EPA
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Environmental Protection
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Bottom-Up Exposomics for Ensuring Chemical Safety

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Office of Research and Development
Center for Computational Toxicology and Exposure

Jan 24, 2020

EPA United States Environmental Protection Agency

Presentation Outline

- The Exposome: State-of-the-Science
- Top-Down vs. Bottom-Up Exposomics
- Bottom-Up Exposomics for Ensuring Chemical Safety
- Non-Targeted Analysis (NTA) within EPA's ORD
- Efforts to Evaluate and Harmonize NTA Methods
- Perspectives for the Future of Exposomics

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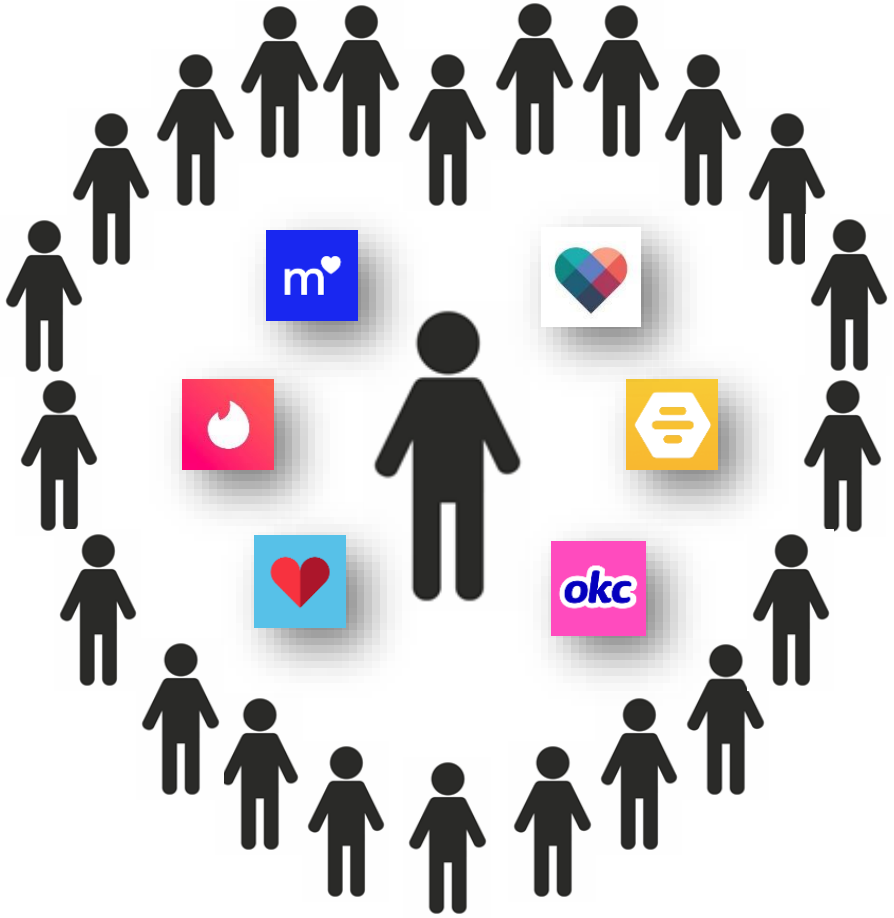
Exposomics:
A Paradigm Shift for Exposure and Health Sciences

Traditional Research : Courtship **::** **Exposomics : Online Dating**



15 Years Later… How Far Have We Come?

Books

Centers

Editorial

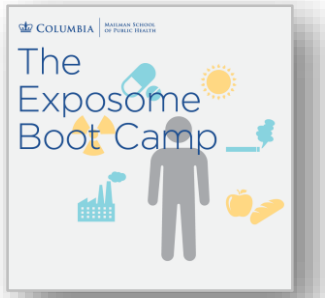
Cancer Epidemiol Biomarkers Prev 2005;14(8). August 2005

Complementing the Genome with an "Exposome": The Outstanding Challenge of Environmental Exposure Measurement in Molecular Epidemiology

