EPA
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Environmental Protection
Agency

High Throughput Assays for Exposure Science

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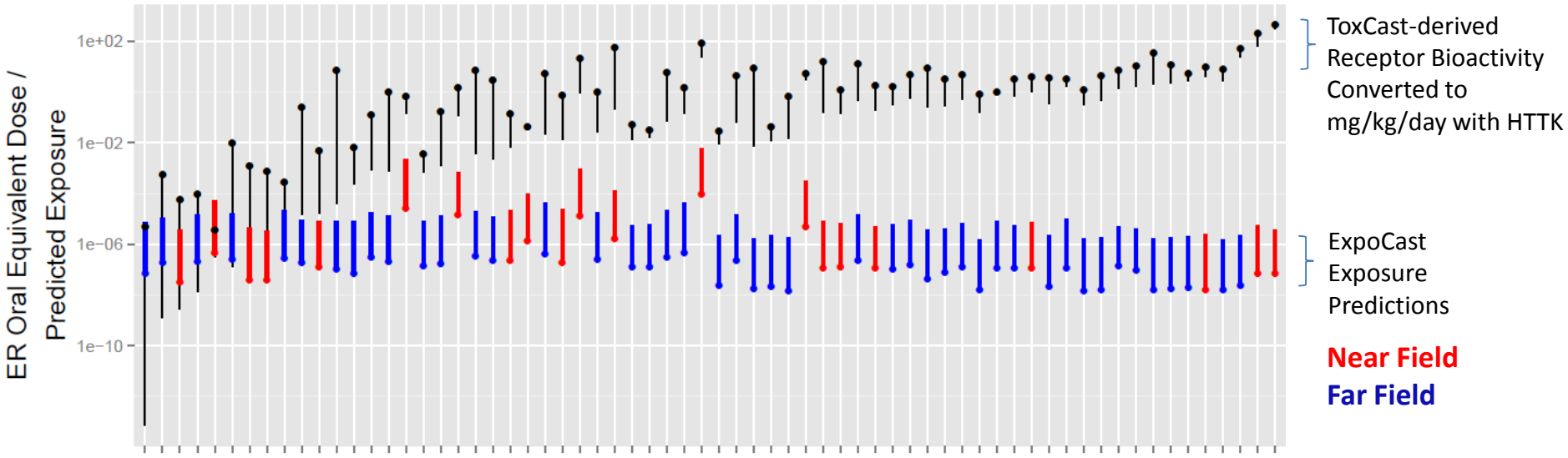
May 11, 2016

High Throughput Risk Prioritization in Practice



ToxCast Chemicals

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

Prioritization as in Wetmore *et al*. (2015) Bioactivity, Dosimetry, and Exposure Paper

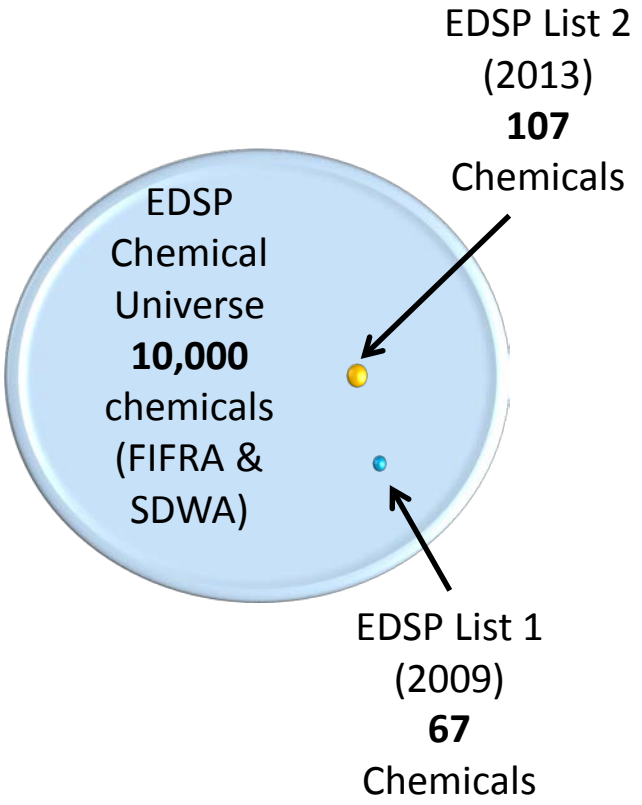
Rapid exposure and dosimetry project helps establish exposure context for ToxCast high throughput screening

Led by Kristin Isaacs and John Wambaugh

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**

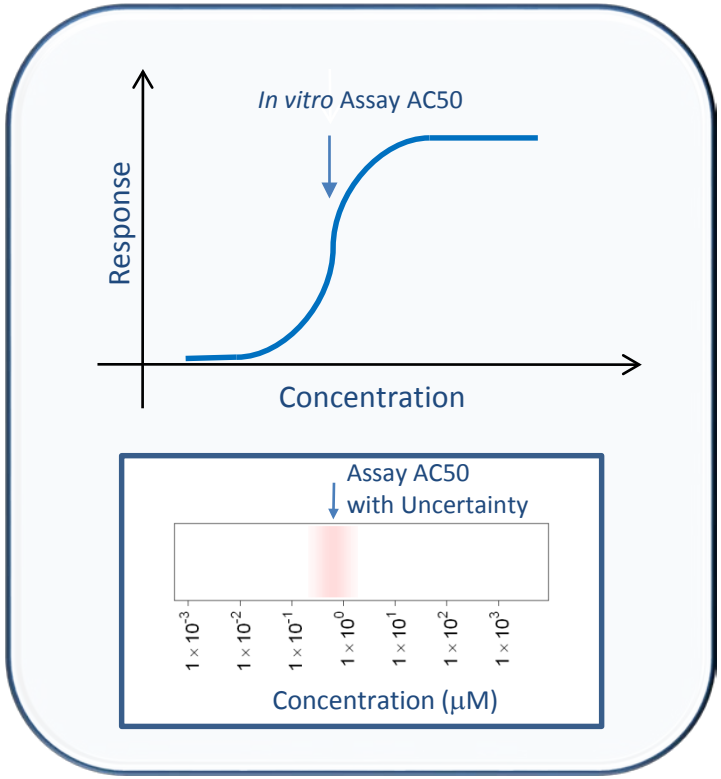


So far 67 chemicals have completed testing and an additional 107 are being tested

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

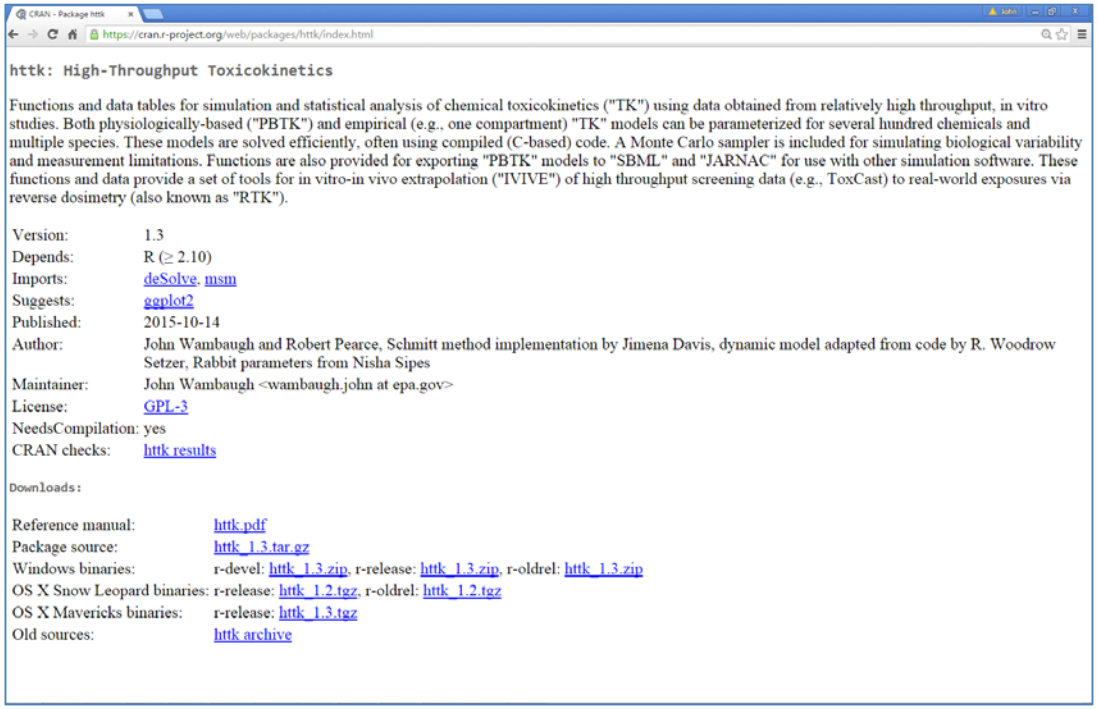
High-Throughput Bioactivity

- **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)
- All data is public: http://actor.epa.gov/



High-Throughput Toxicokinetics

United States Environmental Protection Agency



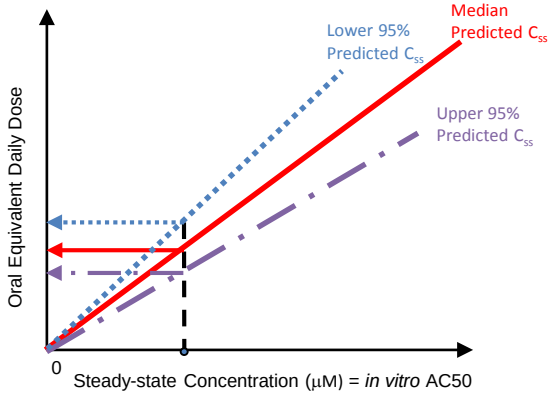
httk: High-Throughput Toxicokinetics

Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") using data obtained from relatively high throughput, in vitro studies. Both physiologically-based ("PBTK") and empirical (e.g., one compartment) "TK" models can be parameterized for several hundred chemicals and multiple species. These models are solved efficiently, often using compiled (C-based) code. A Monte Carlo sampler is included for simulating biological variability and measurement limitations. Functions are also provided for exporting "PBTK" models to "SBML" and "JARNAC" for use with other simulation software. These functions and data provide a set of tools for in vitro-in vivo extrapolation ("IVIVE") of high throughput screening data (e.g., ToxCast) to real-world exposures via reverse dosimetry (also known as "RTK").

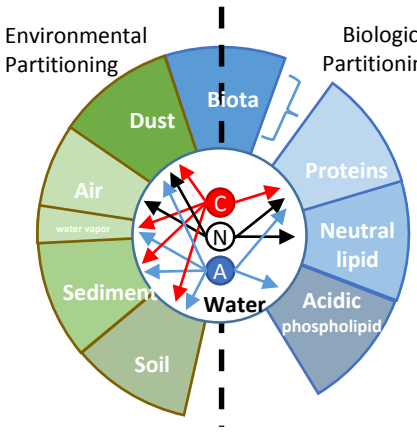
Version:	1.3
Depends:	R (≥ 2.10)
Imports:	deSolve, msm
Suggests:	ggplot2
Published:	2015-10-14
Author:	John Wambaugh and Robert Pearce, Schmitt method implementation by Jimena Davis, dynamic model adapted from code by R. Woodrow Setzer, Rabbit parameters from Nisha Sipes
Maintainer:	John Wambaugh <wambaugh.john at epa.gov>
License:	GPL-3
NeedsCompilation:	yes
CRAN checks:	httk results

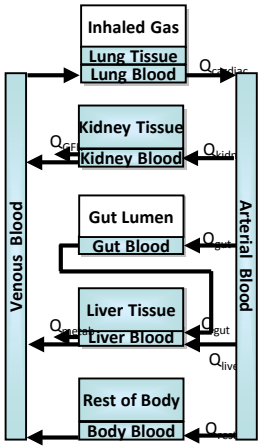
Downloads:

Reference manual:	httk.pdf
Package source:	httk_1.3.tar.gz
Windows binaries:	r-devel: httk_1.3.zip, r-release: httk_1.3.zip, r-oldrel: httk_1.3.zip
OS X Snow Leopard binaries:	r-release: httk_1.2.tgz, r-oldrel: httk_1.2.tgz
OS X Mavericks binaries:	r-release: httk_1.3.tgz
Old sources:	httk archive



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



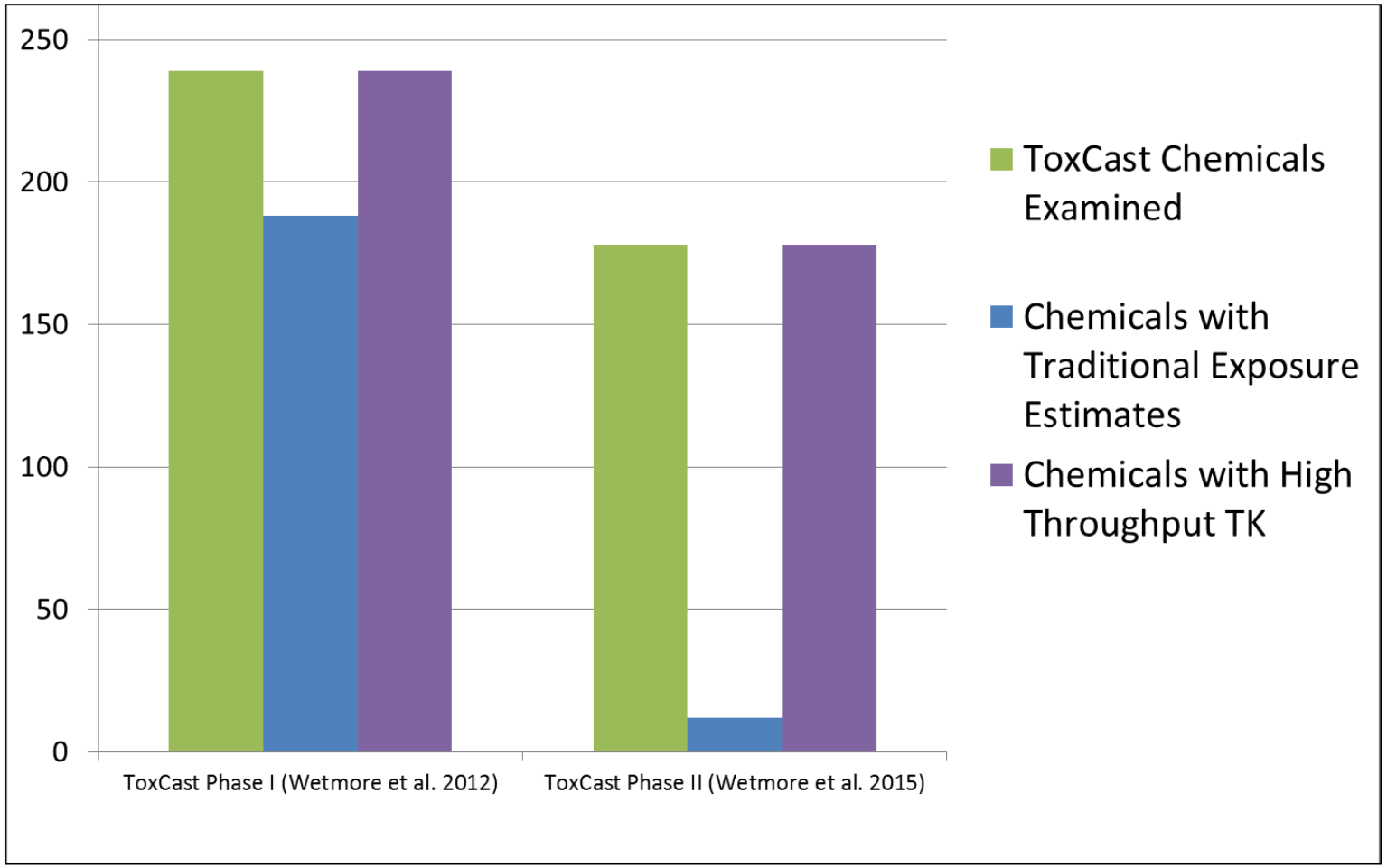


"httk" R Package
543 Chemicals to date
Lead programmer Robert Pearce
Wambaugh *et al.* (2015), Pearce *et al.* submitted

