EPA
United States
Environmental Protection
Agency

High Throughput Assays and Exposure Science

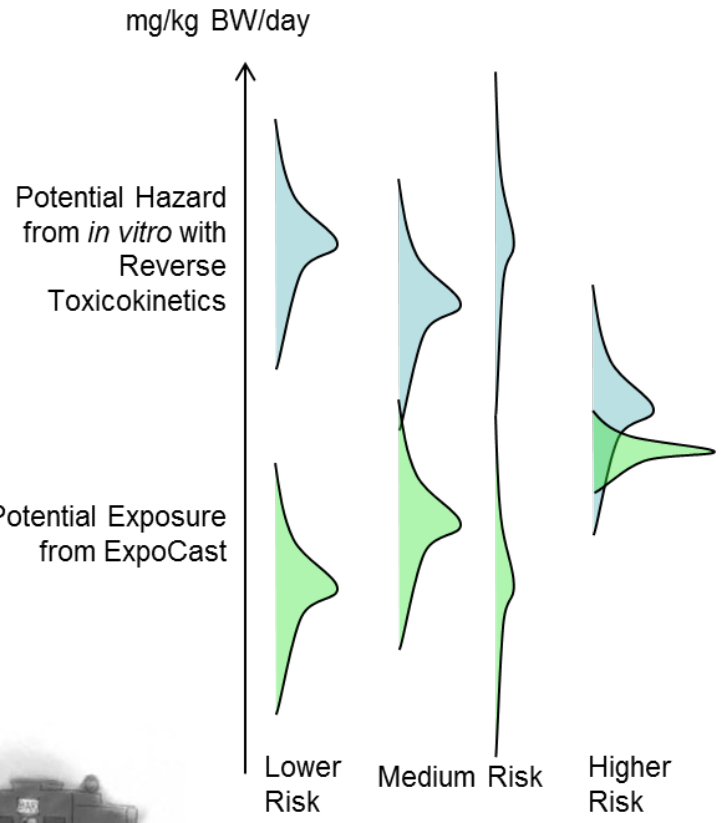
John Wambaugh
National Center for Computational Toxicology
Office of Research and Development
U.S. Environmental Protection Agency

Evolving Analytical Methods
International Society for Exposure Science Annual Meeting
Henderson, NV

October 21, 2015

Introduction

- The timely characterization of the human and ecological risk posed by thousands of existing and emerging commercial chemicals is a critical challenge
- **High throughput risk prioritization** relies on **three components** – high throughput **hazard** characterization, high throughput **exposure** forecasts, and high throughput **pharmacokinetics** (*i.e.*, dosimetry)
- While advances have been made in HT toxicity screening, exposure methods applicable to 1000s of chemicals are needed
- With non-targeted/suspect screening we now have the tools to provide monitoring data greatly beyond the "looking under the lamp post"

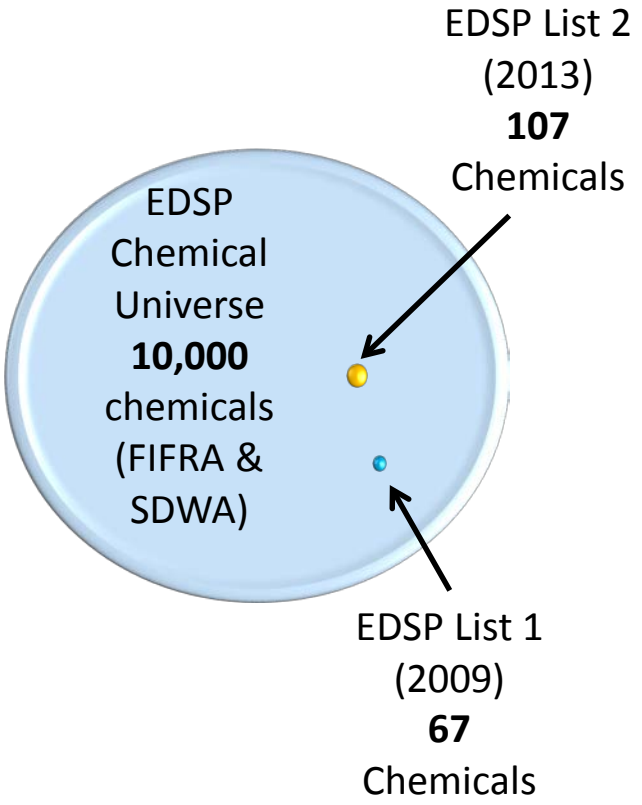


"I'm searching for my keys."

Endocrine Disruptor Screening Program (EDSP)