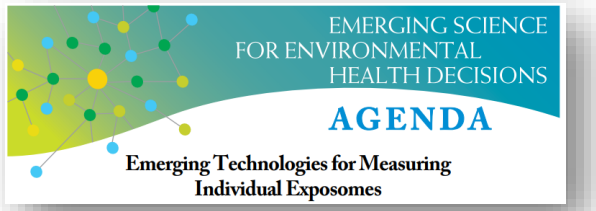
Christopher Paul Wild

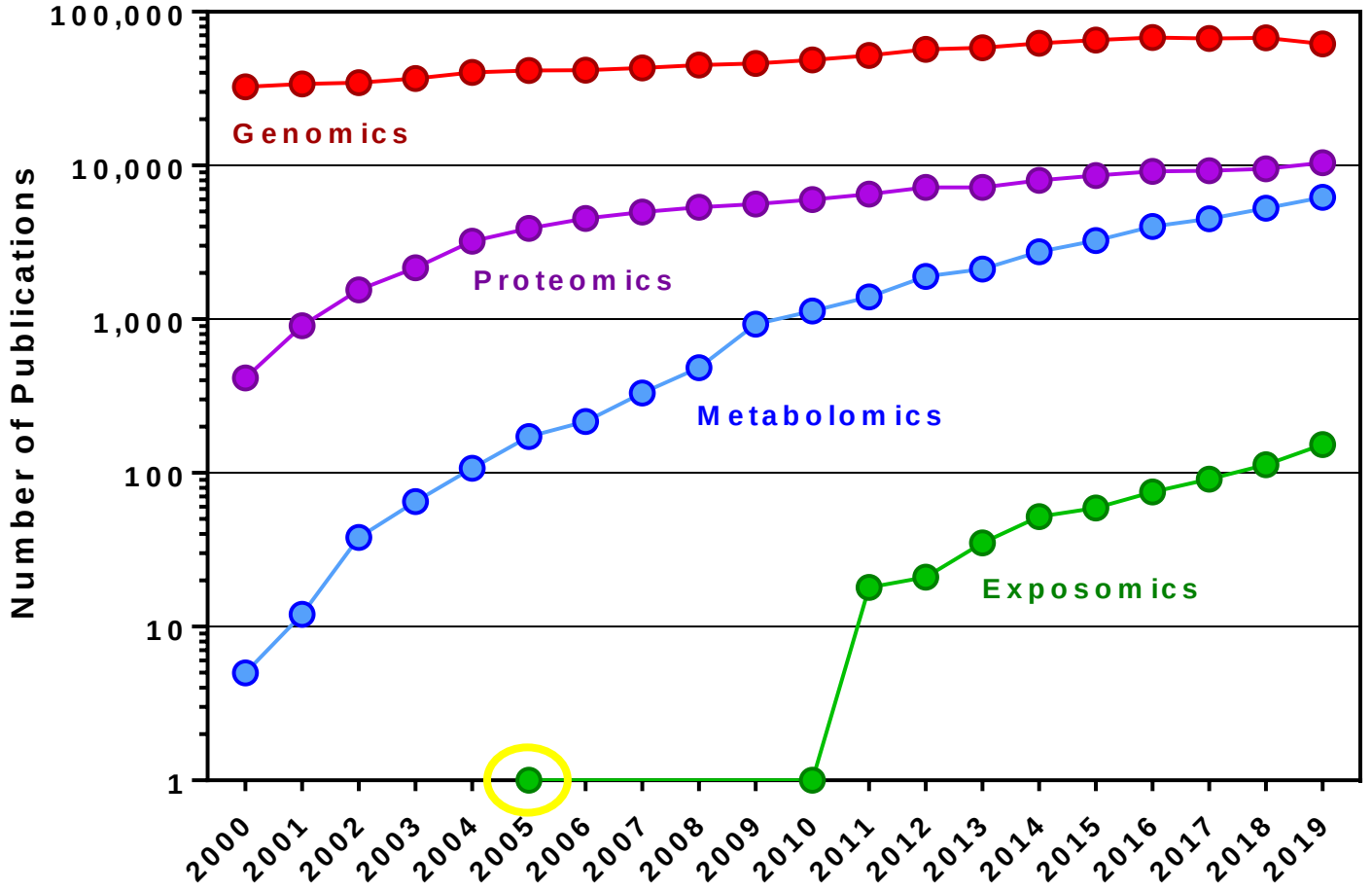
Molecular Epidemiology Unit, Centre for Epidemiology and Biostatistics, Leeds Institute of Genetics, Health and Therapeutics, Faculty of Medicine and Health, University of Leeds, Leeds, United Kingdom