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

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

High Throughput Screening (HTS), HT Toxicokinetics (HTTK), and Exposure



- For non-pesticide chemical space, there is a paucity of data for providing context to HTS data (Egeghy *et al.* (2012))

Thinking About Exposure

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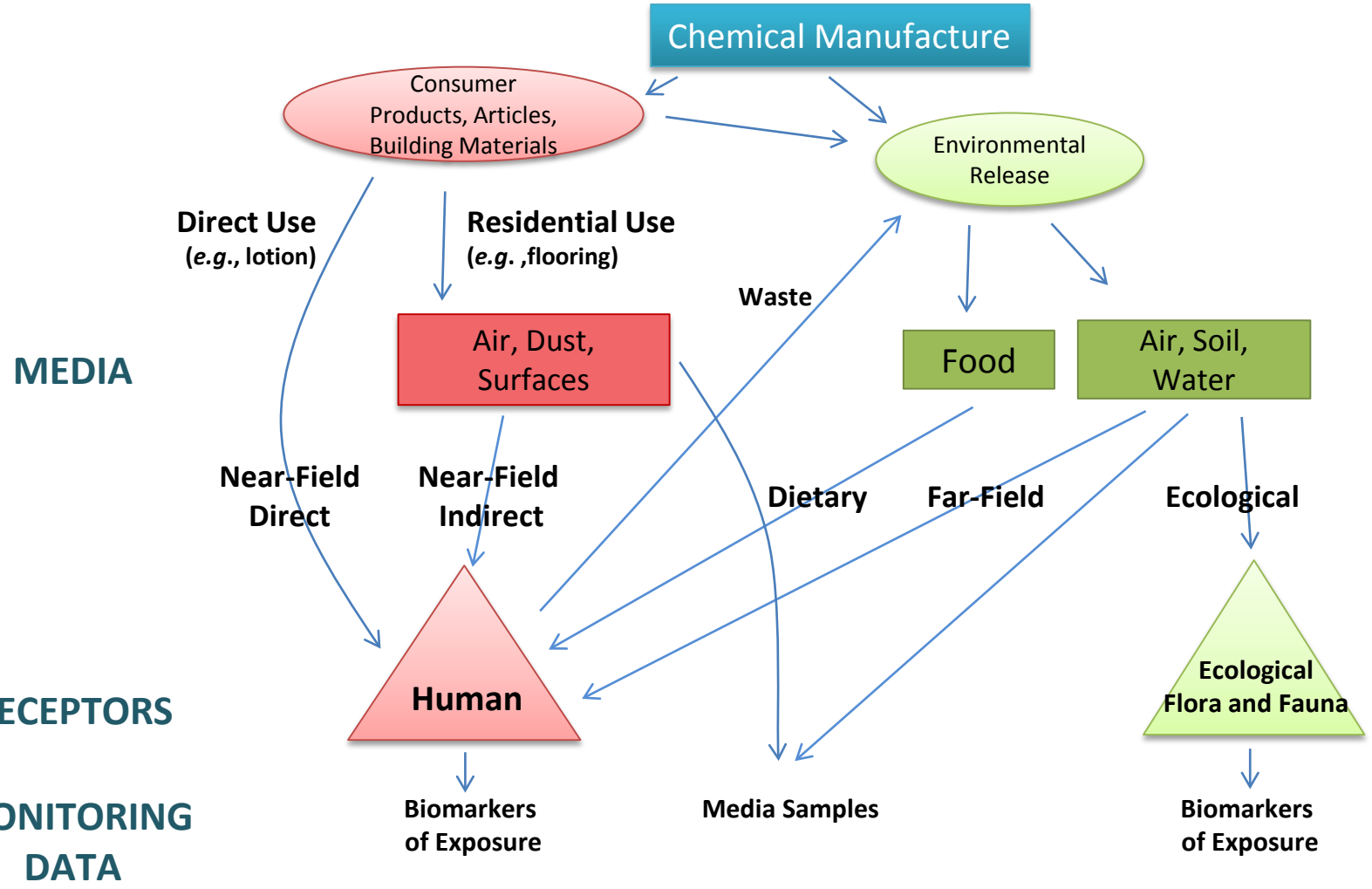


Figure from Kristin Isaacs

Exposure Pathways

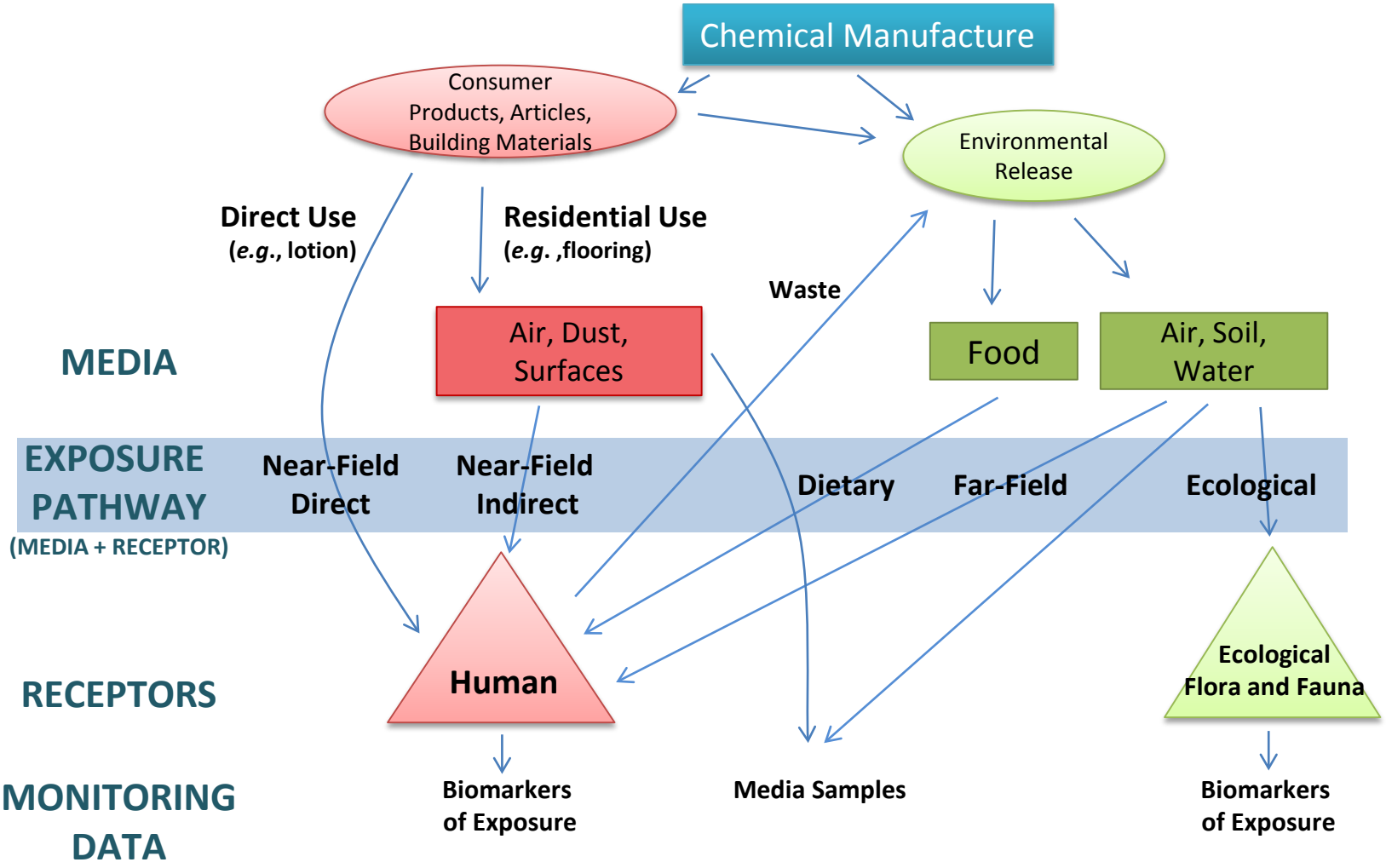
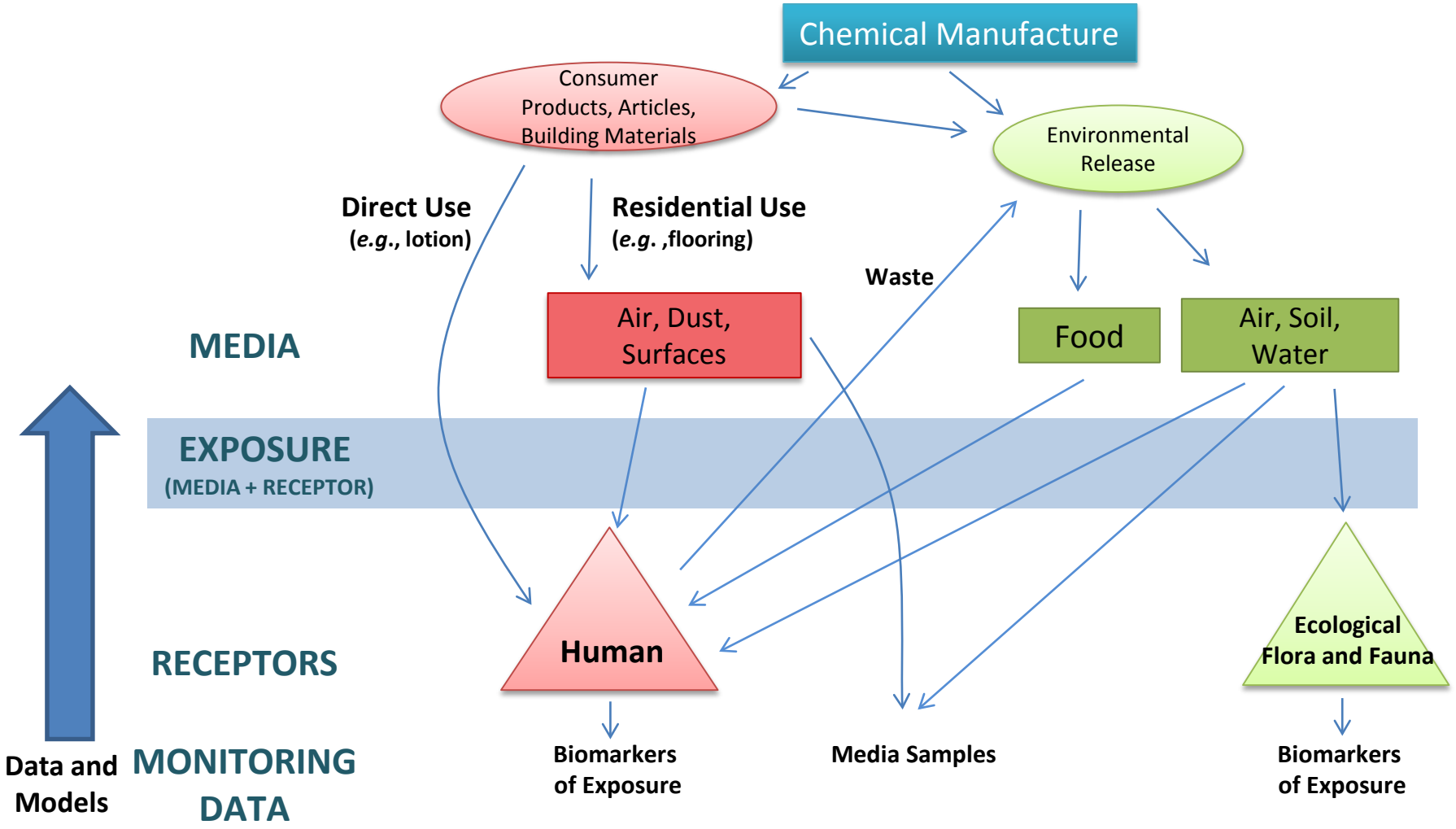


