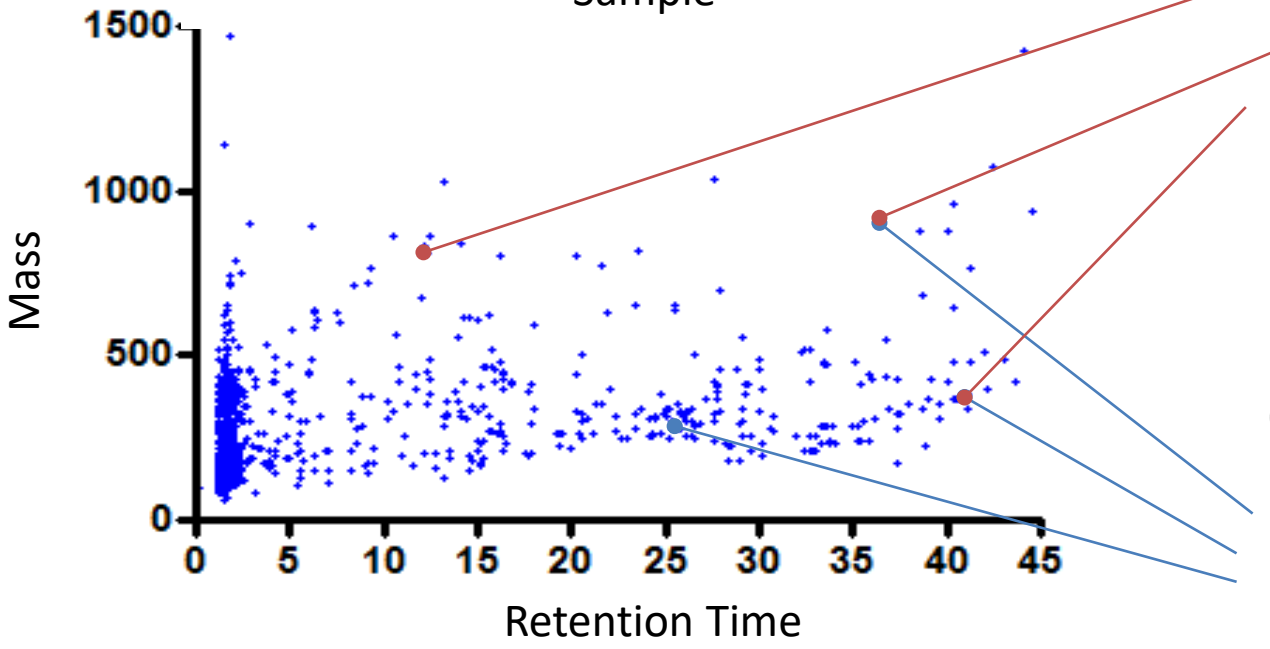
- Potent ER

Phillips et al. (in preparation)

Suspect Screening and Non-Targeted Analytical Chemistry

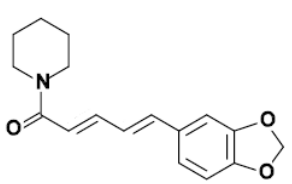
947 Peaks in an American Health Homes Dust Sample

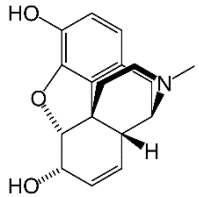


Each peak corresponds to a chemical with an accurate mass and predicted formula:

$C_{17}H_{19}NO_3$

Multiple chemicals can have the same mass and formula:





Is chemical A present, chemical B, both, or some other chemical (neither)?

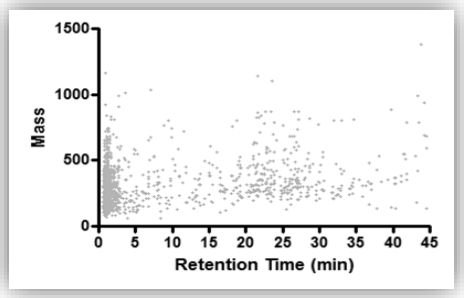
We are expanding our reference libraries using ToxCast chemicals to enable greater numbers and better accuracy of confirmed chemicals

Rager, et al. (2016), Environment International

Application:
HT Exposure "Forensics"

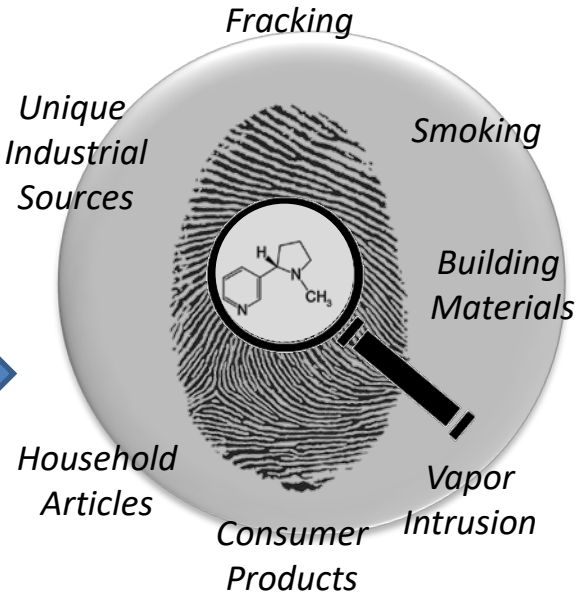
Environmental or Biological Sample

Molecular Features Identified by Non-Targeted Analyses



New Forensic Analysis Tools/Models

- Chemical Use Databases
- Data Mining and Machine Learning
- Chemical Structure Databases
- Analyzed Sample Archives
- Mass Spectra Databases



What sources are present?