Training

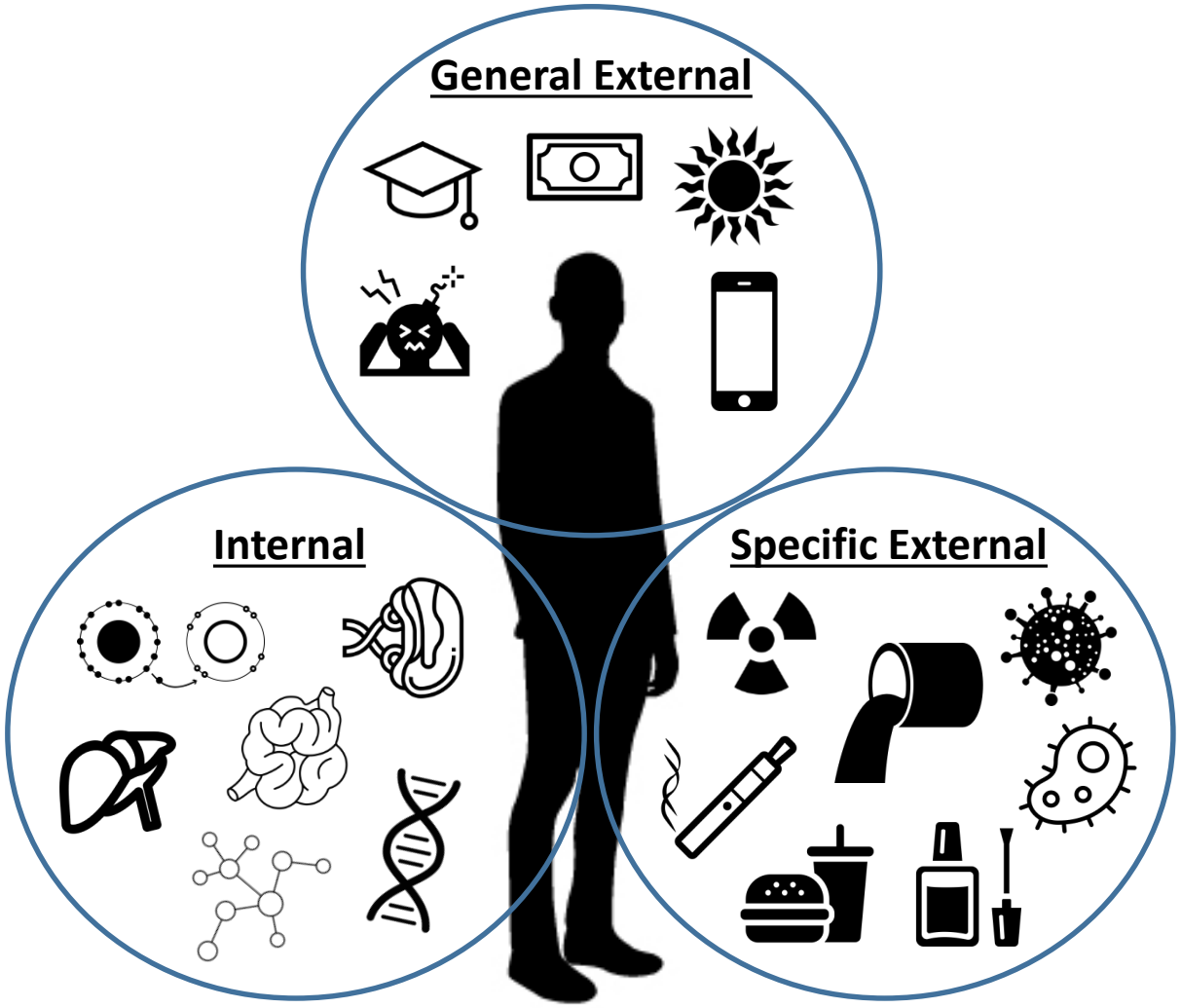


Conferences





What Are Researchers Studying?