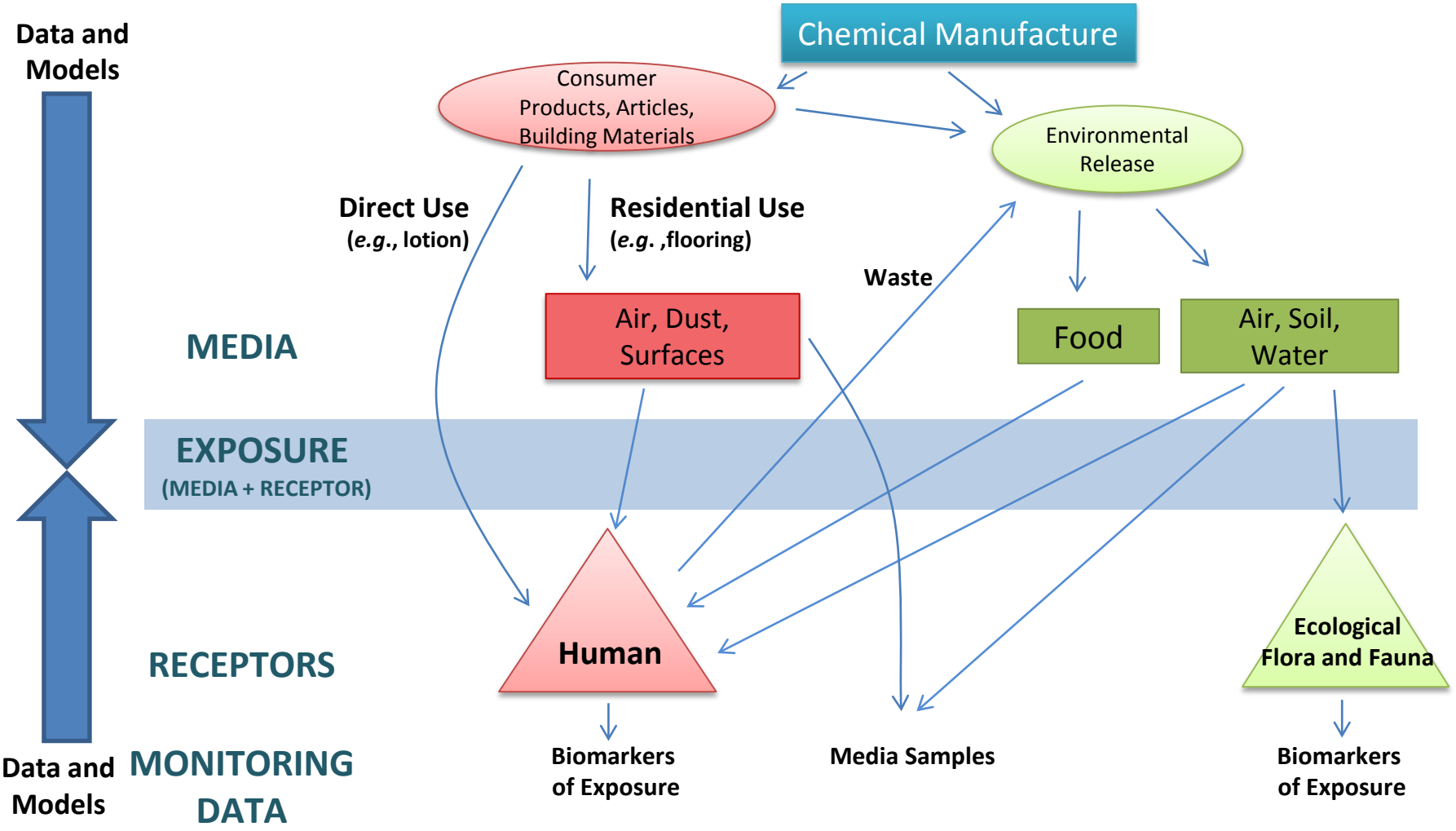
Figure from Kristin Isaacs

Exposure Monitoring



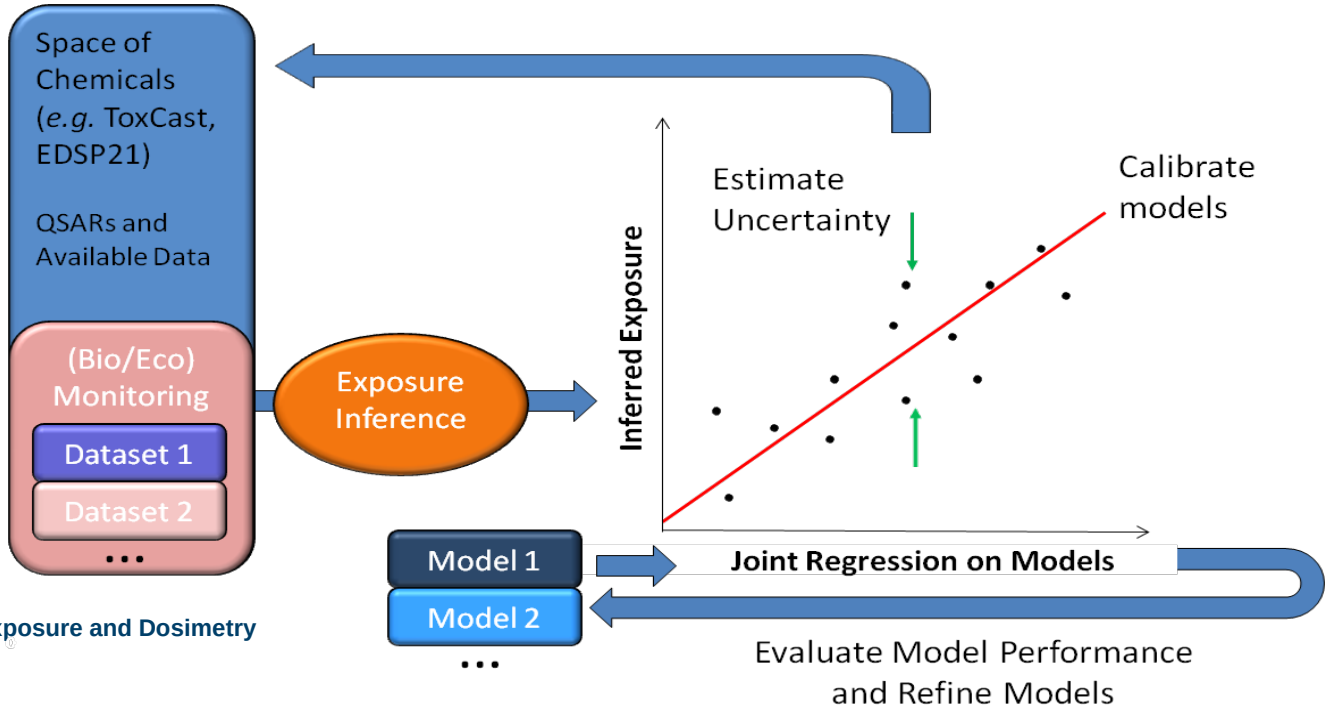
- Centers for Disease Control monitors a few hundred specific chemicals in urine and blood of U.S. citizens

Evaluating Exposure Models



Consensus Exposure Predictions with the SEEM Framework

- Incorporate multiple models into consensus predictions for 1000s of chemicals within the **Systematic Empirical Evaluation of Models (SEEM) framework** (Wambaugh et al., 2013, 2014)
- Evaluate/calibrate predictions with available monitoring data across as many chemical classes as possible to allow extrapolation
- Analogous efforts for both human and ecological exposures

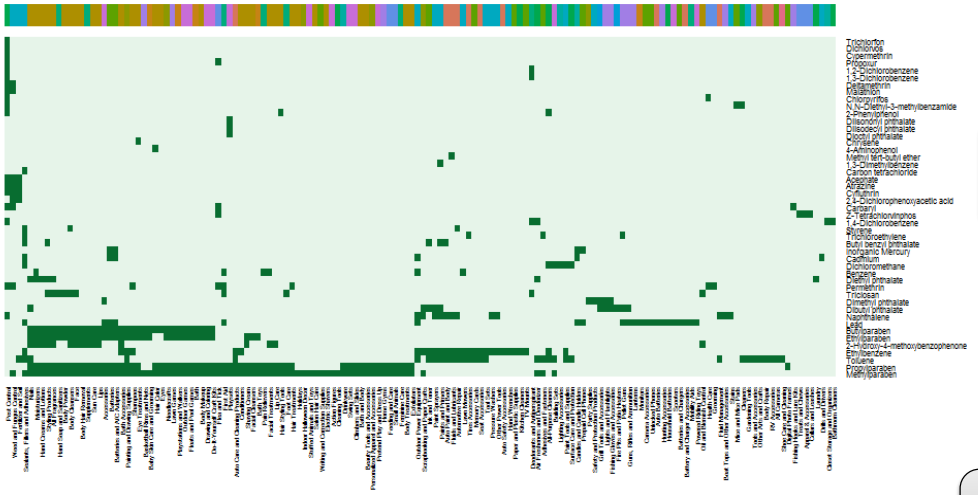


Stochastic Human Exposure Dose Simulator (SHEDS)

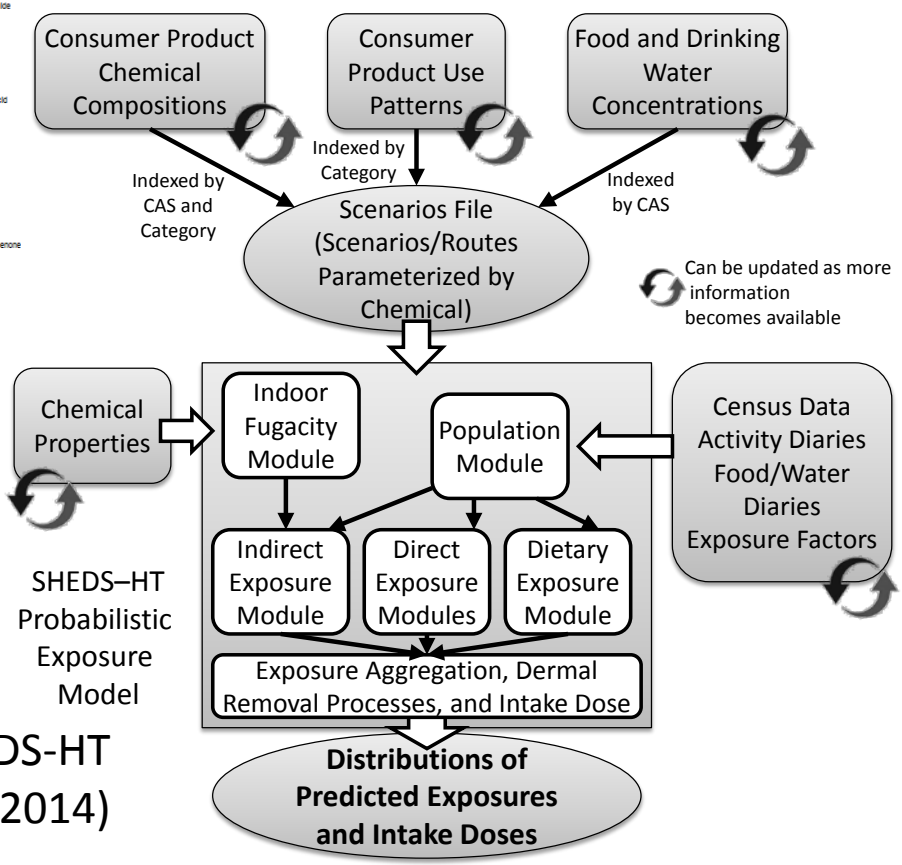
United States Environmental Protection Agency

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

105 NHANES Chemicals



- Apparel
- Auto and Tires
- Baby
- Beauty
- Craft and Party Supply
- Electronics
- Grocery
- Health
- Home
- Home Improvement
- Patio and Garden
- Pets
- Sports and Outdoors
- Toys



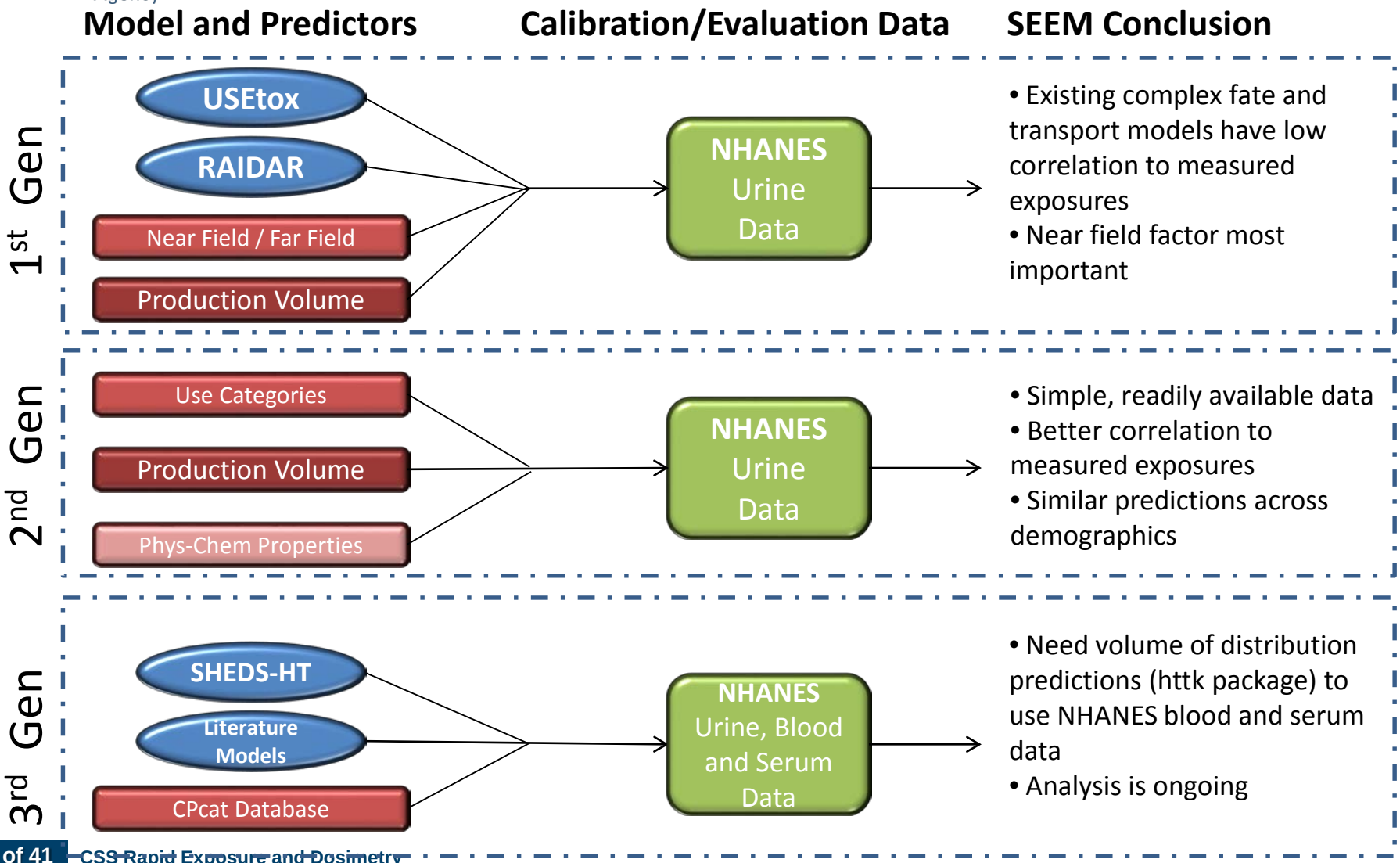
SHEDS–HT Probabilistic Exposure Model

Predictions from SHEDS-HT (Isaacs et al, 2014)