What chemicals comprise the source fingerprint?

Can we identify new sources?

Figure from Kristin Isaacs

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Exposure-Based Screening and Priority-Setting

Using 21st Century Science to Improve Risk-Related Evaluations

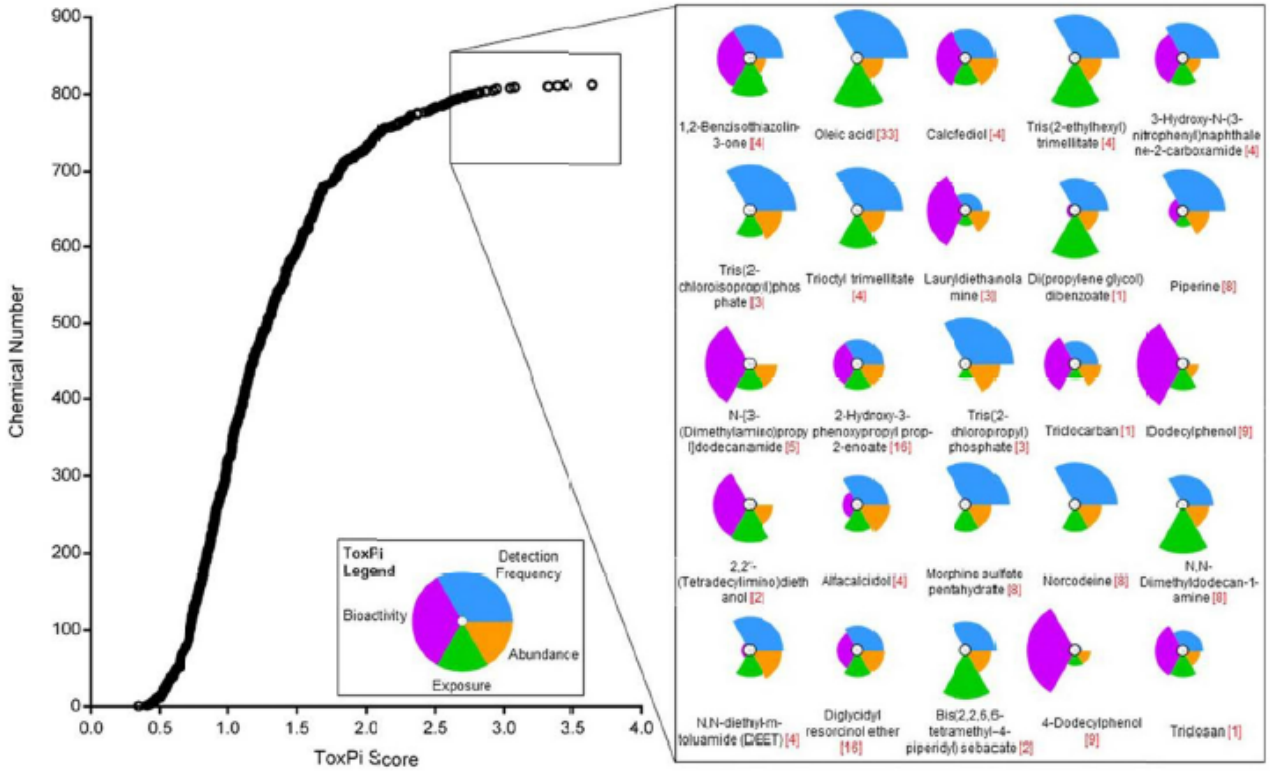
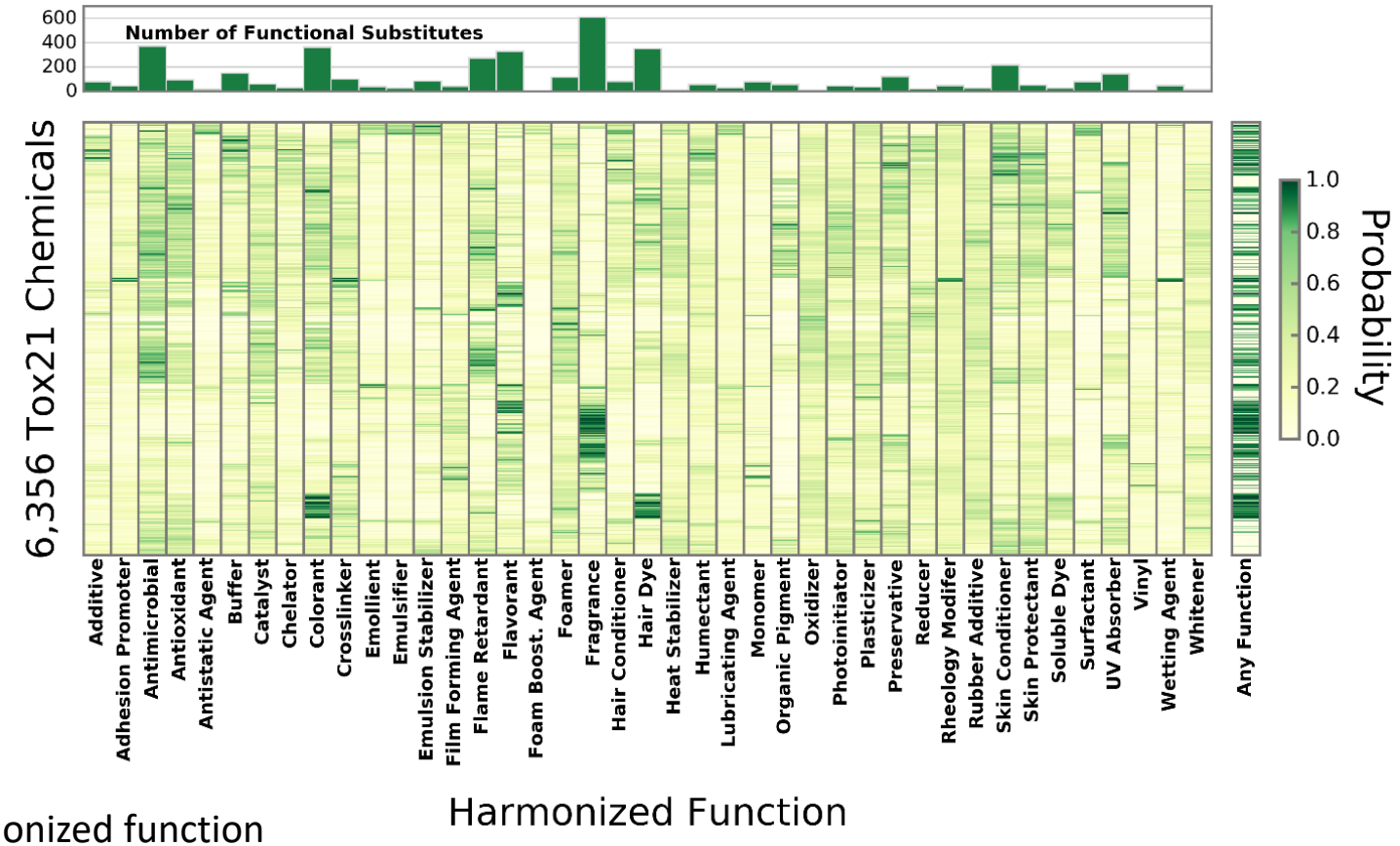