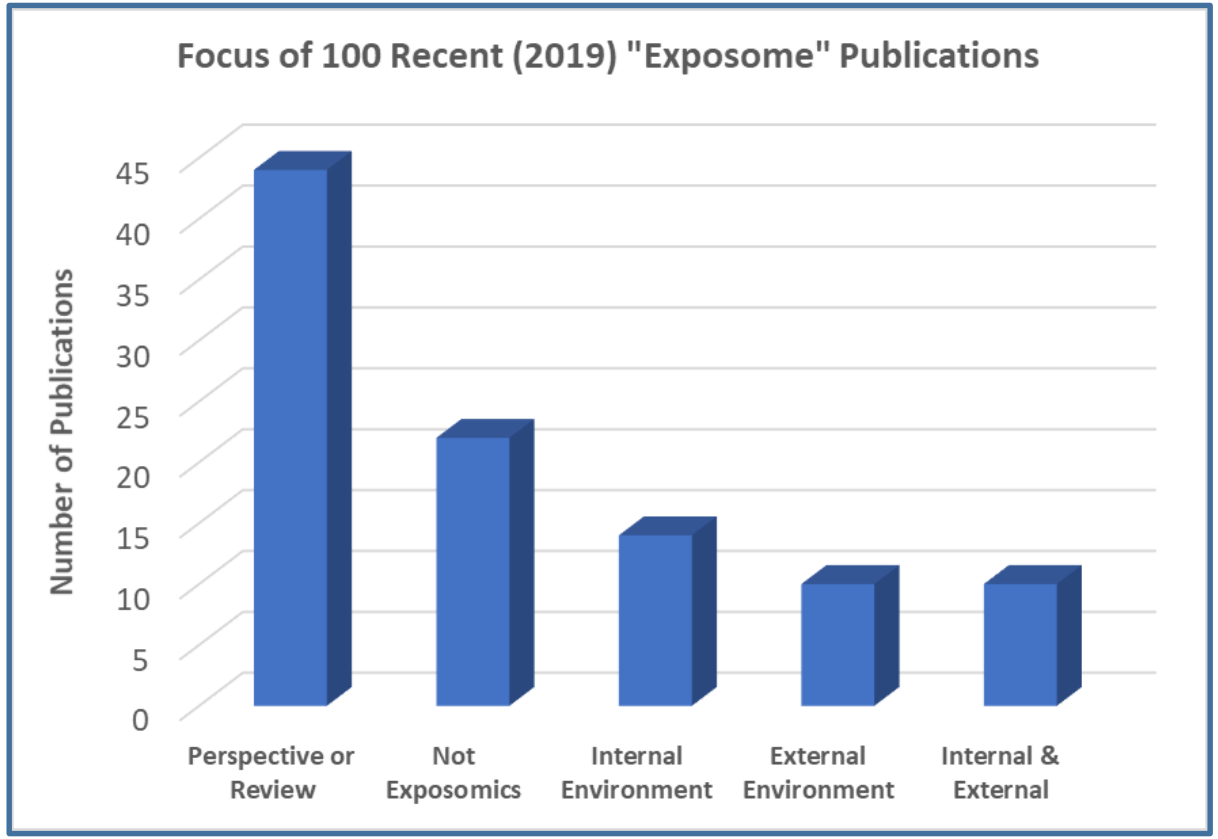
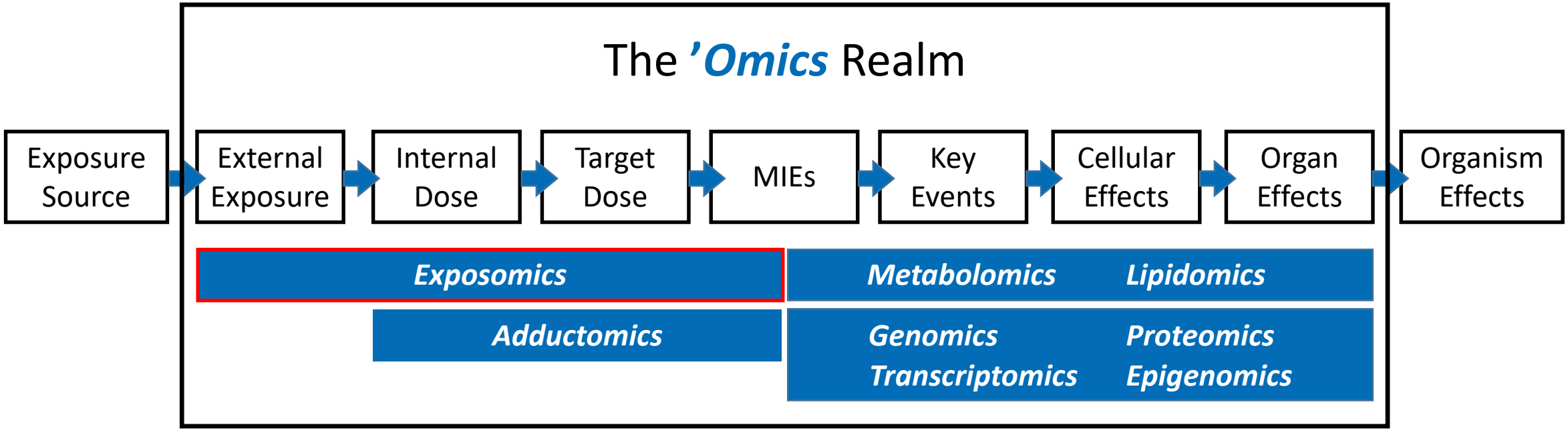