CSS Rapid Exposure and Dosimetry

SEEM Evolution

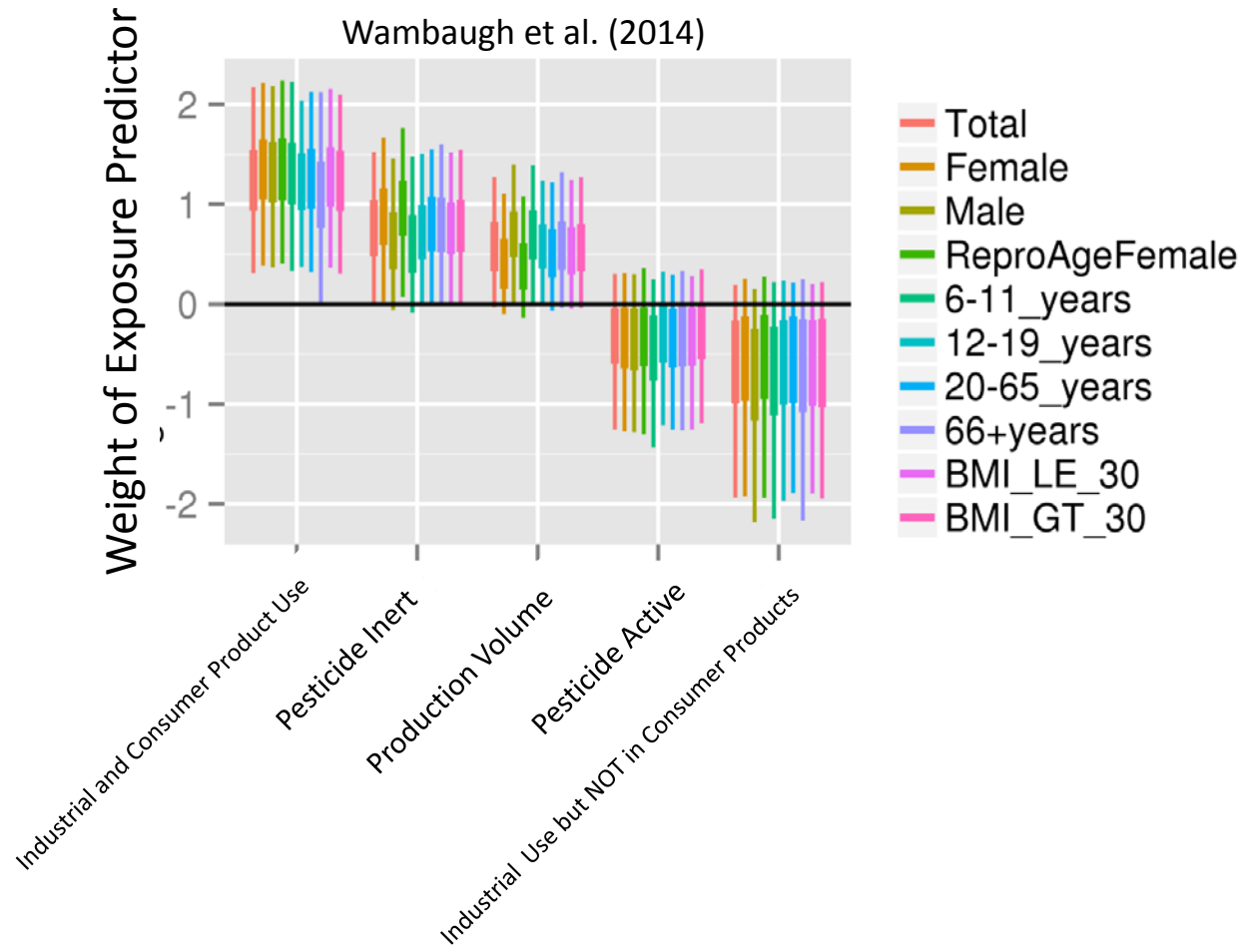
United States Environmental Protection Agency



CSS Rapid Exposure and Dosimetry

Approach described in Wambaugh *et al.* (2013) ExpoCast Framework Paper

Predicting Exposure



The presence of chemicals in consumer products and elsewhere in the home ("near field" sources) is a key driver of high exposure levels in Centers for Disease Control (CDC) National Health and Nutrition Survey (NHANES) biomonitoring data

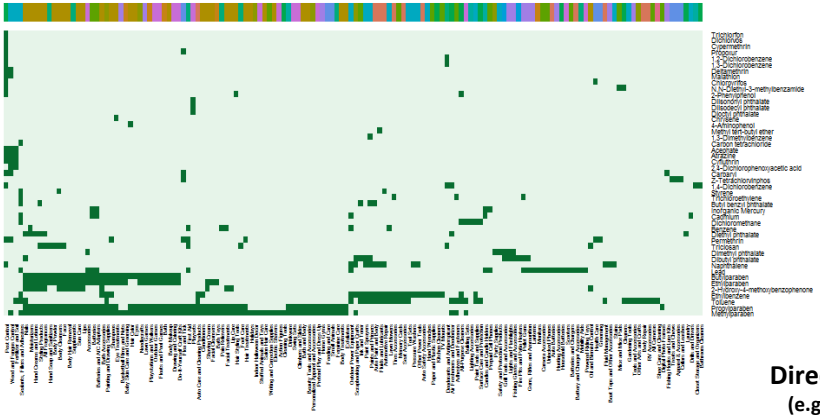
Same five predictors work for all groups analyzed :

- Industrial and Consumer use
- Pesticide Inert
- Pesticide Active
- Industrial but no Consumer use
- Production Volume

Chemical Use Identifies Relevant Pathways

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

105 NHANES Chemicals



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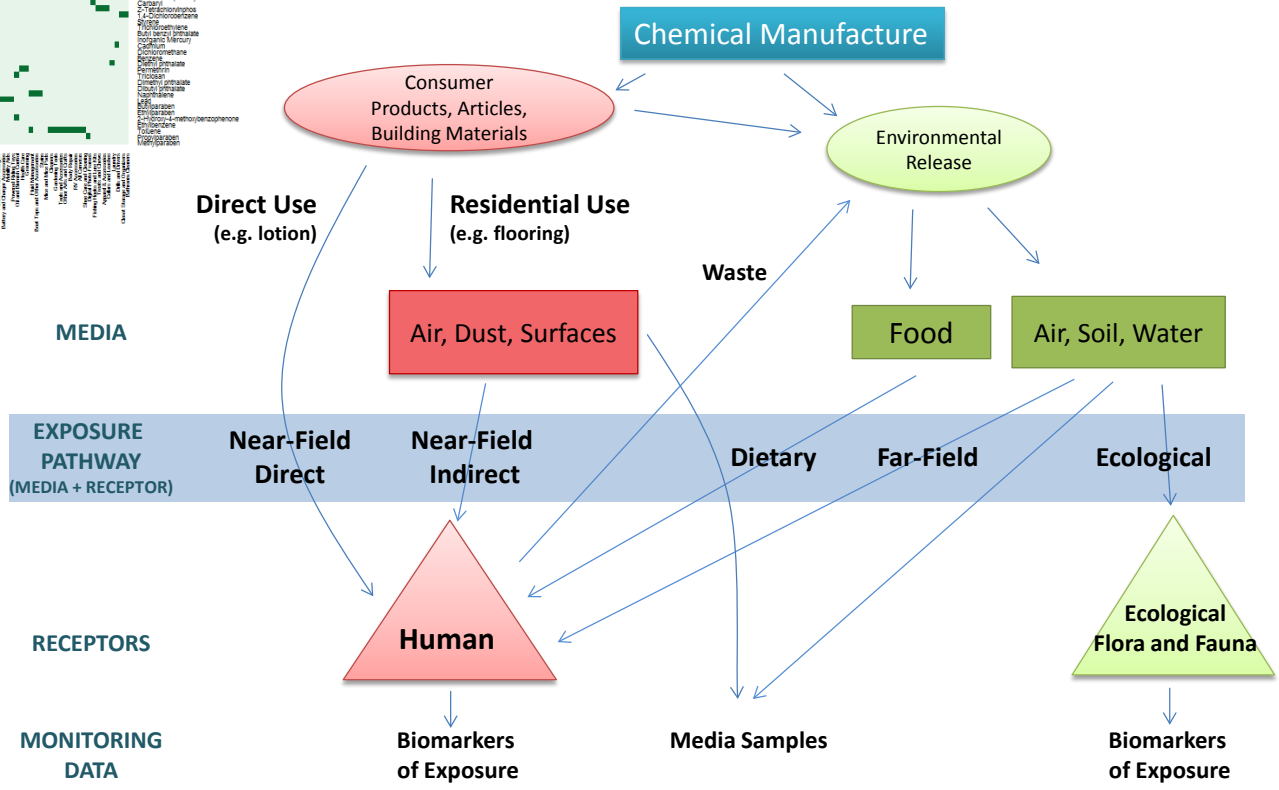
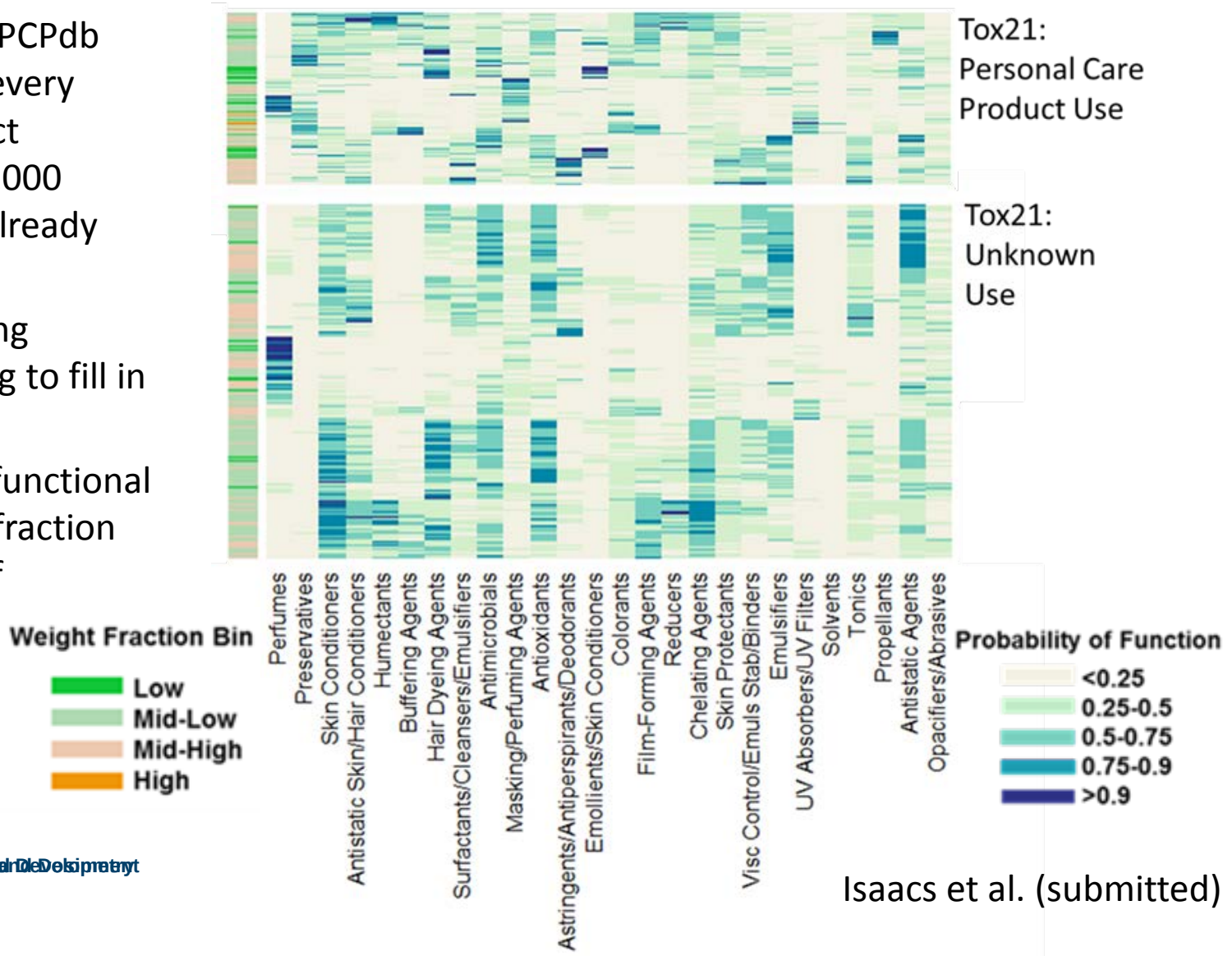


Predicting Chemical Constituents

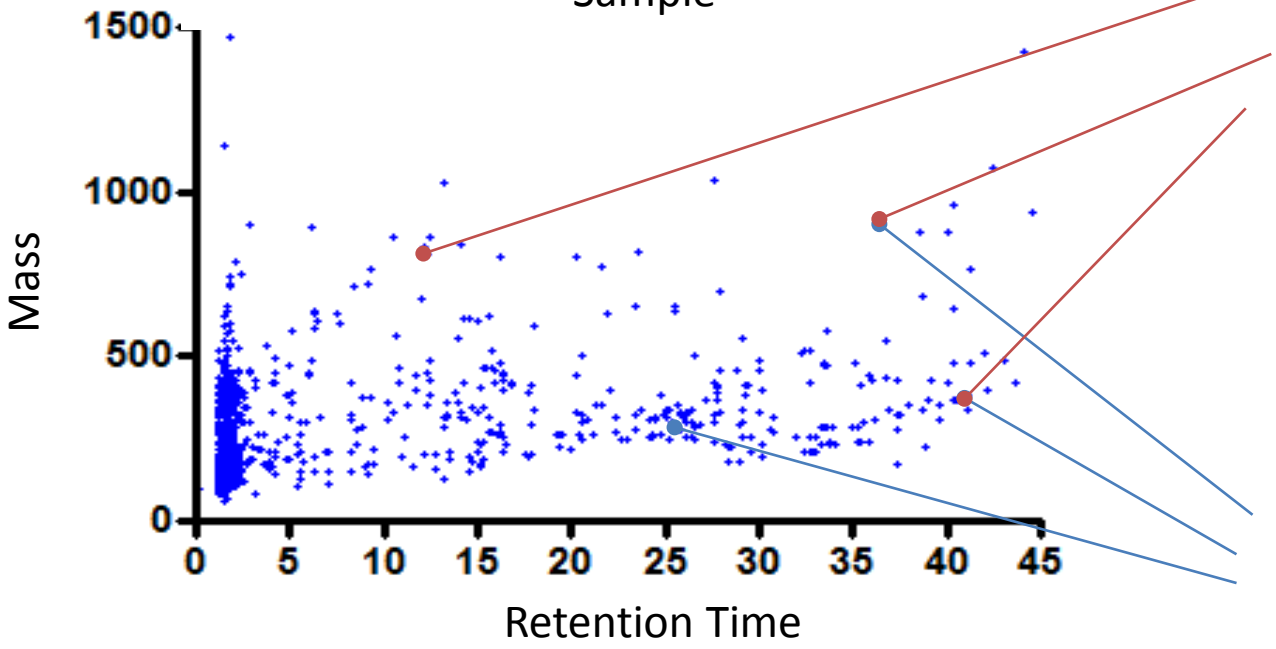
- Unfortunately CPCPdb does not cover every chemical-product combination (~2000 chemicals, but already >8000 in Tox21)
- We are now using machine learning to fill in the rest
- We can predict functional use and weight fraction for thousands of chemicals



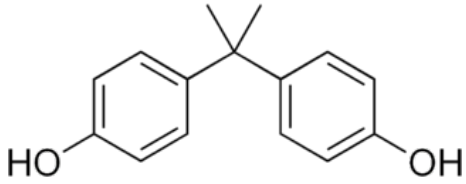
Isaacs et al. (submitted)

Suspect Screening and Non-Targeted Analytical Chemistry

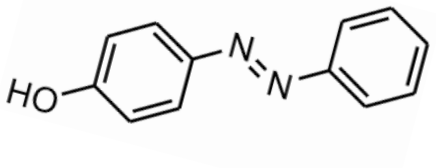
947 Peaks in an American Health Homes Dust Sample



Each peak corresponds to a mass of a chemical or (depending on technique) fragments of that compound



Multiple chemicals can have the same fragments or overall mass



Is chemical A present, chemical B, or both?