FIGURE 2-7 Data from nontargeted and targeted analysis of dust samples were used with toxicity data to rank chemicals for further analysis and testing. Source: Rager et al. 2016. Reprinted with permission; copyright 2016, *Environment International*.

Hazard and Functional Use Prediction Allows Searches for Chemical Alternatives

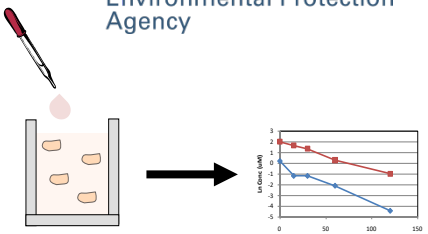
- Some chemicals with may have alternative uses and lesser bioactivity
- Dark green indicates a high probability of a chemical having a function
- The histogram above, indicates how many high-probability predictions were made for each harmonized function



Phillips et al. (2017), Green Chemistry

High-Throughput Toxicokinetics

United States Environmental Protection Agency



Human Hepatocytes (10 donor pool)

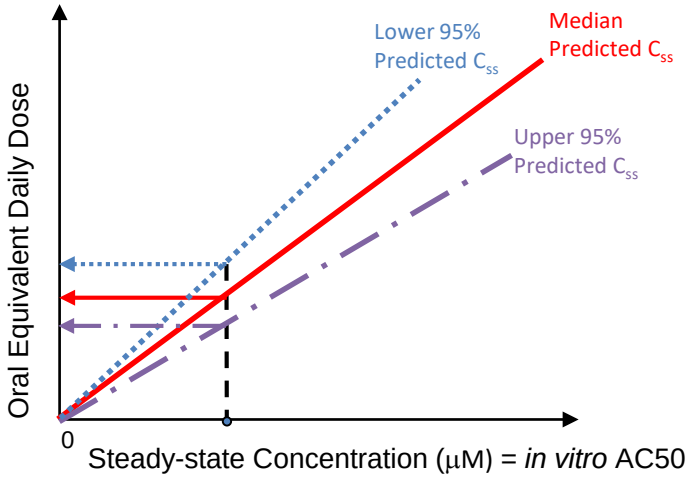
Hepatic Clearance

In Vitro - In Vivo Extrapolation

Steady State Blood Concentrations

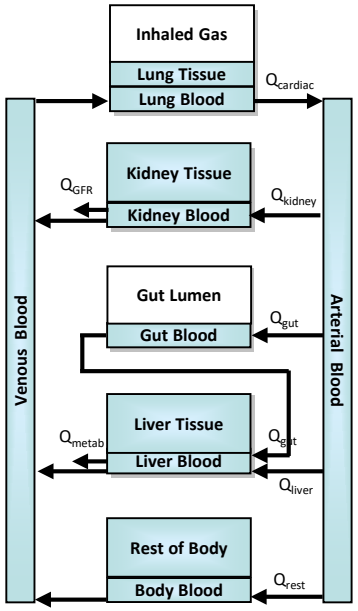
Human Plasma (6 donor pool)

Plasma Protein Binding



543 chemicals published to date

"httk" R Package publicly available



Open source *In Vitro-In Vivo* Extrapolation and Physiological-based Toxicokinetics (PBTK)

EPA United States Environmental Protection Agency

In Vitro - In Vivo Extrapolation (IVIVE)