Figure adapted from: Wild CP. *Int. J. Epidemiol.* 2012 Feb;41(1):24-32

What is Different About Exposomics?

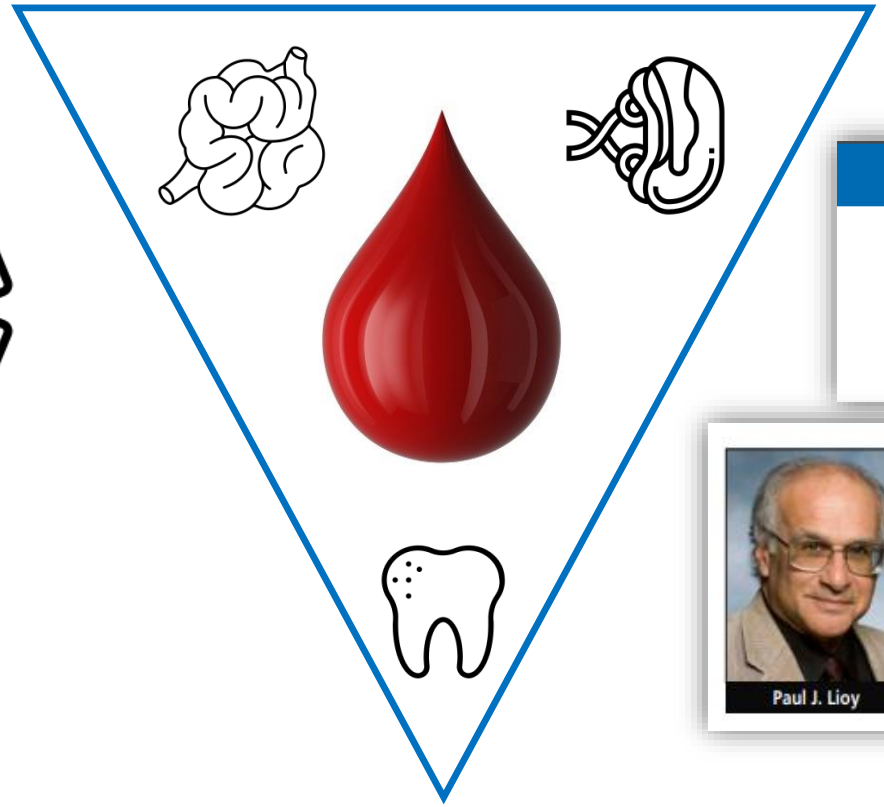


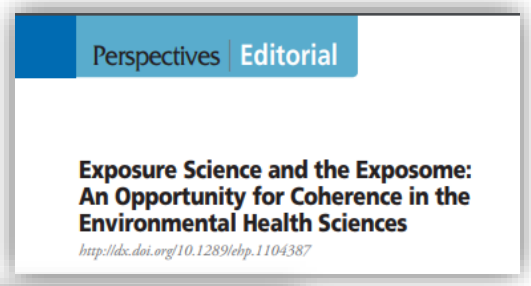
Exposomics is the one 'omics discipline that puts focus on external exposure

The inherent promise of ***Exposomics*** is therefore health protection & disease prevention