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

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**

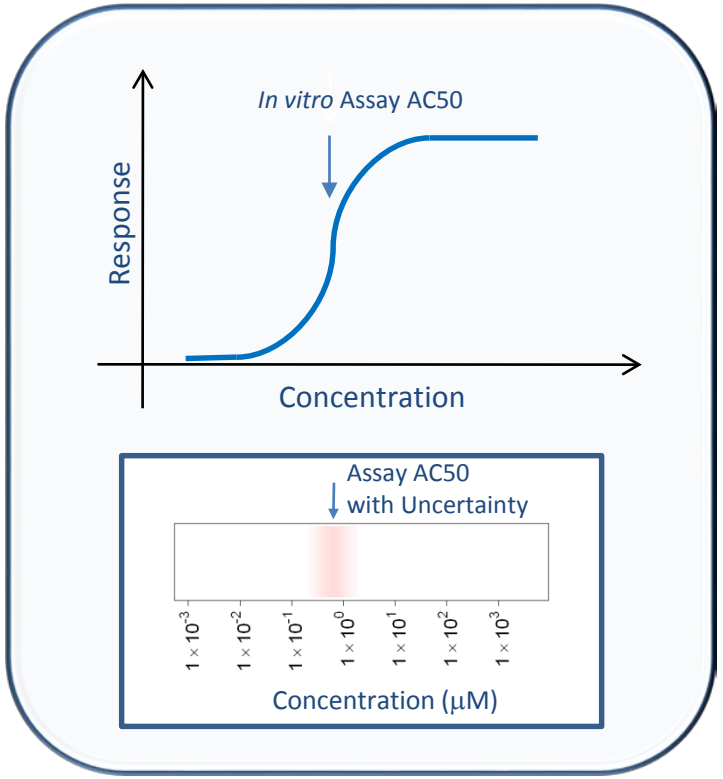


So far 67 chemicals have completed testing and an additional 107 are being tested

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

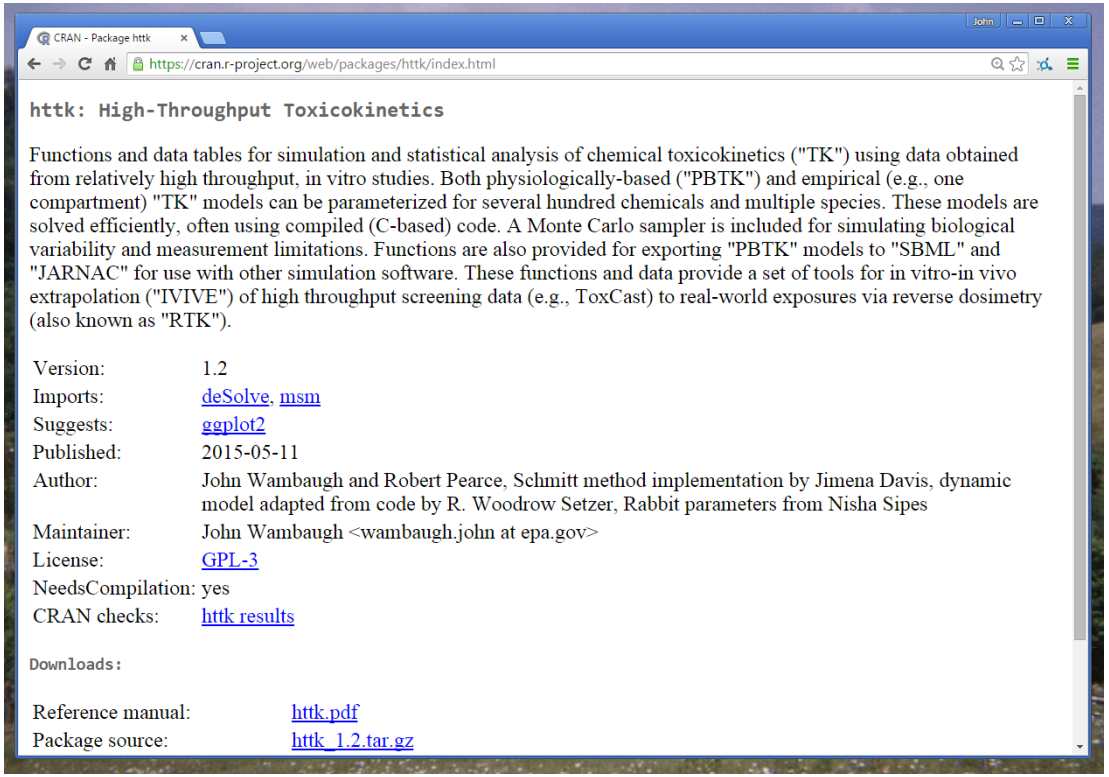
High-Throughput Bioactivity

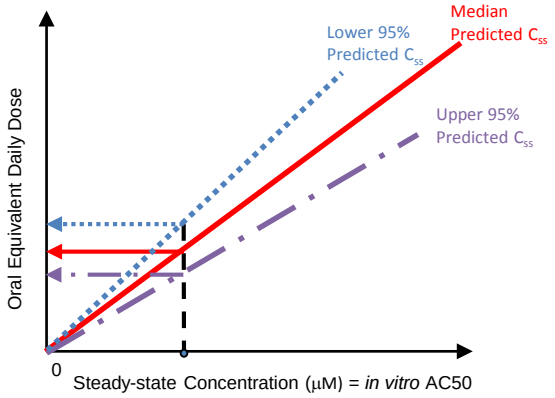
- **Tox21**: Examining >10,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)
- **ToxCast**: For a subset (>1000) of Tox21 chemicals ran >500 additional assays (Judson et al., 2010)
- Most assays conducted in dose-response format (identify 50% activity concentration – AC50 – and efficacy if data described by a Hill function)
- All data is public: http://actor.epa.gov/



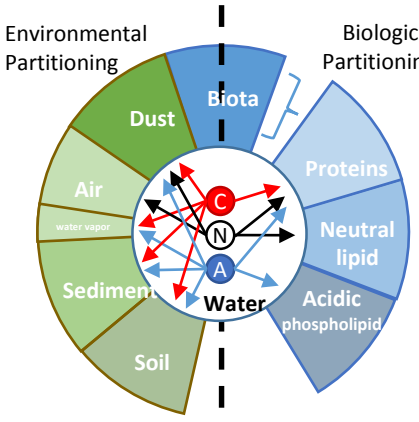
High-Throughput Toxicokinetics

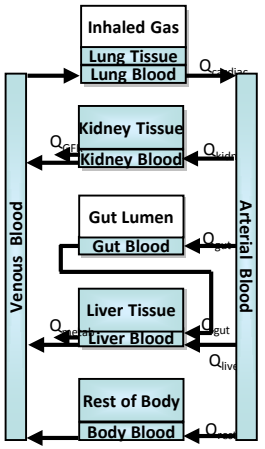
United States Environmental Protection Agency