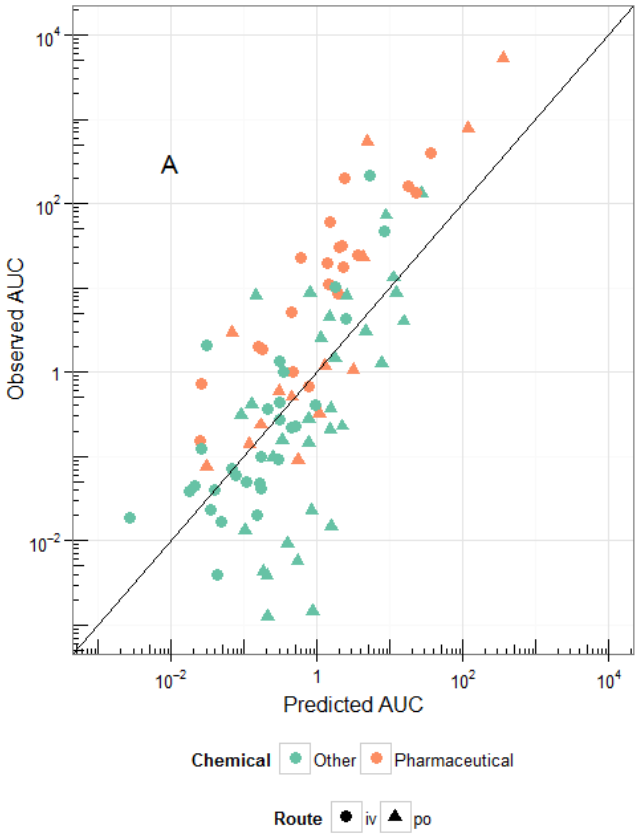
Definition:
IVIVE is the utilization of *in vitro* experimental data to predict phenomena in vivo

- 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

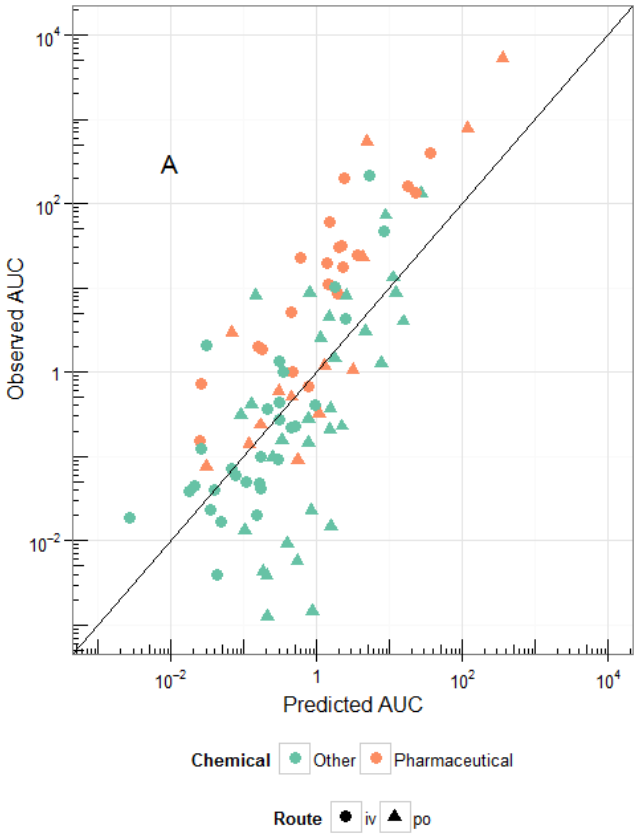
Evaluating *In Vitro* HTTK Predictions with *In Vivo* Data

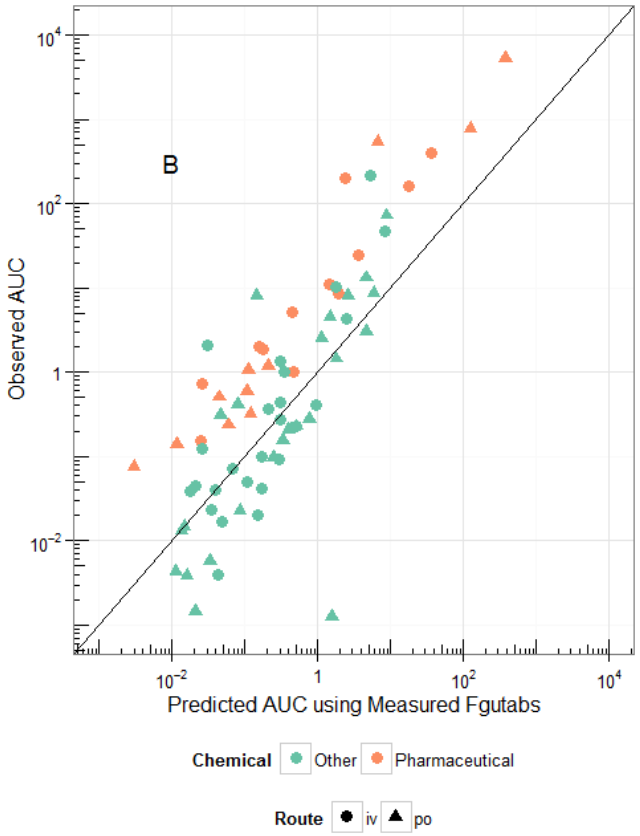


- Collected *in vitro* HTTK data for rat
- Conducted *in vivo* rat TK studies or 26 ToxCast compounds in rat
- Supplemented with published rat *in vivo* TK data (mostly pharmaceuticals)
- Can estimate
  - Fraction absorbed
  - Absorption Rate
  - Elimination Rate
  - Volume of Distribution

with Mike Hughes, Jane-Ellen Simmons, Carolin Ring, Tim Fennell (RTI, and Rusty Thomas