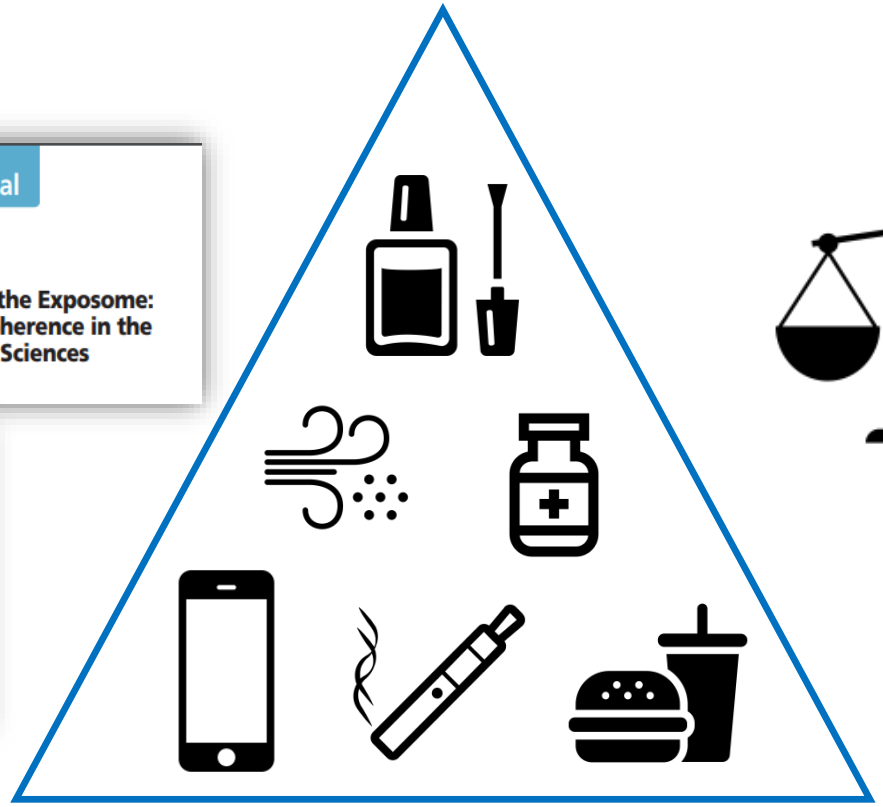
Exposomics Approaches

Top-Down Exposomics





Bottom-Up Exposomics

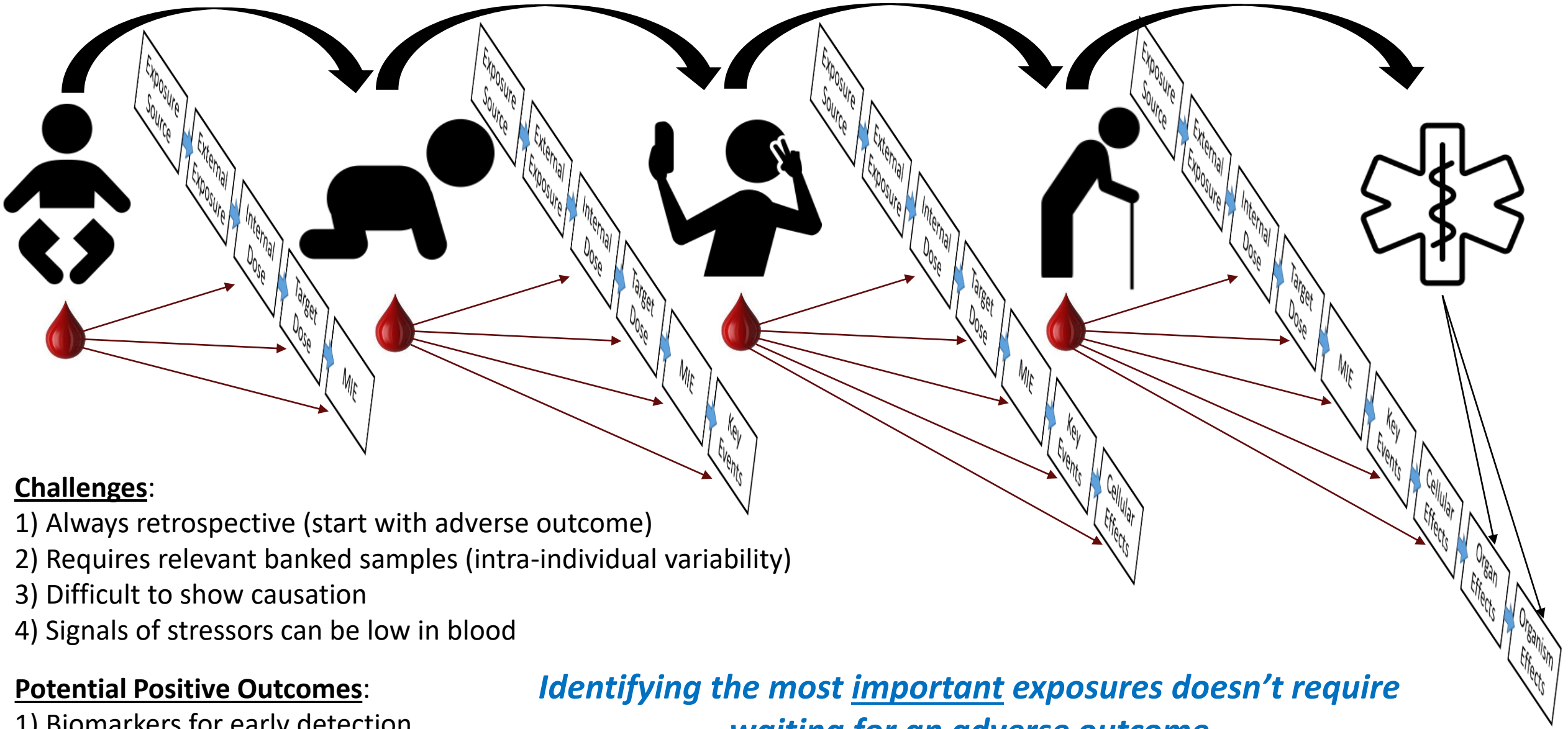


Measure Important Exposures Within the Receptor

Measure Important Exposures in All Relevant Media

Figure adapted from: Rappaport SM. *J Expo Sci Environ Epidemiol.* 2011 Jan-Feb;21(1):5-9