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



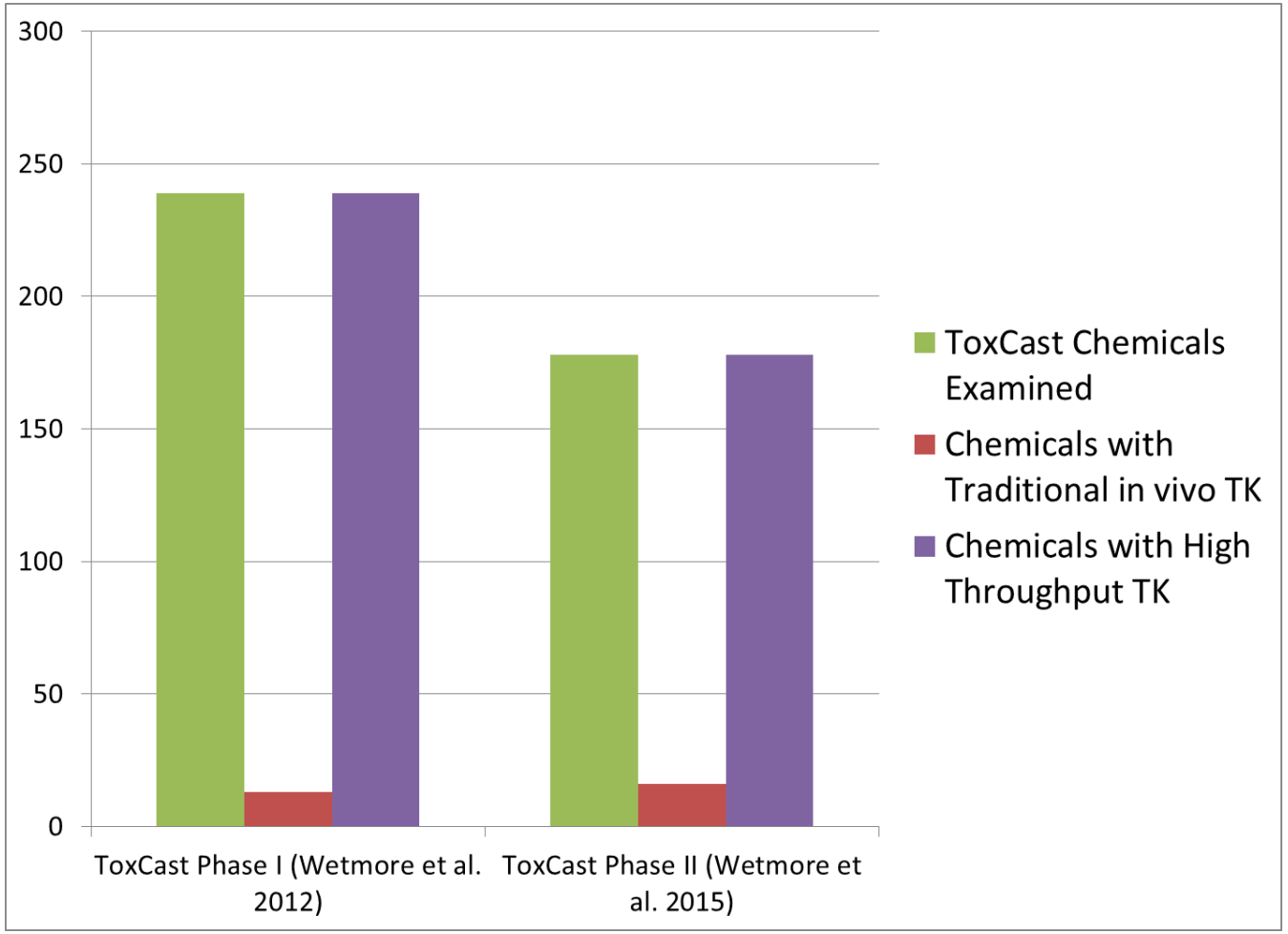


"httk" R Package
Lead programmer Robert Pearce
Wambaugh *et al.* (2015), Pearce *et al.* submitted