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





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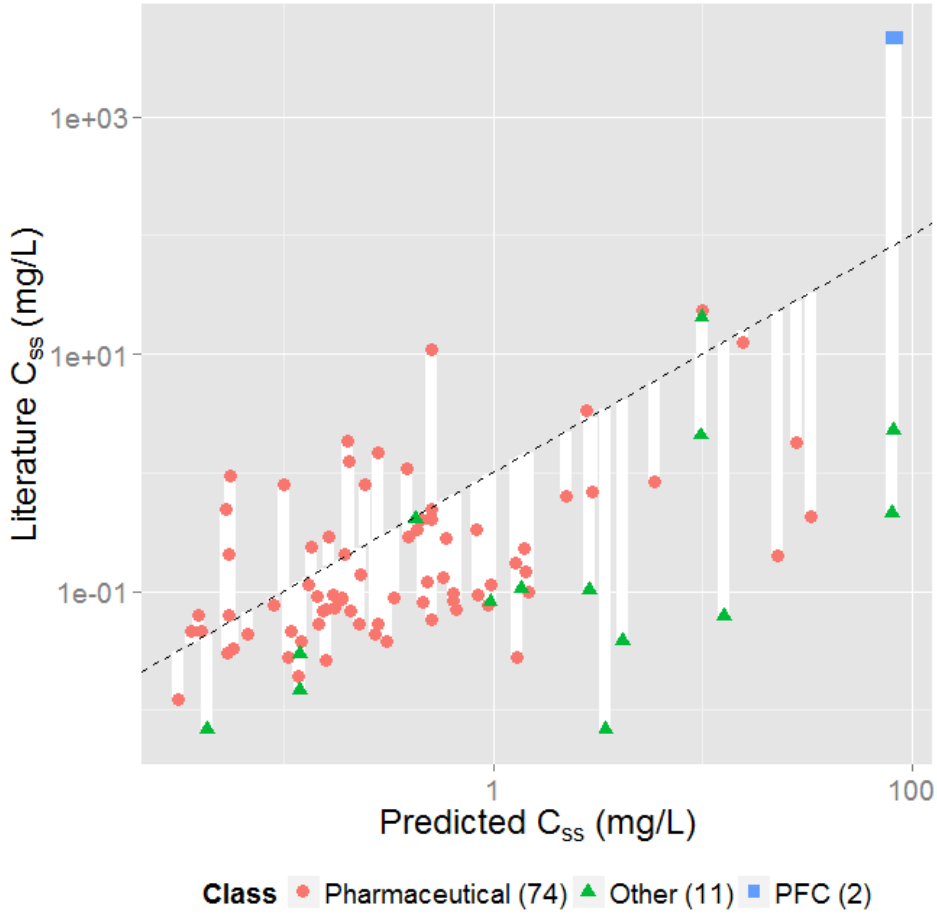
Now measuring bioavailability (CACO2) for all HTTK chemicals

with Mike Hughes, Jane-Ellen Simmons, Carolin Ring, Tim Fennell (RTI, and Rusty Thomas