Challenges with Top-Down Efforts



<u>Challenges</u>:

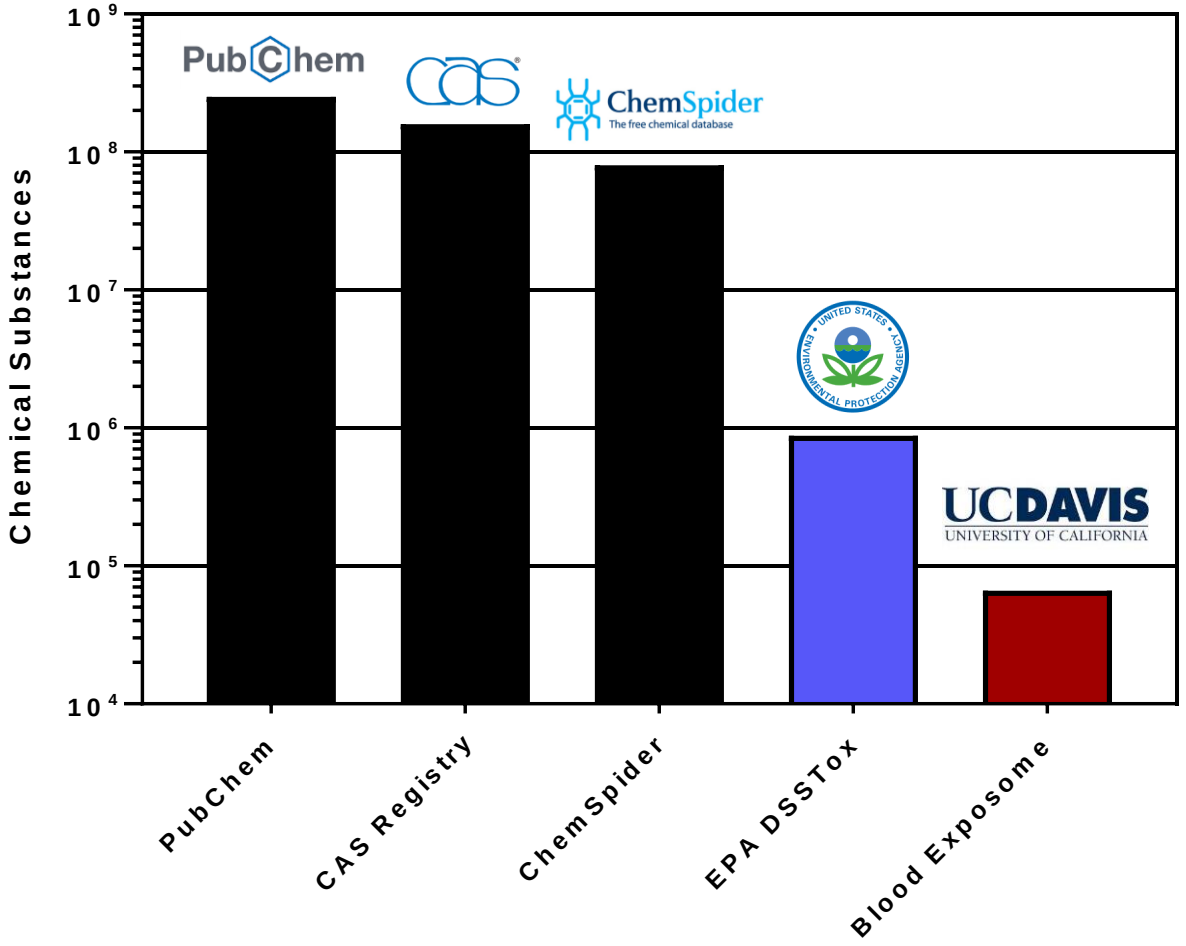
1) Always retrospective (start with adverse outcome)
2) Requires relevant banked samples (intra-individual variability)
3) Difficult to show causation
4) Signals of stressors can be low in blood