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

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

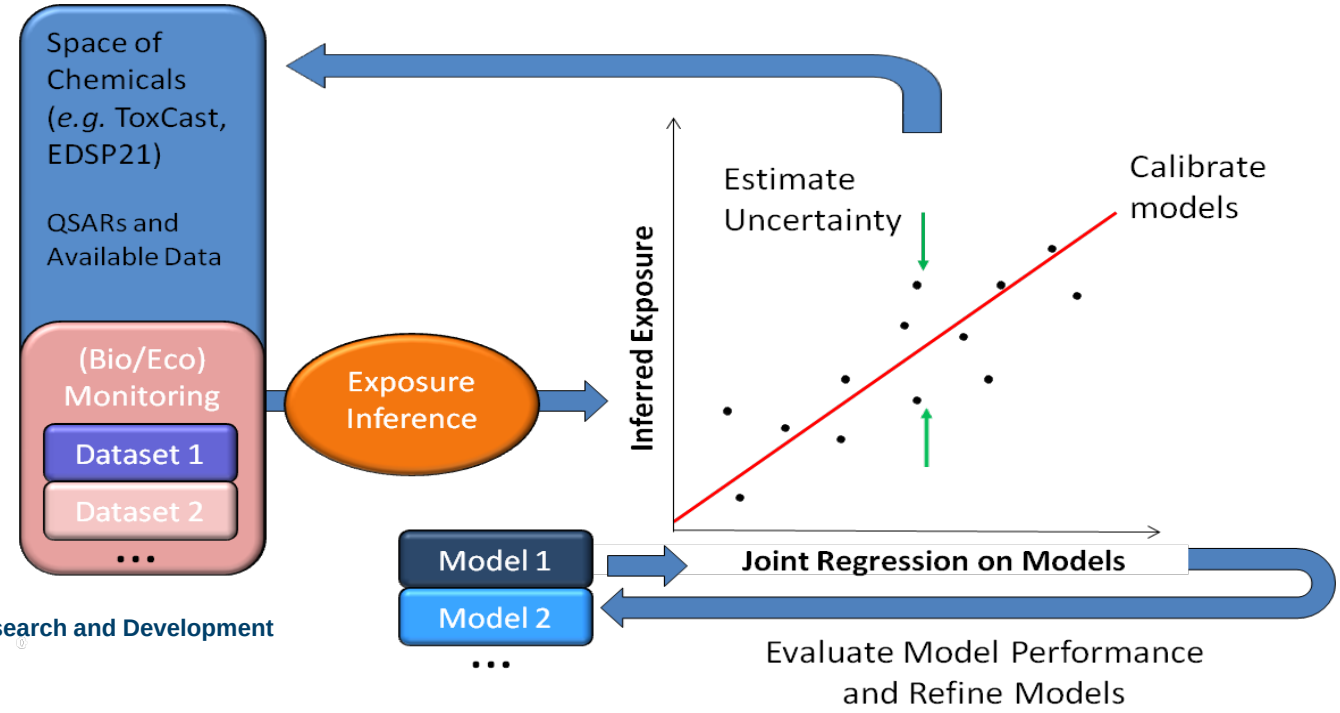
In Vitro Bioactivity, *In Vitro* Toxicokinetics, and Exposure



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

Consensus Exposure Predictions with the SEEM Framework

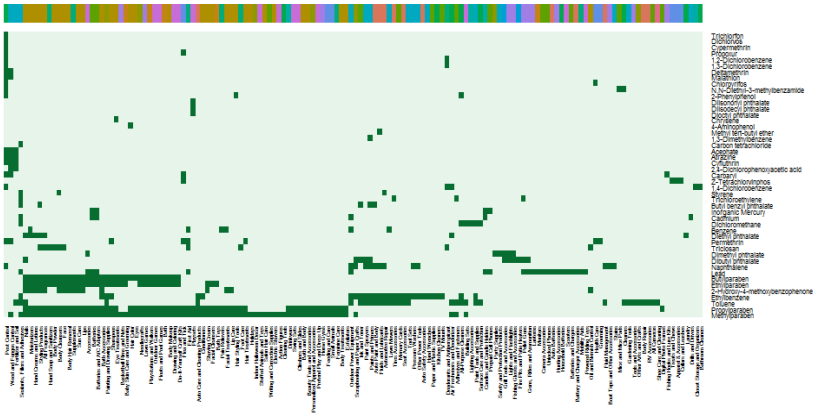
- Incorporate multiple models into consensus predictions for 1000s of chemicals within the **Systematic Empirical Evaluation of Models (SEEM) framework** (Wambaugh et al., 2013, 2014)
- Evaluate/calibrate predictions with available monitoring data across as many chemical classes as possible to allow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