Using *in vivo* Data to Evaluate RTK

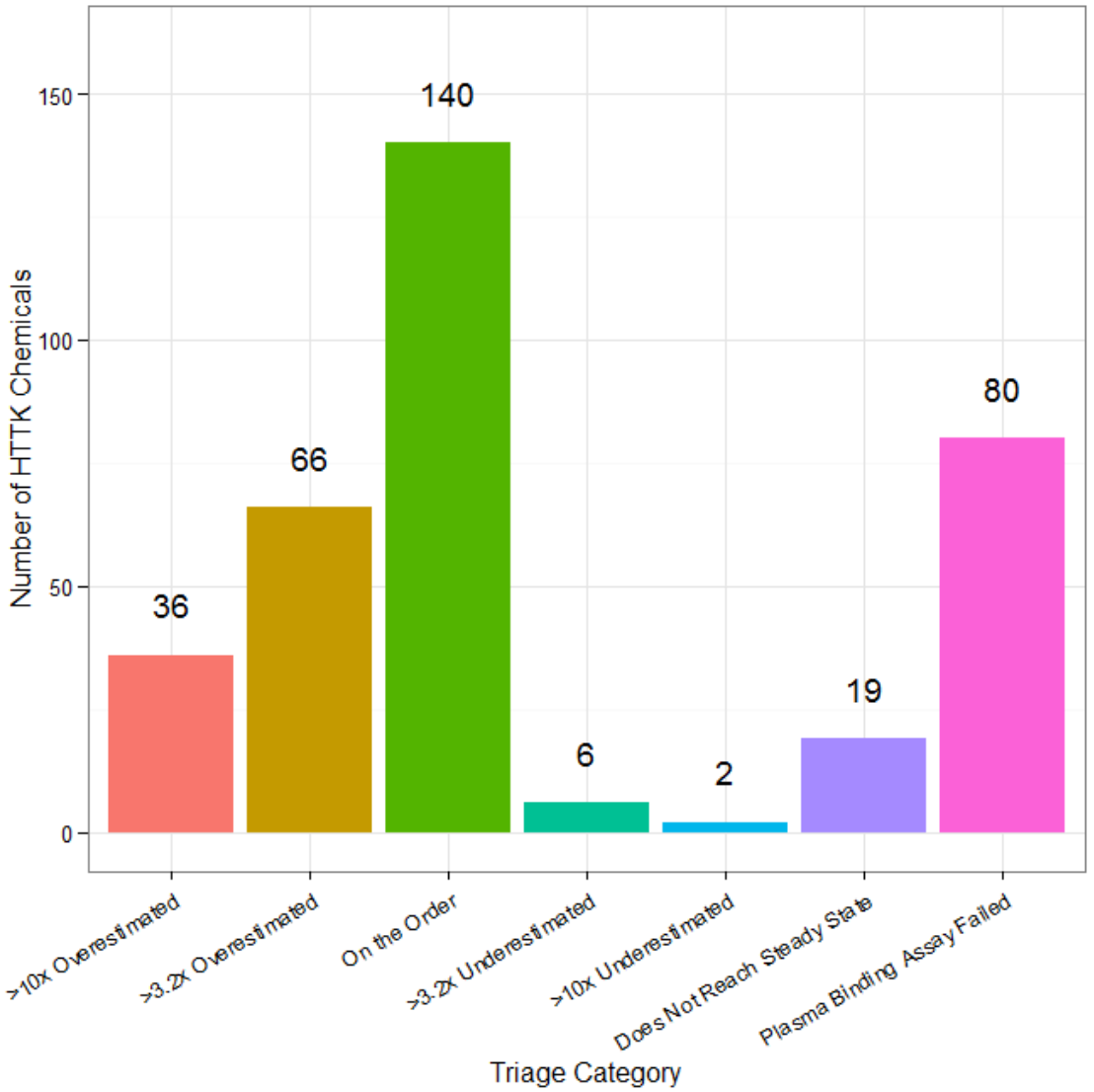


- When we compare the steady-state concentrations (C_{ss}) predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate the discrepancy between measured and predicted values (the residual)

Wambaugh et al. (2015), Tox. Sci.

Toxicokinetic Triage

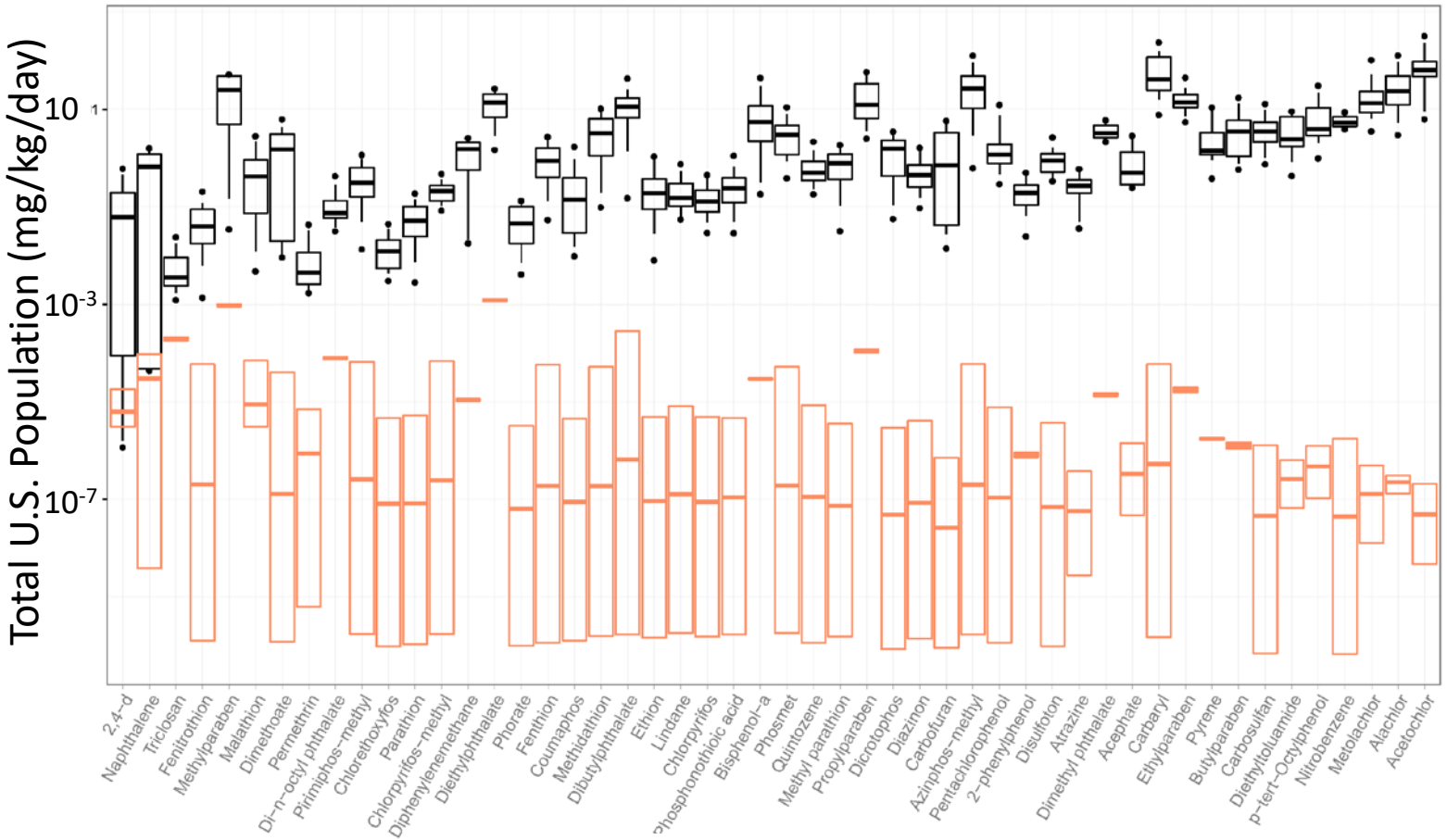
- Through comparison to *in vivo* data, a cross-validated (random forest) predictor of success or failure of HTTK has been constructed
- Add categories for chemicals that do not reach steady-state or for which plasma binding assay fails
- All chemicals can be placed into one of seven confidence categories