Rager, J.E., Strynar, M.J., Liang, S., McMahen, R.L., Richard, A.M., Grulke, C.M., Wambaugh, J.F., Isaacs, K.K., Judson, R., Williams, A.J., Sobus, J.R. "Linking high resolution mass spectrometry data with exposure and toxicity forecasts to advance high-throughput environmental monitoring" Environment International, 88, 269-280 (2016).

Pilot Projects to Reduce Uncertainty and Expand Validation Domain

Project	Pilot Project Scope
High throughput chemical property measurement (e.g., log P)	200 chemicals
Determine the chemical constituents of products, materials, articles	20 classes of product, 5 samples each
Determine chemical emission rate from specific products, materials, articles	100 materials
Screening for occurrence of large numbers of chemicals in blood samples	500 individuals

- Expands application domain of physical chemical property computational models
- Better understanding of what chemicals are associated with household products
- Better understanding of chemicals in the indoor environment
- Expands validation domain of human biomonitoring chemicals

Method for Screening Product Compositions

- Southwest Research Institute conducted analytical chemistry screening for large numbers of chemicals in consumer products and articles of commerce
  - Five sample products were arbitrarily selected from -each of twenty different categories
- Products were analyzed using two dimensional gas chromatograph (GC) x GC Time of Flight Mass Spectrometry
  - Chemical presence and approximate quantitation relative to reference chemicals (internal standards) was determined
  - All dilutions and extractions used Dichloromethane (DCM) *(Hexane:Ether was also examined initially, but had a higher background)*
  - Dilution level and processing were tailored to Mass spectra for some each sample; 1x, 10x and/or 100x
- Data processing
- GC features were matched to NIST 07 spectral database for tentative chemical identification
  - Compounds within some chemical classes are very similar, making definitive identifications difficult
  - Some peaks have a large, unresolved region of hydrocarbons in the C17-C32 range
    - Classifications used to manage hydrocarbon regions were ambiguous

Results from Kristen Favela and Alice Yau (SWRI)

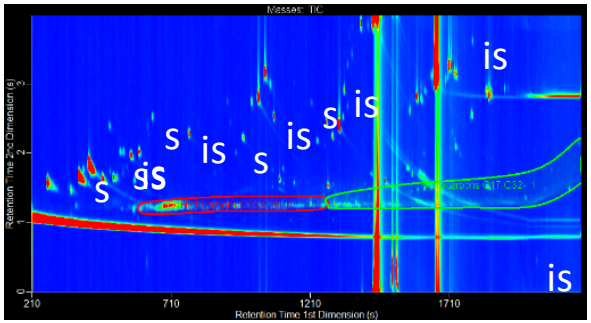
Caveats to Non-Targeted Screening

- Chemical presence in an object does not necessarily mean that it is bioavailable
- Samples are being homogenized (e.g., grinding)
- Chemicals are extracted with a solvent (CDM)
- Varying degrees of intimacy of exposure– carpet padding to shampoo to cereal
- Chemical presence in an object does not mean that exposure occurs
- We are not assessing toxicity of chemical exposure here – exposure alone is not risk

Example Chromatograms for Baby Toys

United States Environmental Protection Agency

Product 1



1x

10x

100x

DCM Dilution

Retention Time
First Dimension (s)

Retention Time
Second Dimension (s)

is = internal standard
s = surrogate

Results from Kristen Favela and Alice Yau (SWRI)

Example Chromatograms for Baby Toys

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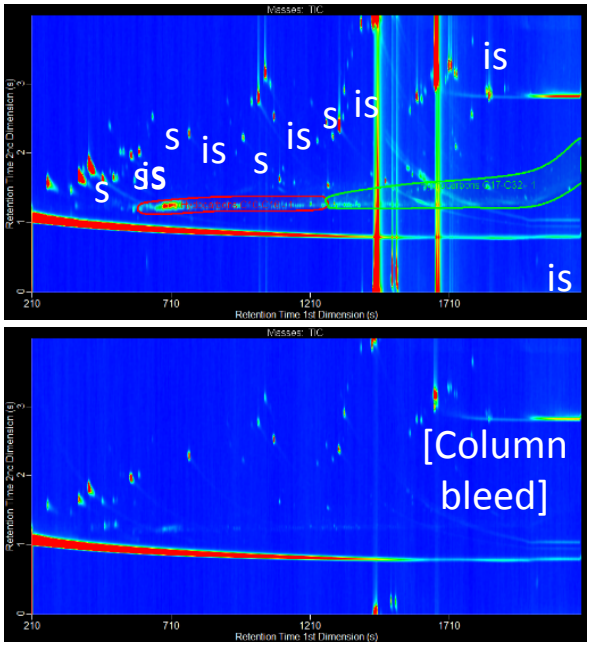

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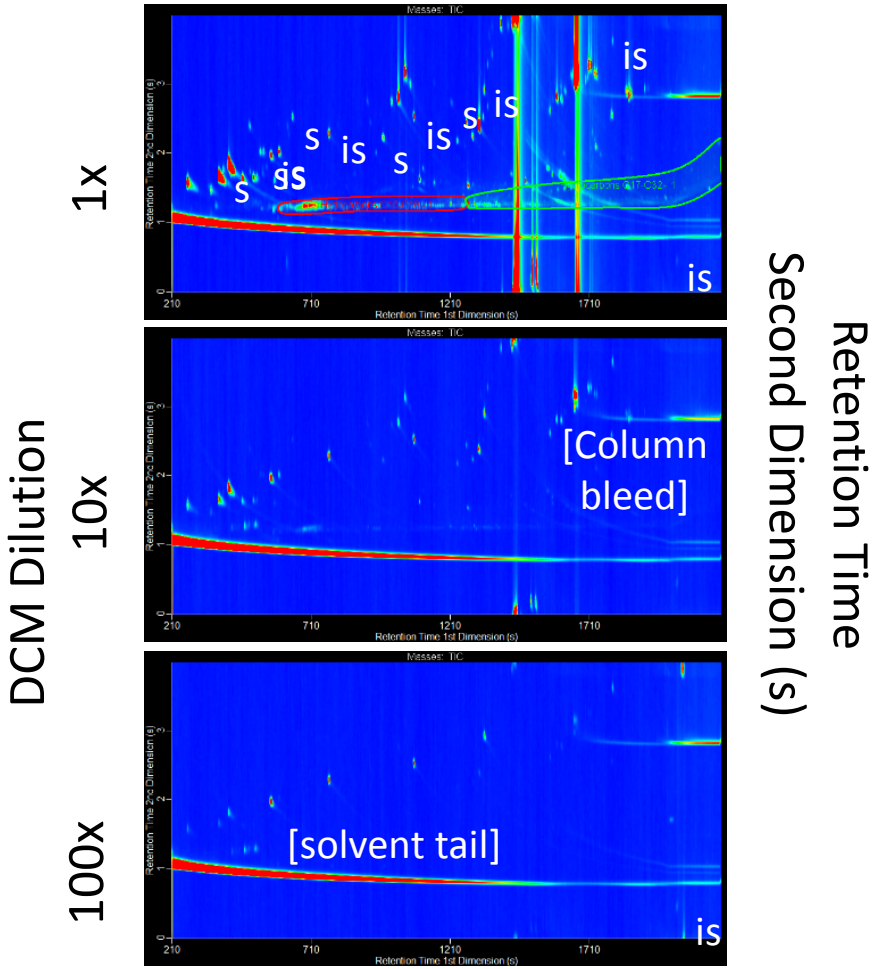

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Example Chromatograms for Baby Toys

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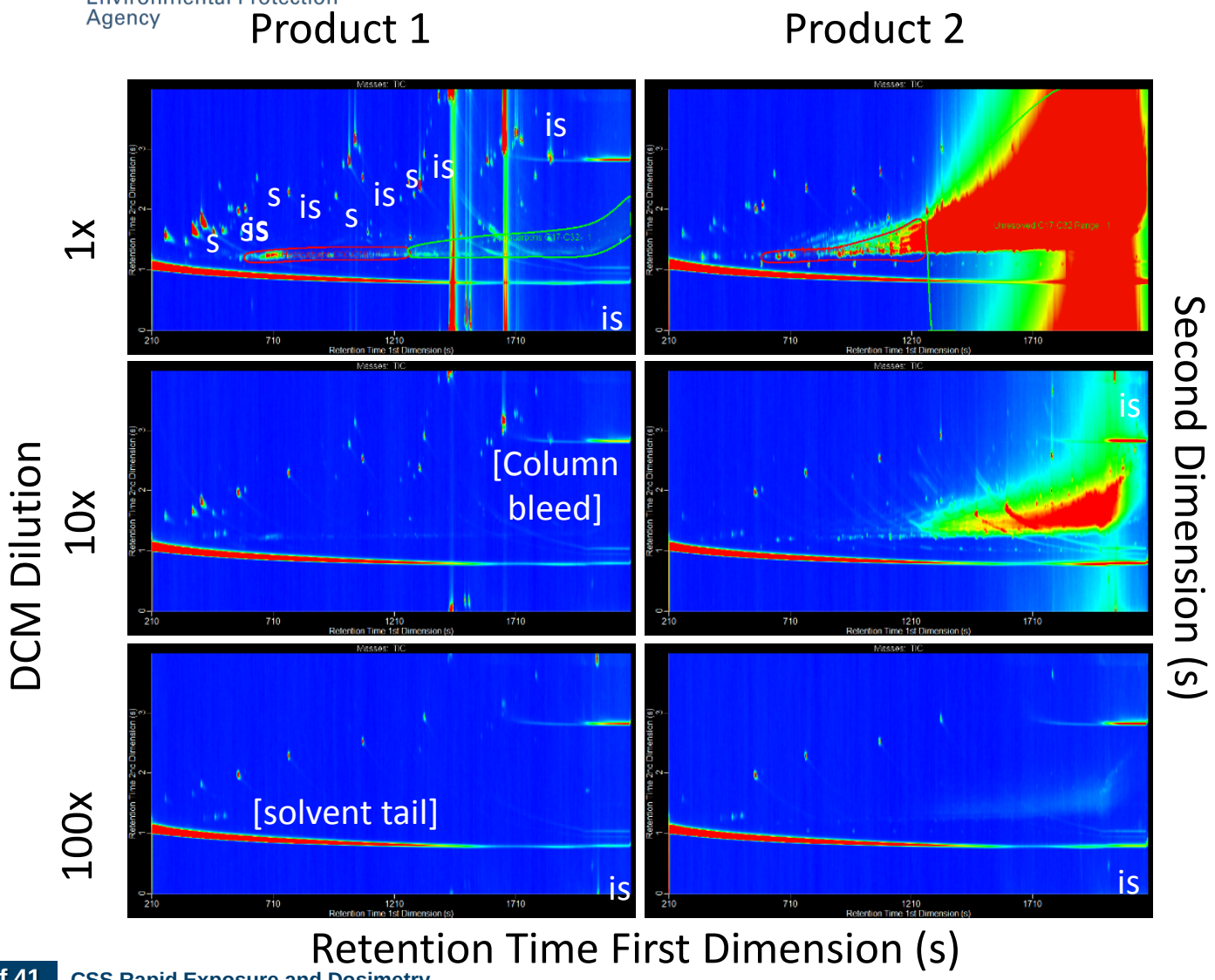

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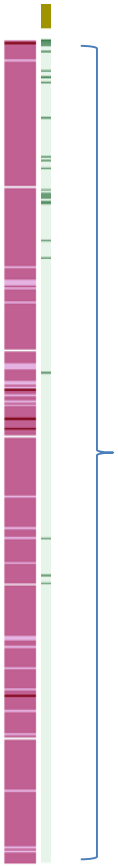
Results from Kristen Favela and Alice Yau (SWRI)

ExpoCast Consumer Product Scan

United States Environmental Protection Agency



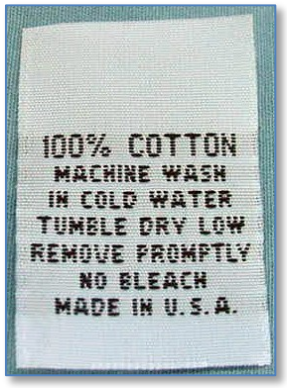
Scanned 5 examples each of 20 class of consumer products

Found 3803 chemical signature across the 100 products

1606 have tentative identifications

41 had confirmed chemical identities

The chemicals found in a cotton shirt



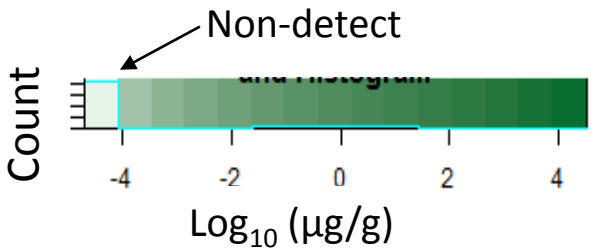
GC-MS with DCM Extraction

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

Results from Kristen Favela and Alice Yau (SWRI)