Chemical Use Identifies Relevant Pathways

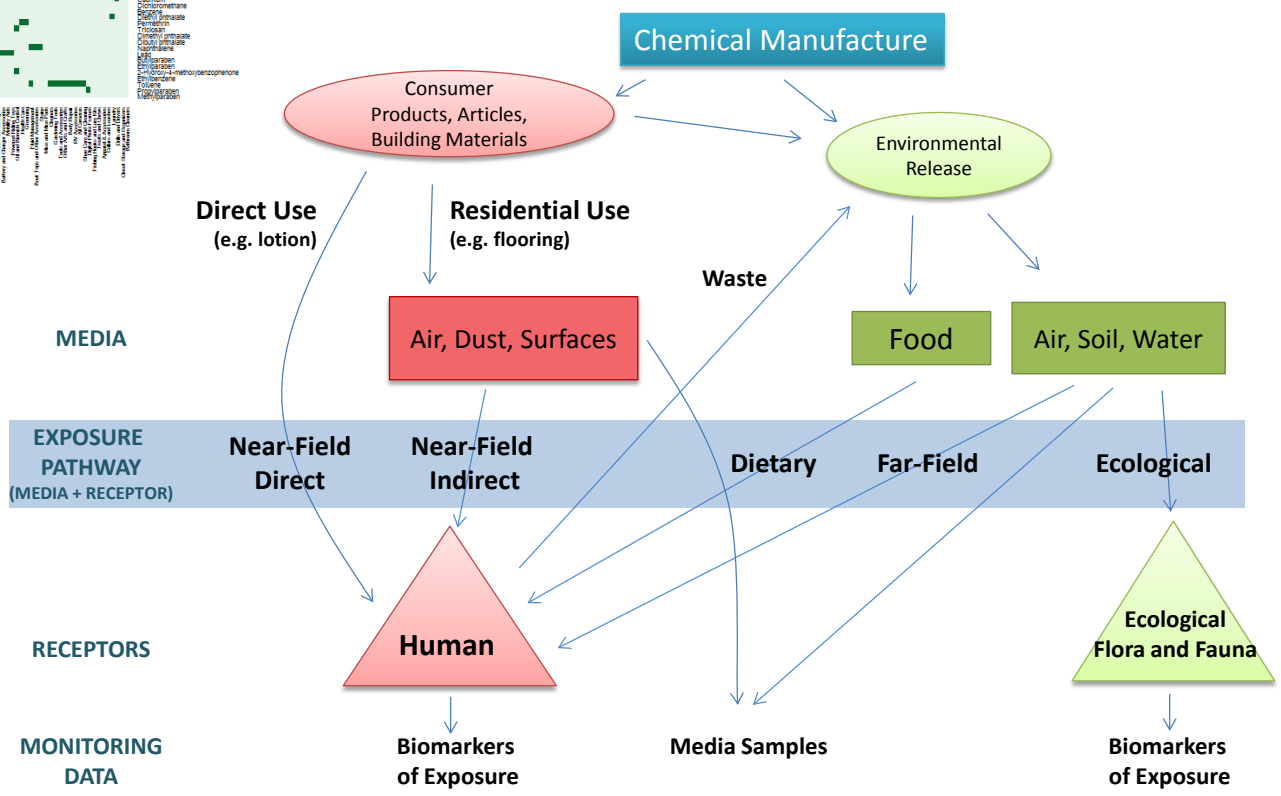
>2000 chemicals with Material Safety Data Sheets (MSDS) in CPCPdb (Goldsmith *et al.*, 2014)

105 NHANES Chemicals

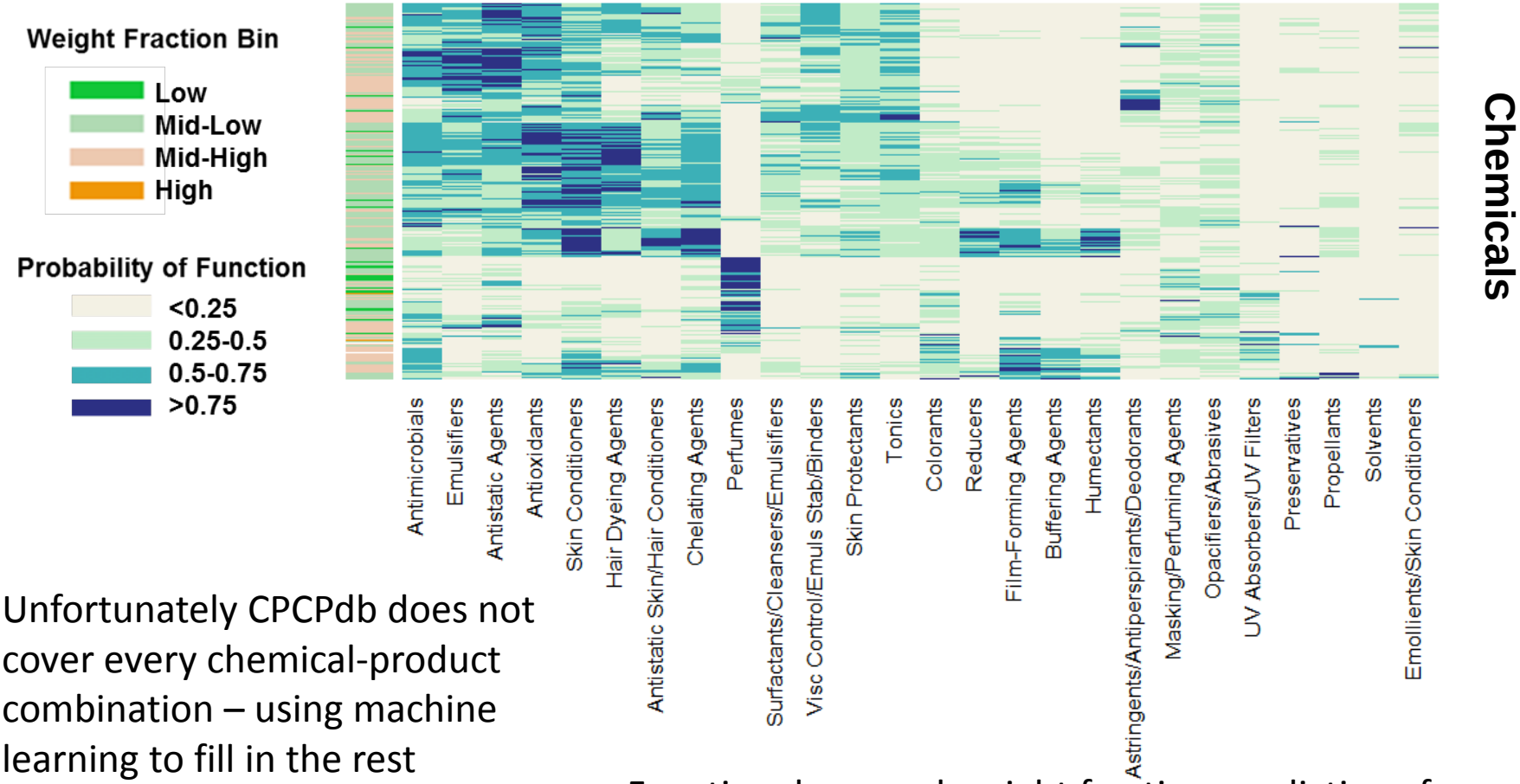


- Apparel
- Auto and Tires
- Baby
- Beauty
- Craft and Party Supply
- Electronics
- Grocery
- Health
- Home
- Home Improvement
- Patio and Garden
- Pets
- Sports and Outdoors
- Toys