Wambaugh et al. (2015), Tox. Sci.

High Throughput Risk Prioritization in Practice

United States Environmental Protection Agency



Ring *et al.*, submitted

Monte Carlo Population simulator for HTTK

Sample NHANES quantities

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

Predict physiological quantities

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

Correlated sampling of physiological model parameters

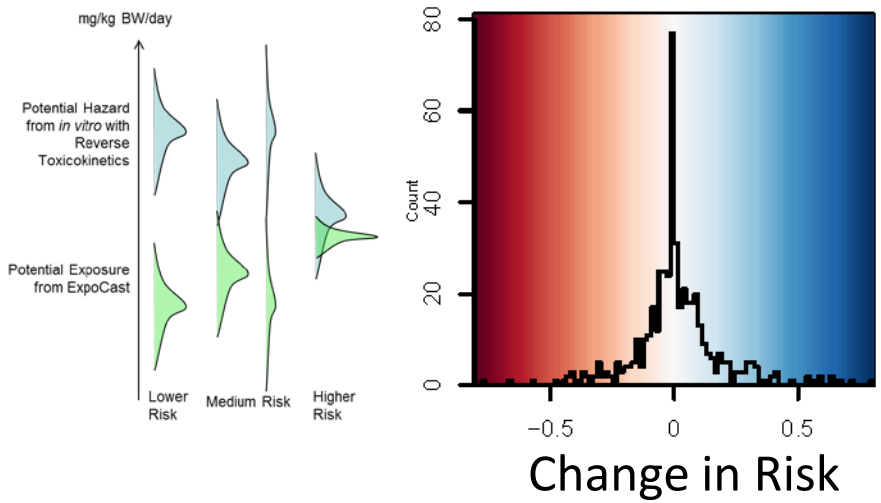
(Similar approach used in SimCYP [Jamei et al. 2009], GastroPlus, PopGen [McNally et al. 2014], P3M [Price et al. 2003], physB [Bosgra et al. 2012], etc.)

Ring *et al.*, submitted

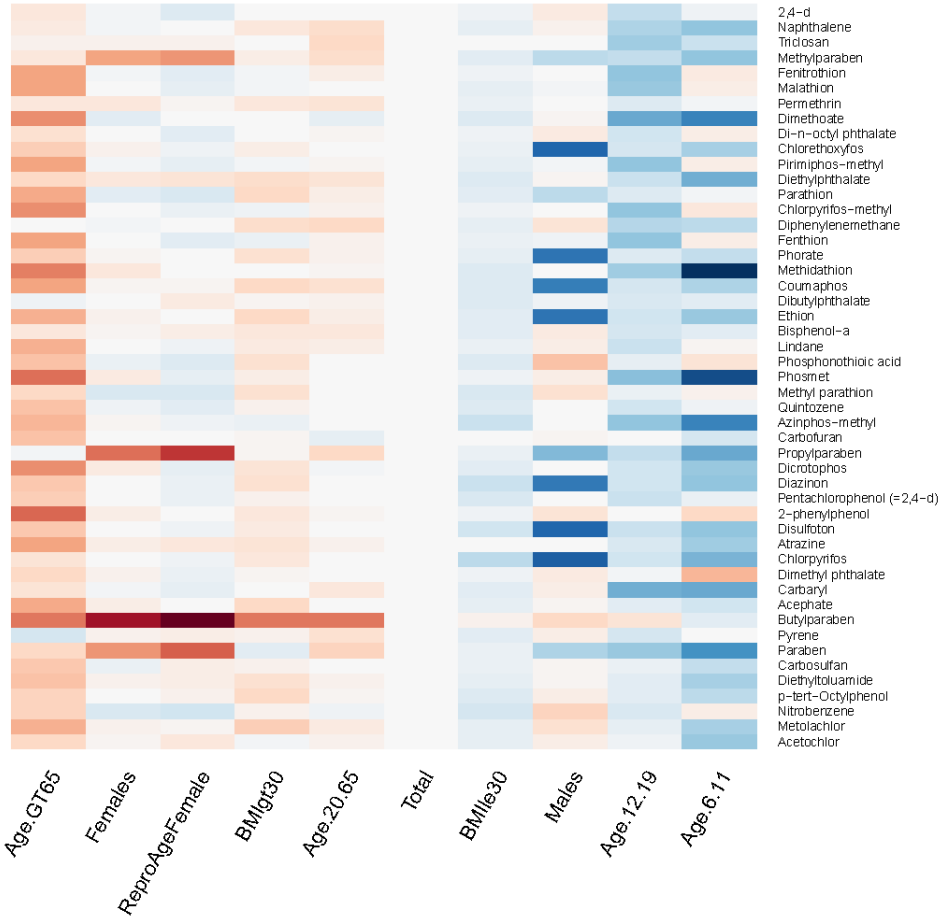
Life-stage and Demographic Specific Predictions