ExpoCast Consumer Product Scan

United States Environmental Protection Agency



Scanned 5 examples each of 20 class of consumer products

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Dark green is a high concentration

Light green is not detected

GC-MS with DCM Extraction

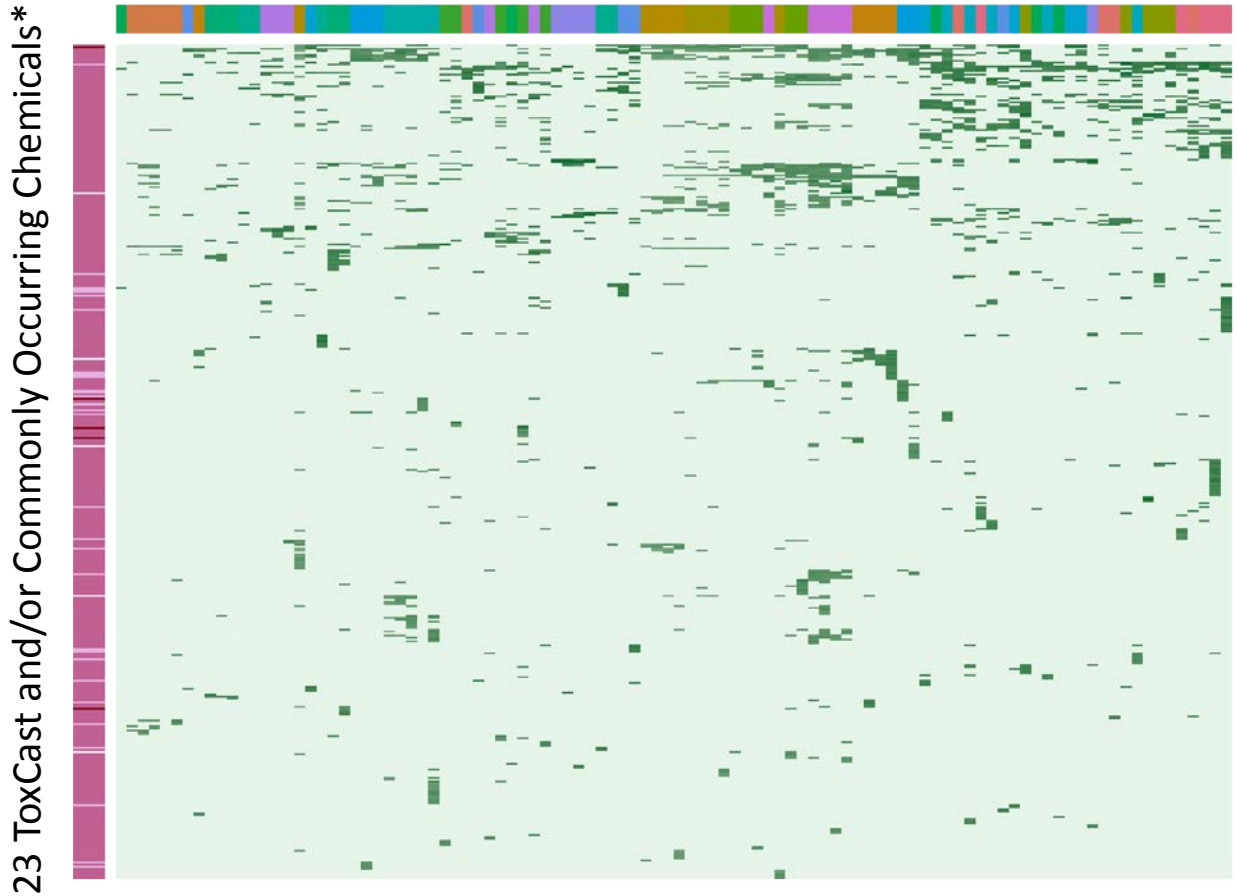
Common Chemical (n>19)
ToxCast
Flame Retardant
Potent ER

Results from Kristen Favela and Alice Yau (SWRI)

Five arbitrary products in each of twenty categories

United States Environmental Protection Agency

100 Consumer Products and Articles of Commerce



GCXGC-MS with DCM Extraction

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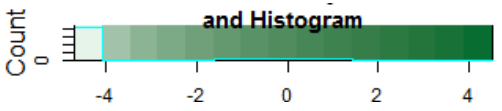


Log_{10} (µg/g)

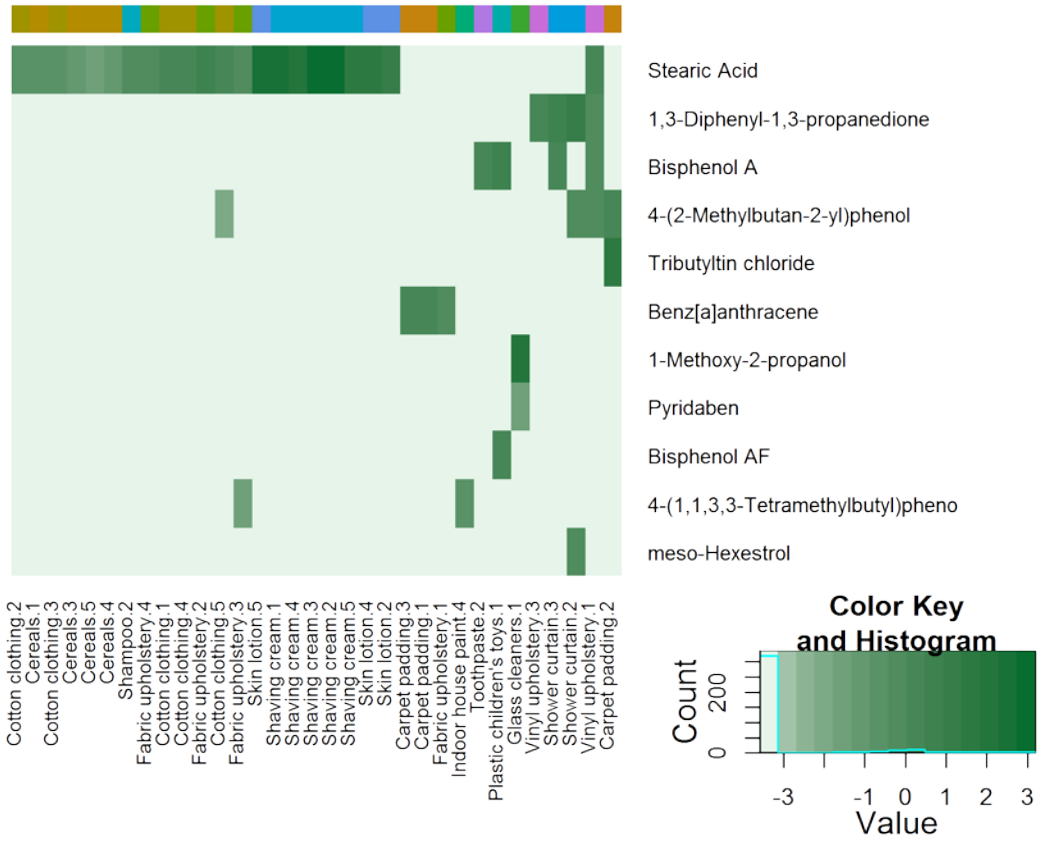
- Air freshener
- Baby soap
- Carpet
- Carpet padding
- Cereals
- Cotton clothing
- Deodorant
- Fabric upholstery
- Glass cleaners
- Hand soap
- Indoor house paint
- Lipstick
- Plastic children's toys
- Shampoo
- Shaving cream
- Shower curtain
- Skin lotion
- Sunscreen
- Toothpaste
- Vinyl upholstery

Results from Kristen Favela and Alice Yau (SWRI)

1606 tentative and confirmed chemical identifications

- 184 of 1797 chemicals with previously known consumer product use (CPCPdb)
- 520 of 8948 Tox21 chemicals
- 393 of 3805 ToxCast chemicals
- 11 of 96 ToxCast ER active chemicals
- 17 of 178 EDSP List 1 and 2 chemicals
- 94 of 1172 ToxRefDB chemicals
- 32 of 452 NHANES chemicals
- 1 of 670 pharmaceuticals (Obach, 2008)
- 9 of 67 flame retardants

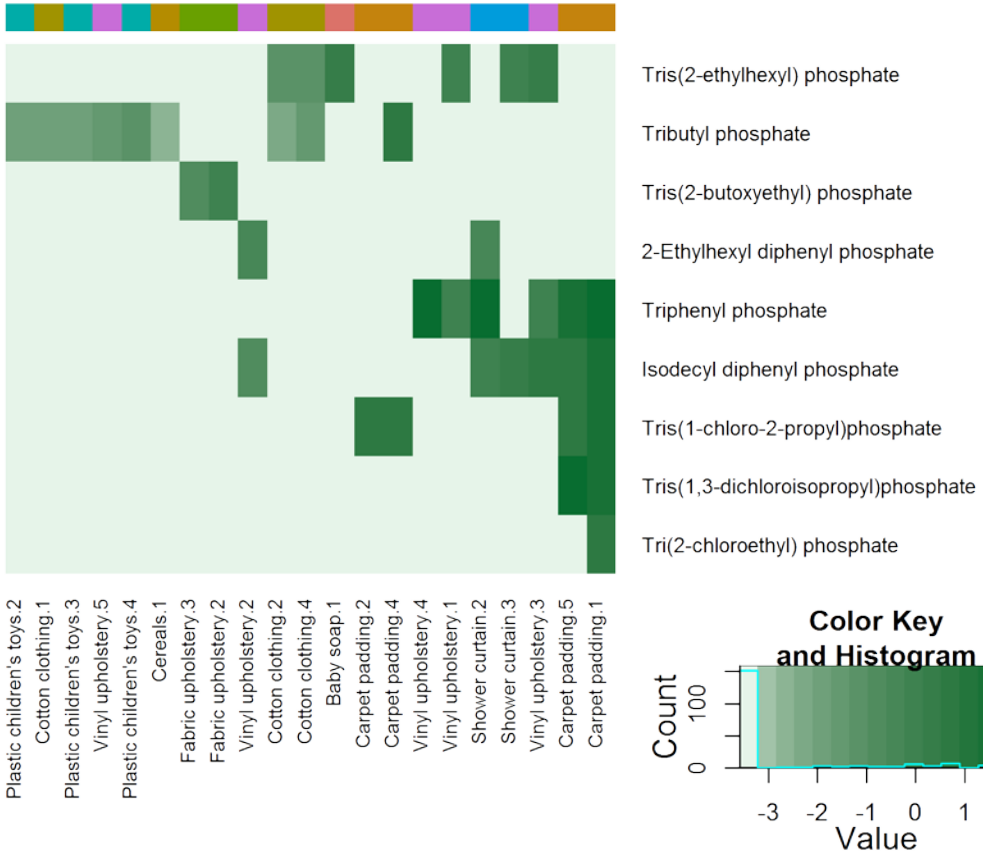
In Vitro Estrogen active chemicals



- Here we examine those chemicals that were among the top 25% most potent ER active chemicals in Judson et al., (2015)
- Stearic acid is a naturally occurring fatty acid known to be used in many detergents, soaps, shampoos, and shaving creams[10] Other estrogen active chemicals were found in articles like shower curtains, upholstery, and carpet padding
- Bisphenol A was found in toothpaste and one children's toy, while a replacement for Bisphenol A, Bisphenol AF, was found in another toy

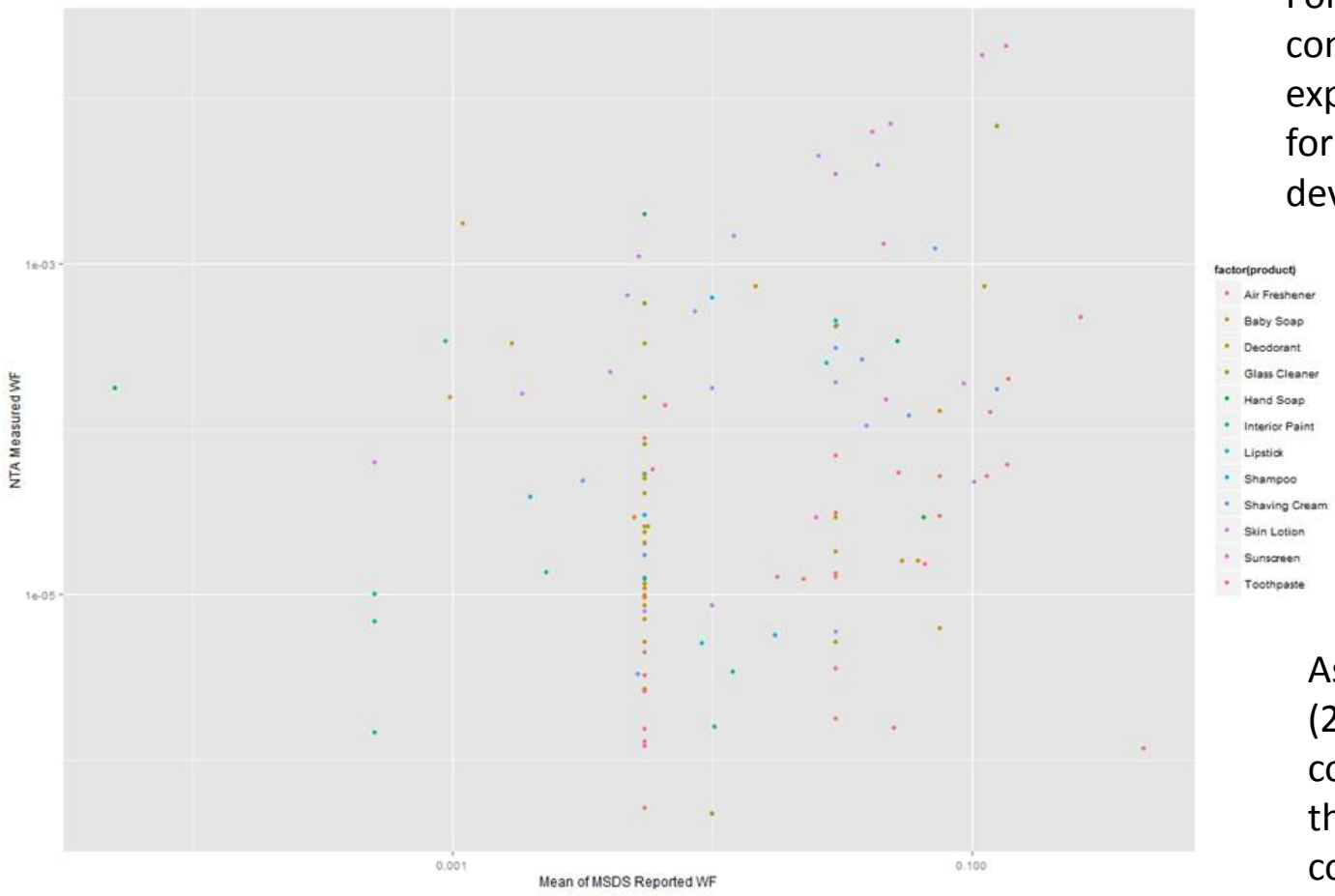
Flame retardant chemicals

United States Environmental Protection Agency



- We used ToxCast chemical annotation and public information to identify chemicals that are sometimes used as flame retardants
- Chemicals with flame retardant application were indicated in most fabric and vinyl upholsteries, carpet paddings, cotton clothing, shower curtains, and children's toys, as well as in one hand soap, baby soap, and breakfast cereal* (*likely used as an anti-foaming agent)