Predictions with High Throughput Stochastic Human Exposure Dose Simulator (SHEDS-HT) (Isaacs *et al.*, 2014)



Predicting Chemical Constituents



- Unfortunately CPCPdb does not cover every chemical-product combination – using machine learning to fill in the rest
- Functional use and weight fraction predictions for Tox21 chemical library

Isaacs et al. (submitted)

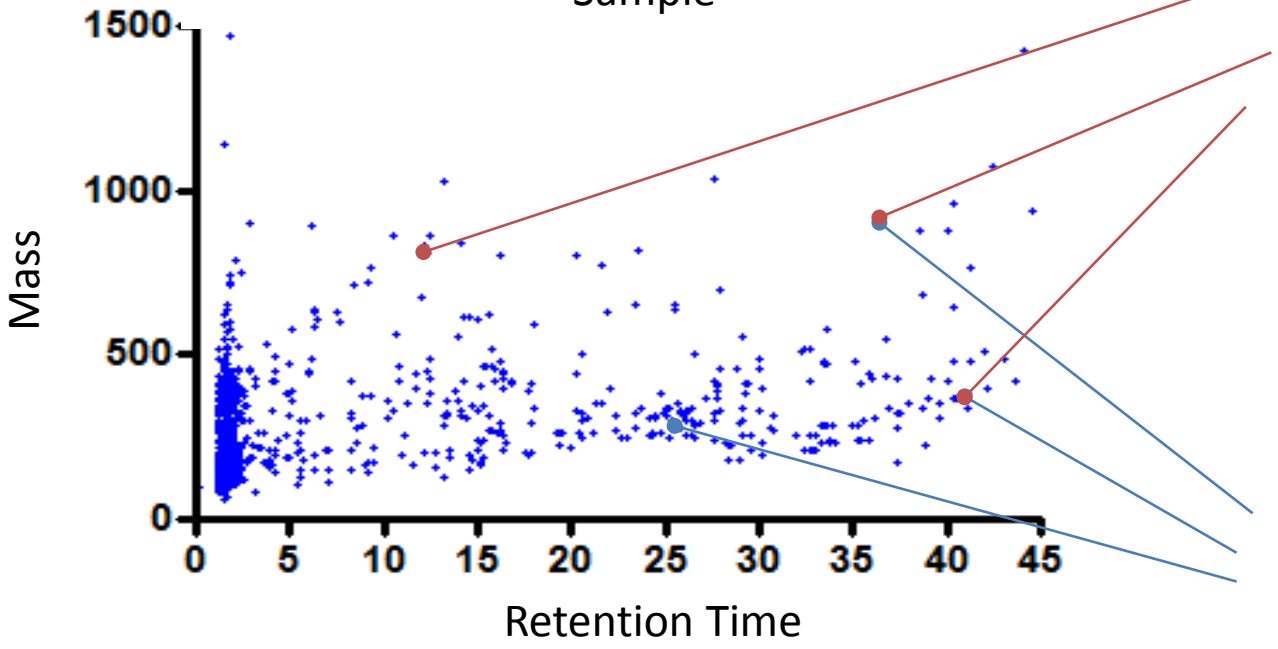
EPA United States Environmental Protection Agency

Analytical Chemistry Methods

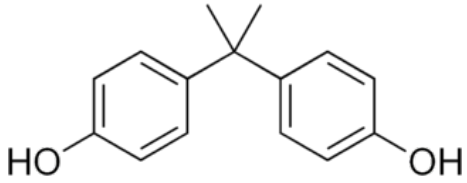
- At least two methods are typically needed to quantify concentration of ToxCast chemicals
  - Liquid chromatography (LC) and gas chromatography (GC) mass spectrometry (MS)
- Typically would have a calibration curve to relate MS signal to concentration
  - HTTK *in vitro* assays designed to work using only ratios of signal, so no calibration needed (in theory)
- Different non-targeted methods have been developed
  - Can try to relate everything to signal for known standard chemical concentration
- Different machines require different calibrations, but many aspects for a chemical should generalize (e.g., GC vs. LC)
  - Need to develop a methods database

Suspect Screening with Non-Targeted Analytical Chemistry

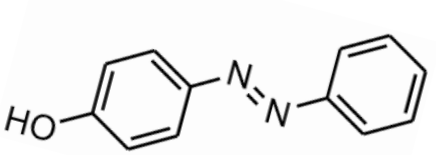
947 Peaks in an American Health Homes Dust Sample



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?