- Wambaugh *et al.* (2014) predictions of exposure rate (mg/kg/day) for various demographic groups

- Can use HTTK to calculate margin between bioactivity and exposure for specific populations



Change in Activity:Exposure Ratio

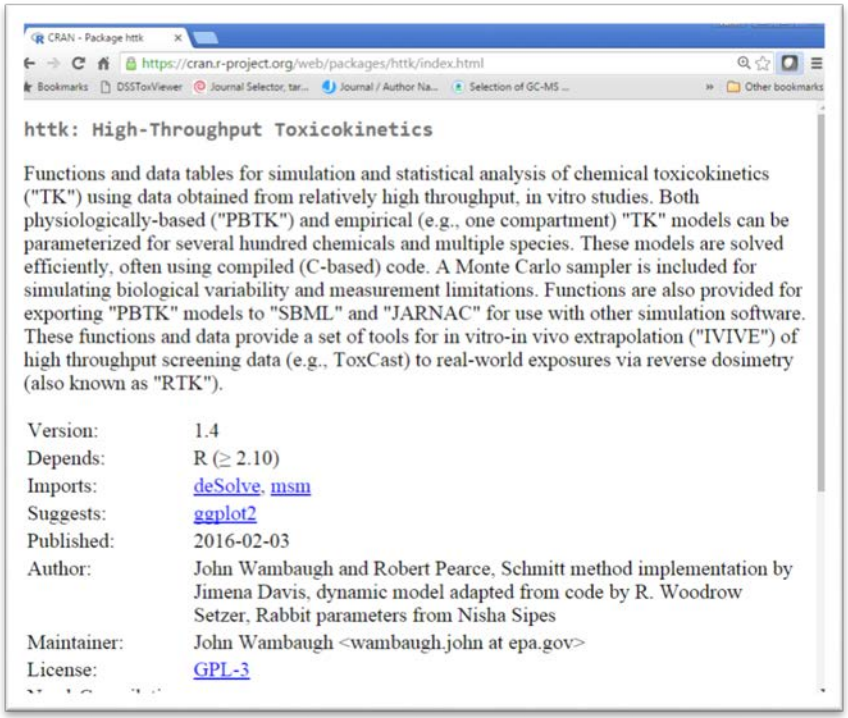


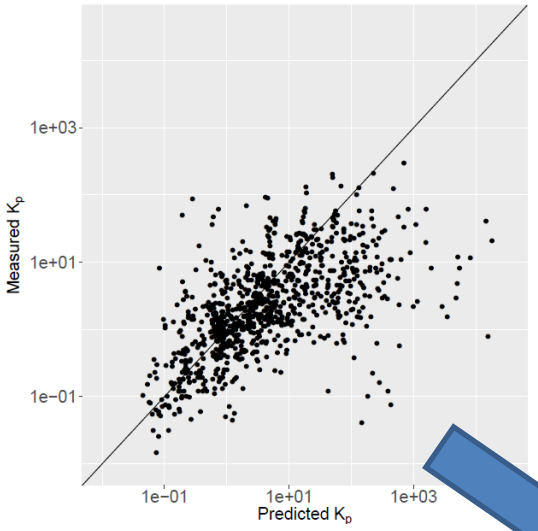
Ring *et al.*, submitted

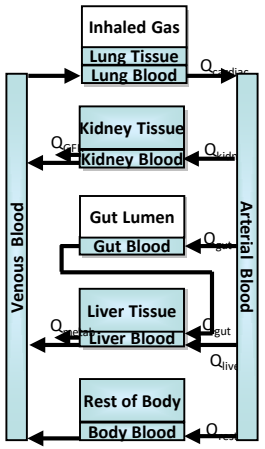
httk: An Public, Open Source Tool

United States Environmental Protection Agency

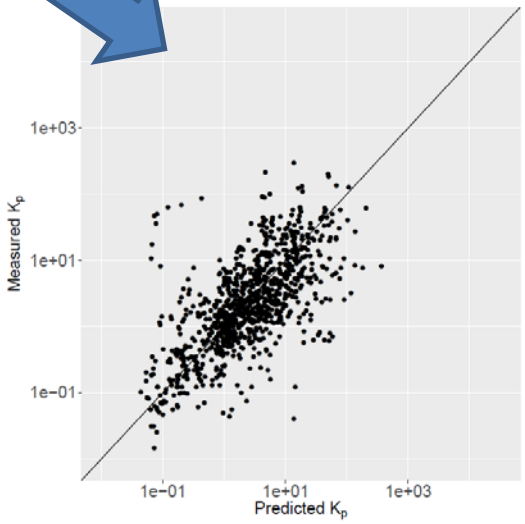
Old versions are archived







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



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

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

Can access this from the R GUI: "Packages" then "Install Packages"

Conclusion

- We would like to know more about the risk posed by thousands of chemicals in the environment – which are most worthy of further study?
  - High throughput screening (HTS) provides a path forward for identifying potential hazard
  - Exposure and dosimetry provide real world context to hazards indicated by HTS
- 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
- Using high throughput exposure approaches we can make coarse predictions of exposure
  - We are actively refining these predictions with new models and data
  - In some cases, upper confidence limit on current predictions is already many times lower than predicted hazard,

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

EPA United States Environmental Protection Agency

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