Evaluation – Measured Chemical Concentrations vs. Formulation



For some chemicals we can compare to concentrations expected in generic formulations that were developed for SHEDS-HT

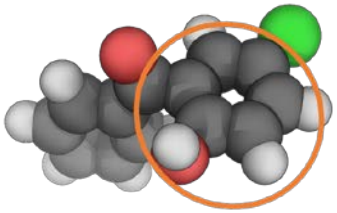
As seen in Rager et al. (2016), we underestimate concentration of chemicals that occur at high concentrations

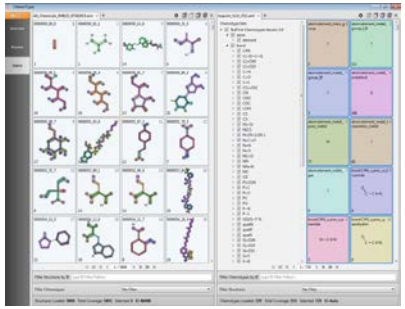
Figure from Kristin Isaacs

Evaluation – Predicting Function in Products Based on Structure

United States Environmental Protection Agency

Chemical Structure and Property Descriptors





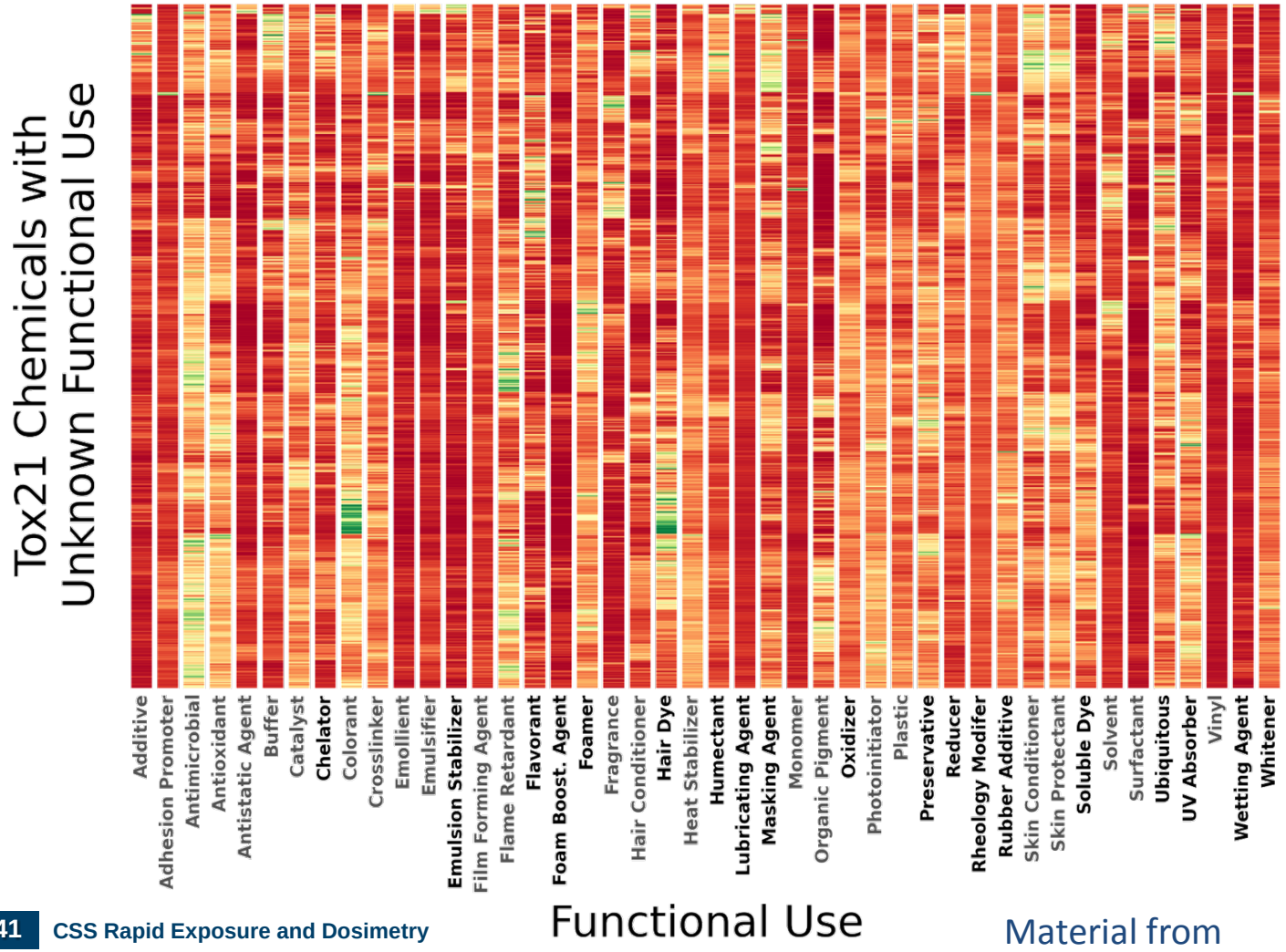
Use Database (FUSE)

Machine-Learning Based Classification Models

Prediction of Of Potential Alternatives from Chemical Libraries

Material from Katherine Phillips

Screening for Alternatives By Function and Bioactivity

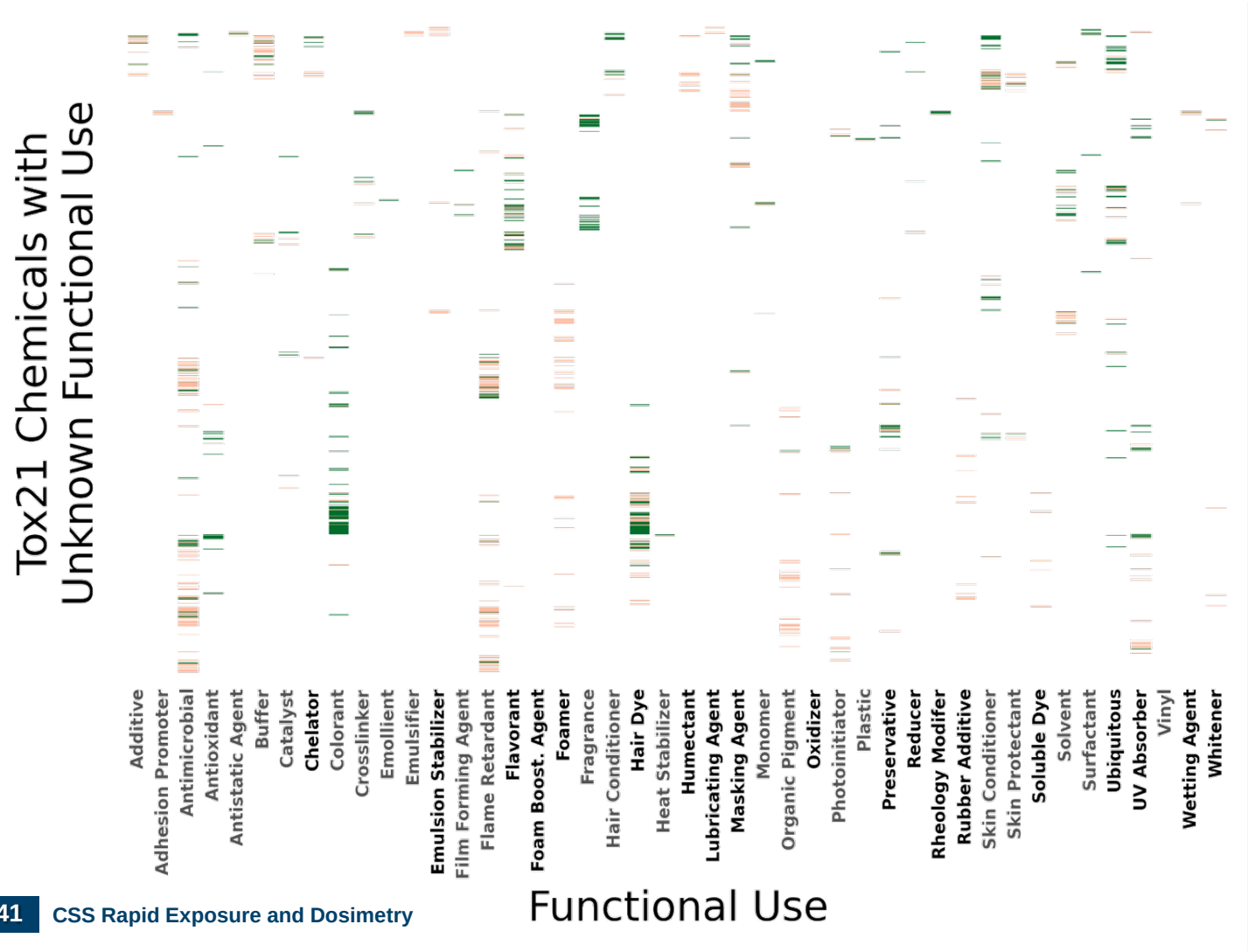


- Unfortunately CPCPdb does not cover every chemical-product combination – using machine learning to fill in the rest

Material from Katherine Phillips

CSS Rapid Exposure and Dosimetry

Screening for Alternatives By Function and Bioactivity



Yes
No

Lower Bioactivity Metric?

Material from Katherine Phillips

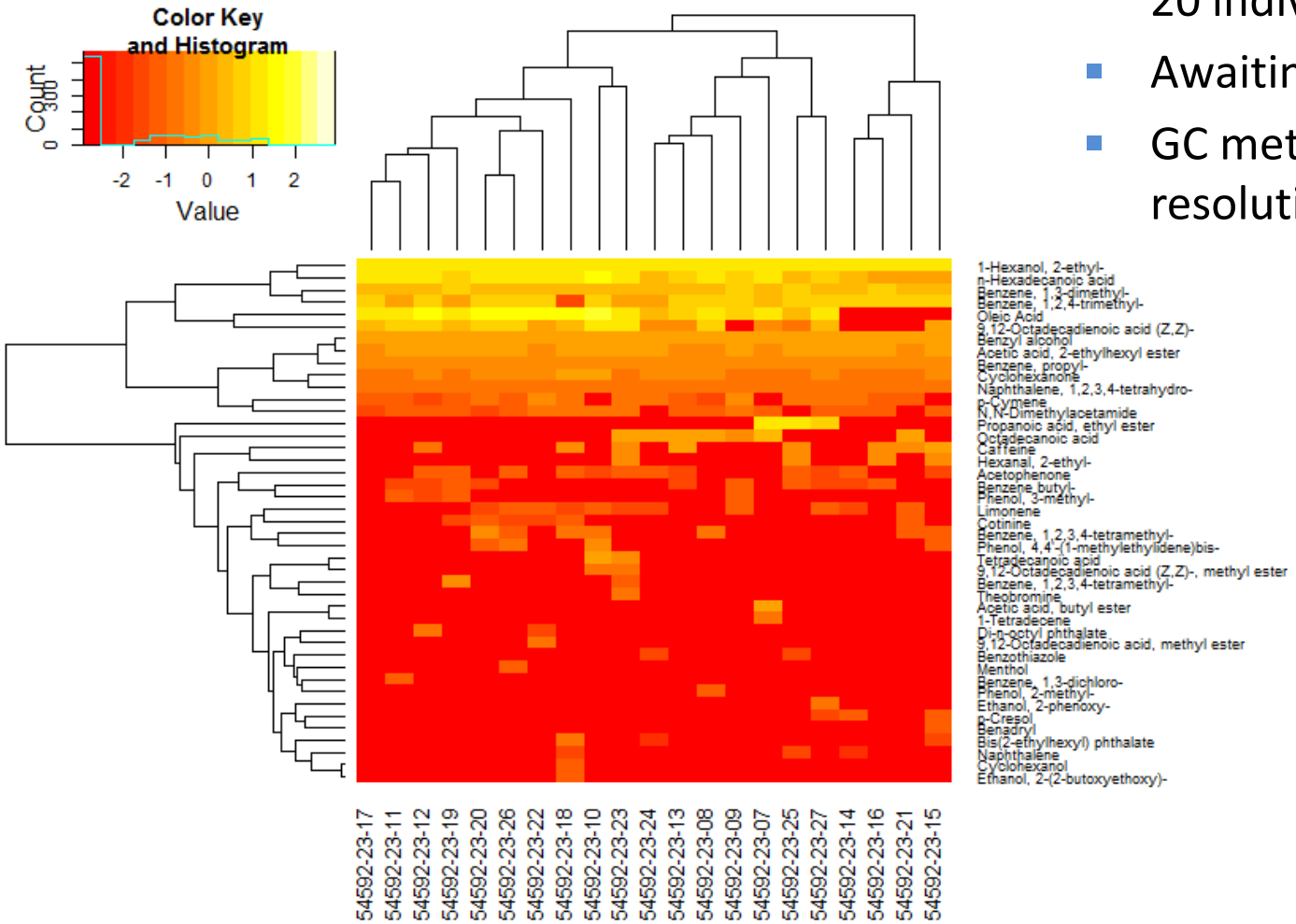
ExpoCast Pilot 4: Biomonitoring

- Screening for occurrence of large numbers of chemicals in sample acquired by contractor (biological media)
- Research Conducted by Battelle Memorial Institute (Anne Gregg)
- Cohort is a mixed gender and race group of adults from Indianapolis
- Sample Screening
  - One extraction method resulting in two aliquots for analysis
  - Two analysis methods GCxGC TOFMS and LC-TOFMS
- In addition to 200 priority ToxCast chemicals, we will look for NHANES chemicals as reference