We are now expanding our identity libraries using reference samples of ToxCast chemicals

Rager et al. (submitted) with Jon Sobus and Mark Strynar

Exposure Screening Tools for Accelerated Chemical Prioritization (ExpoCast)

- Contracts were awarded (December, 2014) to Southwest Research Institute and Battelle
- Phase I (Pilot) Examining capabilities and feasibility

Assay	Unit	Pilot Order	Contractor Lead Researcher	Lead EPA Post-Docs
High Throughput Screening-Level Physico-Chemical Properties Measurement (VP, pKa, Henry's Law, Kow)	compound	200	Alice Yau (SWRI)	Chantel Nicolas and Kamel Mansouri
Determine Chemical Constituents of products, materials, articles (screening level)	test object	20 classes of product, 5 samples each	Alice Yau (SWRI)	Katherine Phillips
Determine chemical emission rate from specific products, materials, articles	test object	100	Anne Louise Sumner and Tom Kelly (Battelle)	Chantel Nicolas
Screening for occurrence of large numbers of chemicals in sample acquired by contractor (biological media)	sample	500 blood samples (likely from Indianapolis)	Anne Gregg (Battelle)	Caroline Ring

Pilot 2: Determining Chemical Constituents

- Broad screening for ToxCast chemical library compounds in consumer products. Test objects will consist of five products selected by contractor in each of the following twenty consumer products and article of commerce categories
- Research conducted by Southwest Research Institute (Alice Yau)

1.Cotton Clothing (new shirt)
2.Shampoo
3.Toothpaste
4. Skin Lotion
5. Vinyl upholstery
6. Fabric upholstery
7. Hand Soap
8. Baby Soap
9. Shaving cream
10. Lipstick
11. Indoor house paint
12. Plastic children's toys
13. Glass cleaner
14. Air freshener
15. Deodorant
16. Shower curtains
17. Breakfast cereal
18. Carpet
19. Carpet Padding
20. Sunscreen

- Two Extraction Methodologies:
  - DCM and Hexane:Ether
- One sample in each category processed in duplicate
- Surrogates (s) and internal standards (is) spiked into each sample

Plastic Baby Toy Preliminary Results

	Product 1		Product 2		Product 3		Product 4		Product 5	
Category	Number	Peak Area	Number	Peak Area	Number	Peak Area	Number	Peak Area	Number	Peak Area
Reported peaks with reviewed library matches	98	0.0037	106	0.0018	114	0.62	67	0.42	56	0.24
Bisphenol A	0	0.0000	1	0.0000	0	0.00	0	0.00	0	0.00
Reported unknowns (>500,000)	11	0.0012	40	0.0010	27	0.20	0	0.00	1	0.00
Confirmed Hydrocarbons (n-alkanes)*	7	0.0000	5	0.0012	20	0.01	20	0.06	21	0.08
Unconfirmed Hydrocarbons C10-C16	171	0.0008	245	0.0143	141	0.05	117	0.11	109	0.22
Unconfirmed Hydrocarbons C17-C32	--	--	--	--	181	0.05	261	0.35	243	0.38
Unresolved C17-C32	2457	0.9942	1934	0.9813	--	--	--	--	--	--
Excluded unknowns (<500,000)	37	0.0001	120	0.0003	66	0.00	52	0.02	48	0.02
Excluded non-specific (<500,000)	1	0.0000	0	0.0000	11	0.00	17	0.00	14	0.00
Excluded trace (<100,000) and similarity < 850	36	0.0000	32	0.0000	118	0.00	116	0.02	123	0.02
Excluded artifacts	1	0.0000	4	0.0000	34	0.07	16	0.02	7	0.03
Total	2819	1.0000	2486	1.0000	712	1.00	666	1.00	622	1.00

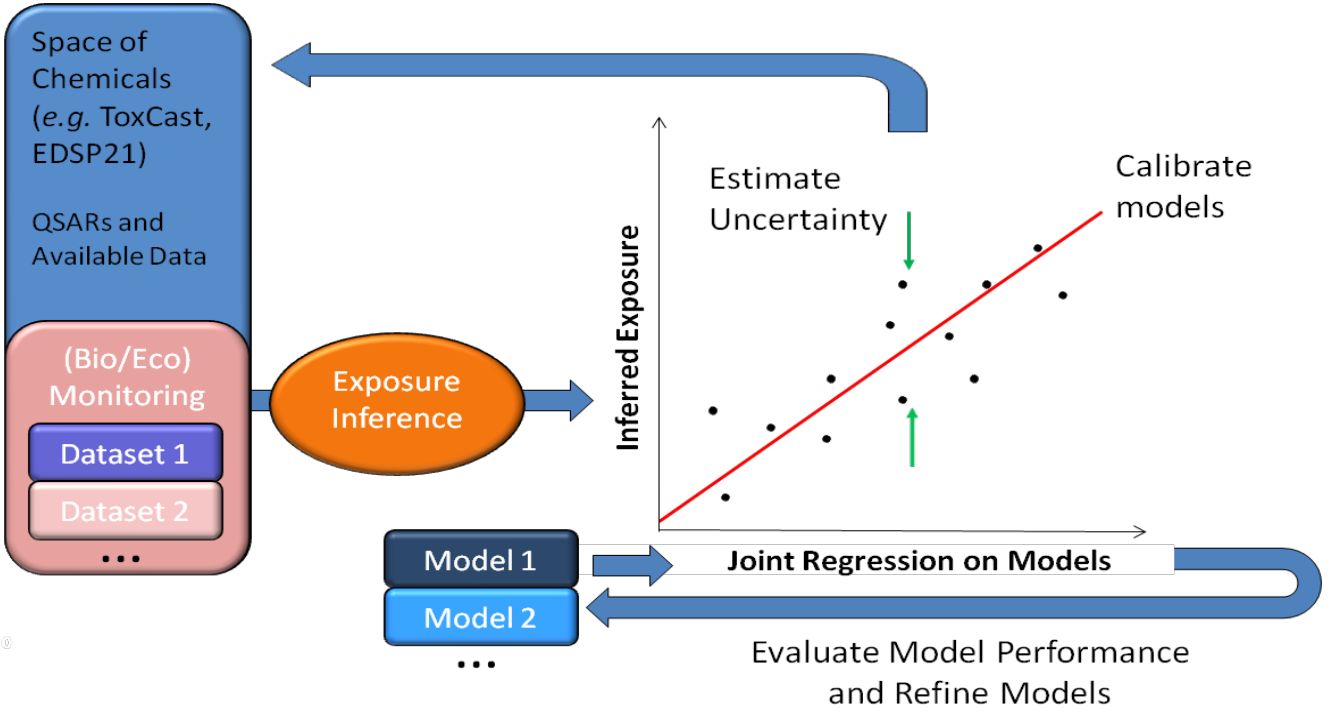
Results from Alice Yau (SWRI)