ExpoCast Pilot 4: Biomonitoring

United States Environmental Protection Agency

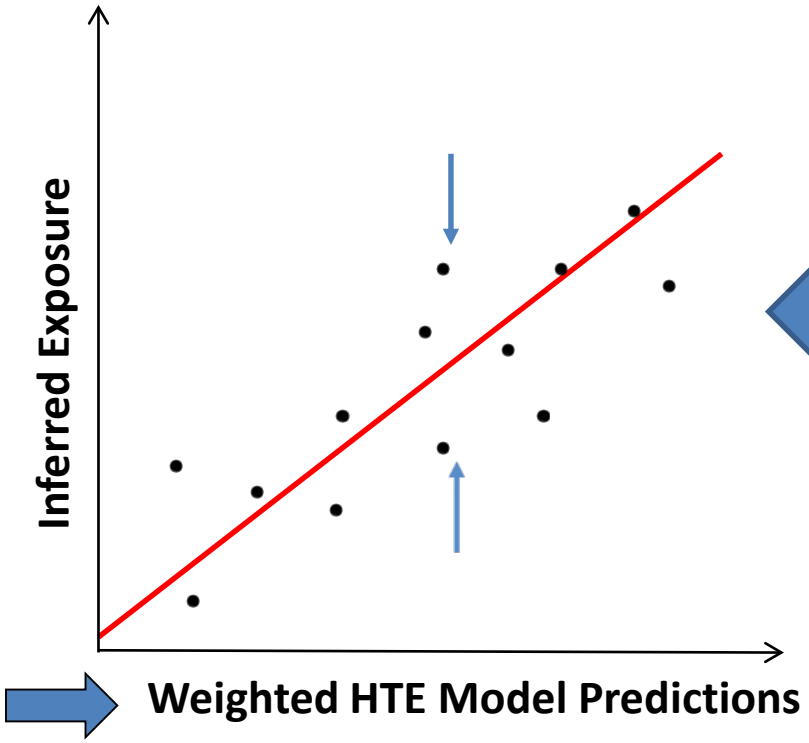
- GCxGC TOFMS data for first 20 individuals
- Awaiting LC-TOFMS
- GC method is not high resolution, but LC method is



Results from Anne Gregg (Battelle)

SEEM is a Linear Regression

$$\text{Log(Parent Exposure)} = a + m * \log(\text{Model Prediction}) + b * \text{Near Field} + \varepsilon$$



$\varepsilon \sim N(0, \sigma^2)$
Residual error, unexplained by the regression model

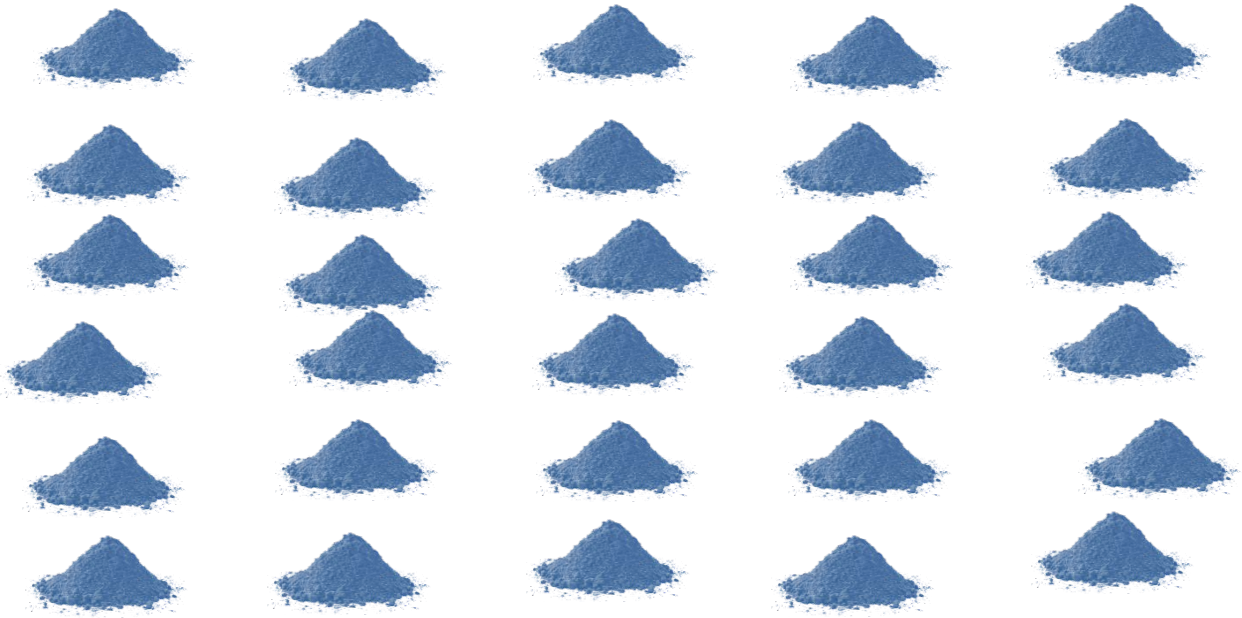
- Product composition and physico-chemical property measurements inform model predictions
- Expanded monitoring (more chemicals) data allow further evaluation

CSS Rapid Exposure and Dosimetry

Suspect-Screening Challenge

A “collaborative trial”: What methods are available (“multiple technologies/multiple preparations”)? What is the coverage of chemical universe? Do the methods differ in their coverage?

The Chemical Universe



Challenge led by Jon Sobus and Elin Ulrich

Images from Thinkstock

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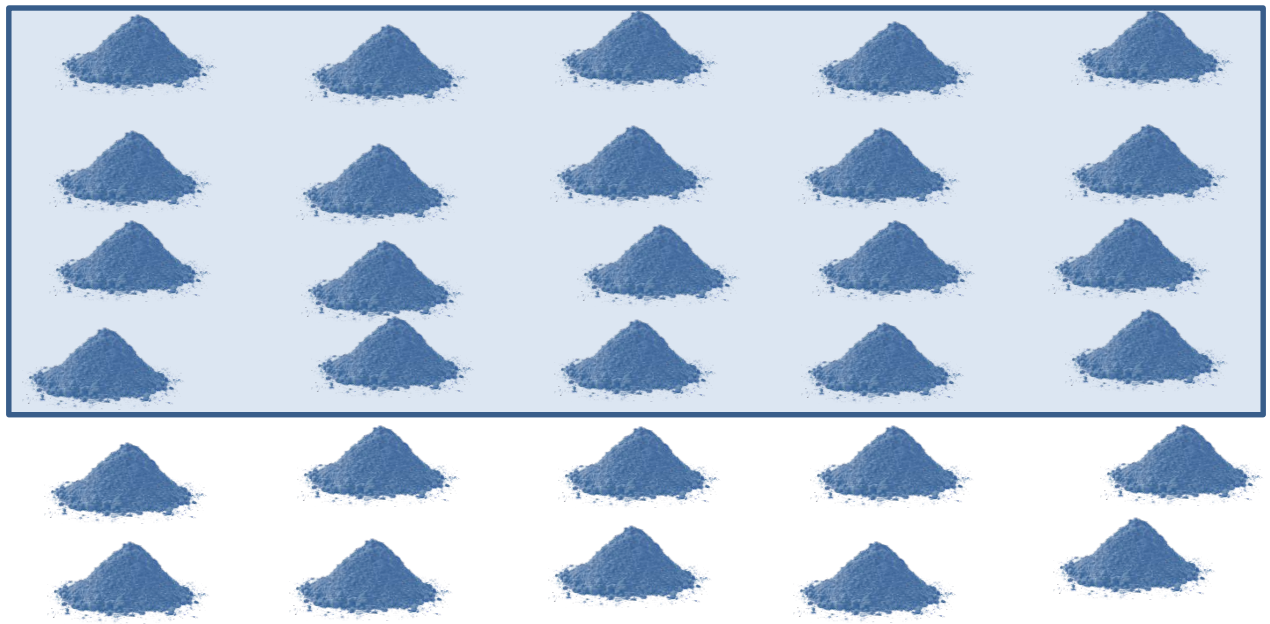
The Chemical Universe



Method 1

Challenge led by Jon Sobus and Elin Ulrich

Images from Thinkstock

Suspect-Screening Challenge

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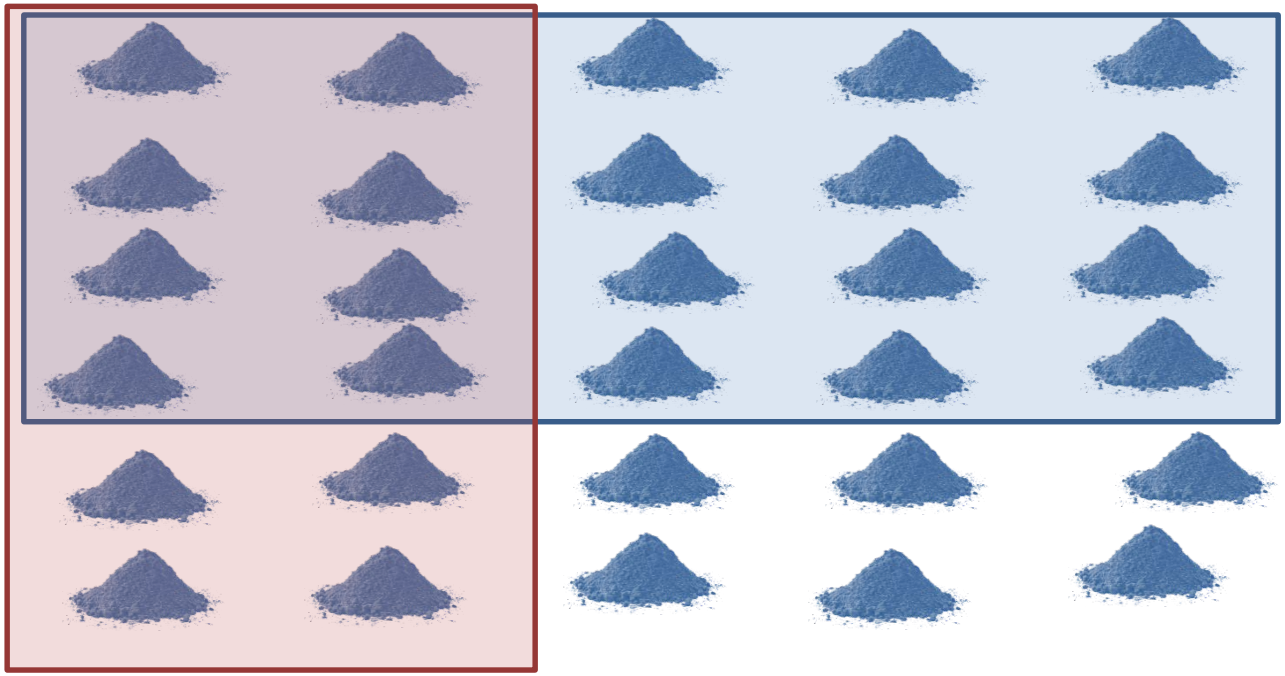
The Chemical Universe



Challenge led by Jon Sobus and Elin Ulrich

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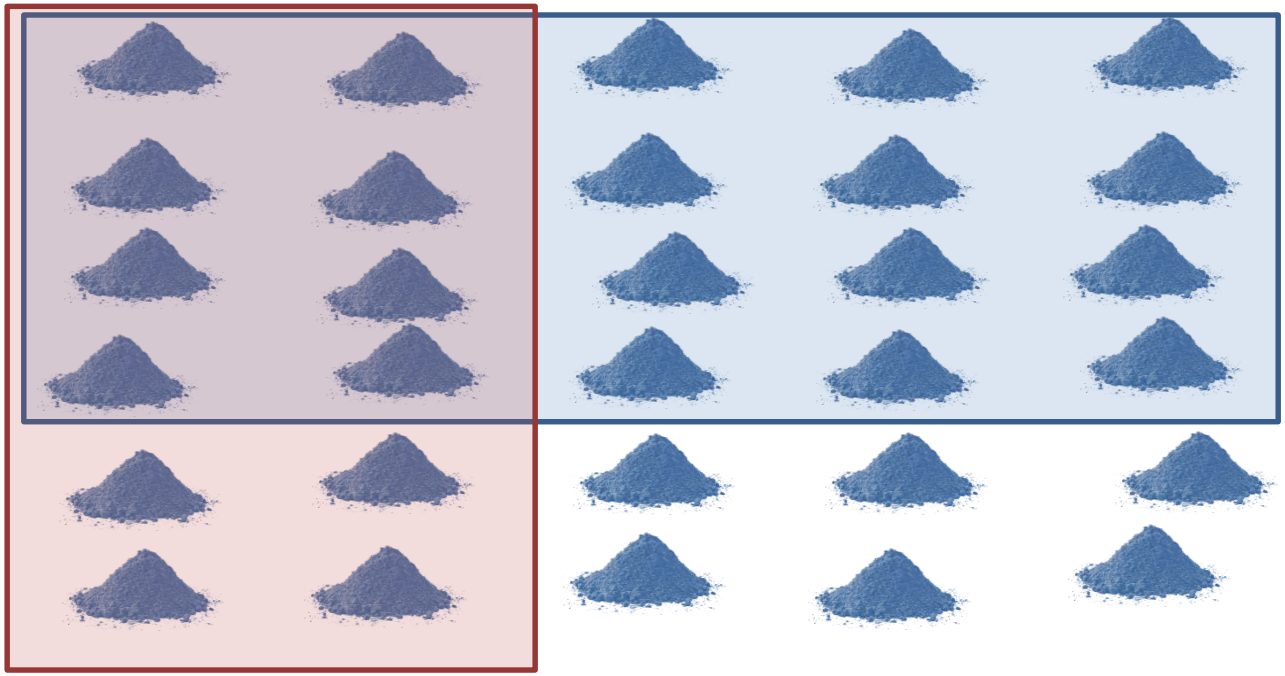

Method 1

Method 2

EPA has mechanisms to provide reference samples for 1000’s of chemicals for cross-lab evaluation

EPA has team devoted to developing and maintaining public databases

Challenge led by Jon Sobus and Elin Ulrich

Images from Thinkstock

High Throughput Exposure

- There are low levels of thousands of xenobiotic chemicals present in the metabolome, relating these to exposures and health effects is an important unsolved problem

- Pathways provide a means to address the complexity of exposure as a system

- Can use a combination of forward modeling and reverse inference from biomarkers to predict exposure pathways and rates
  - Broader monitoring data informs evaluation of those predictions
  - Better chemical use data informs models predicting exposure

- Non-targeted and suspect screening provides an important new tool for both evaluating models and broadening applicable chemistries
  - Important limitations must be noted at all times

EPA United States Environmental Protection Agency

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

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