Pilot 4: Biomonitoring

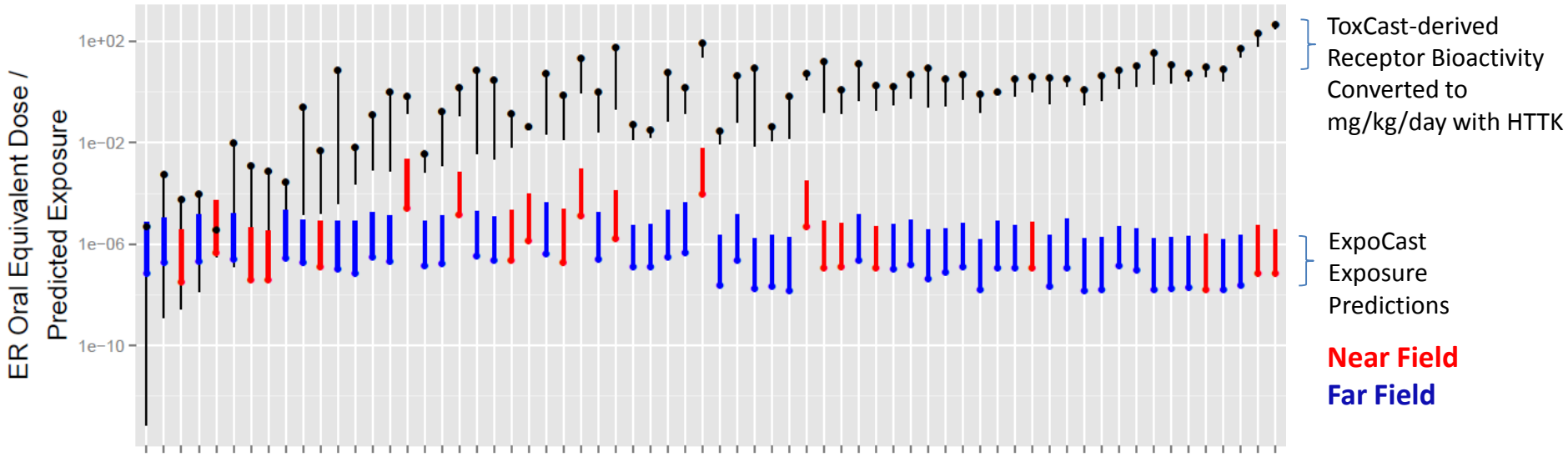
- Screening for occurrence of large numbers of chemicals in sample acquired by contractor (biological media)
- Research Conducted by Battelle Memorial Institute (Anne Gregg)
- Cohort is a mixed gender and race group of adults from Indianapolis
- Sample Screening
  - One extraction method resulting in 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

Consensus Exposure Predictions with the SEEM Framework

- Better chemical use data informs models predicting exposure
- Broader monitoring data informs evaluation of those predictions



Conclusion: Exposure for High Throughput Risk Prioritization



Prioritization as in Wetmore *et al*. (2012) Bioactivity, Dosimetry, and Exposure Paper

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

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

NCCT
Chris Grulke
Richard Judson
Dustin Kapruan*
Chantel Nicolas*
Robert Pearce*
James Rabinowitz
Ann Richard
Caroline Ring*
Woody Setzer
Rusty Thomas
John Wambaugh
Antony Williams

NRMRL
Yirui Liang*
Xiaoyu Liu

NHEERL
Jane Ellen Simmons
Marina Evans
Mike Hughes

*Trainees

NERL
Craig Barber
Brandy Beverly*
Derya Biryol*
Kathie Dionisio
Peter Egeghy
Kim Gaetz
Brandall Ingle*
Kristin Isaacs
Katherine Phillips*
Paul Price
Mark Strynar
Jon Sobus
Mike Tornero-Velez
Elin Ulrich
Dan Vallero

Collaborators

Arnot Research and Consulting
Jon Arnot
Battelle Memorial Institute
Anne Louise Sumner
Anne Gregg
Chemical Computing Group
Rocky Goldsmith
Hamner Institutes
Barbara Wetmore
Cory Strope
National Institute for Environmental Health Sciences (NIEHS)
Mike Devito
Nisha Sipes
Kyla Taylor
Kristina Thayer
Netherlands Organisation for Applied Scientific Research (TNO)
Sieto Bosgra
Research Triangle Institute
Timothy Fennell
Silent Spring Institute
Robin Dodson
Southwest Research Institute
Alice Yau
Kristin Favela
University of California, Davis
Deborah Bennett
University of Michigan
Olivier Jolliet
University of North Carolina, Chapel Hill
Alex Tropsha