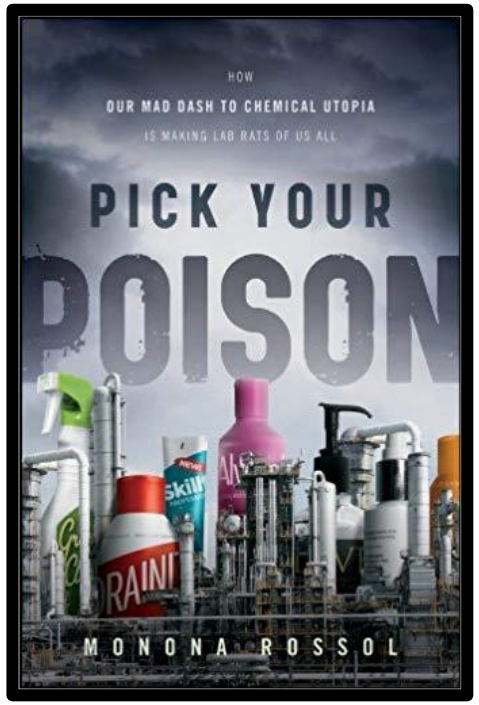
<u>Potential Positive Outcomes</u>:

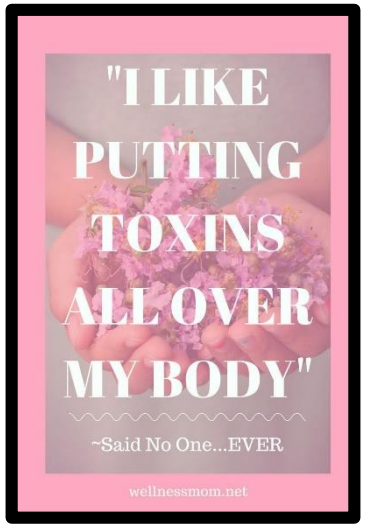
1) Biomarkers for early detection
2) Drugs for early treatment

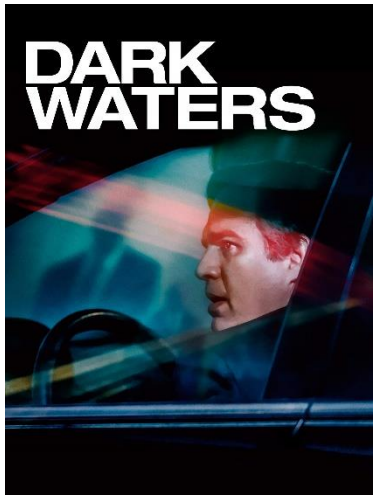
Identifying the most <u>important</u> exposures doesn't require waiting for an adverse outcome

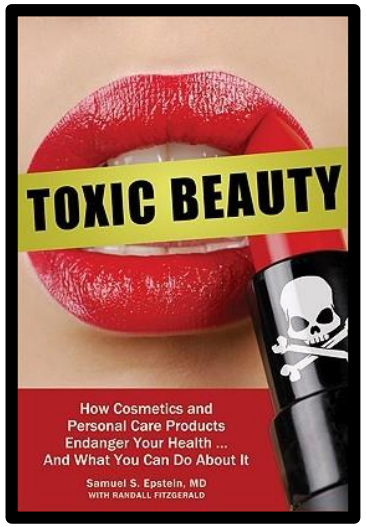
Rationale for Bottom-Up Efforts











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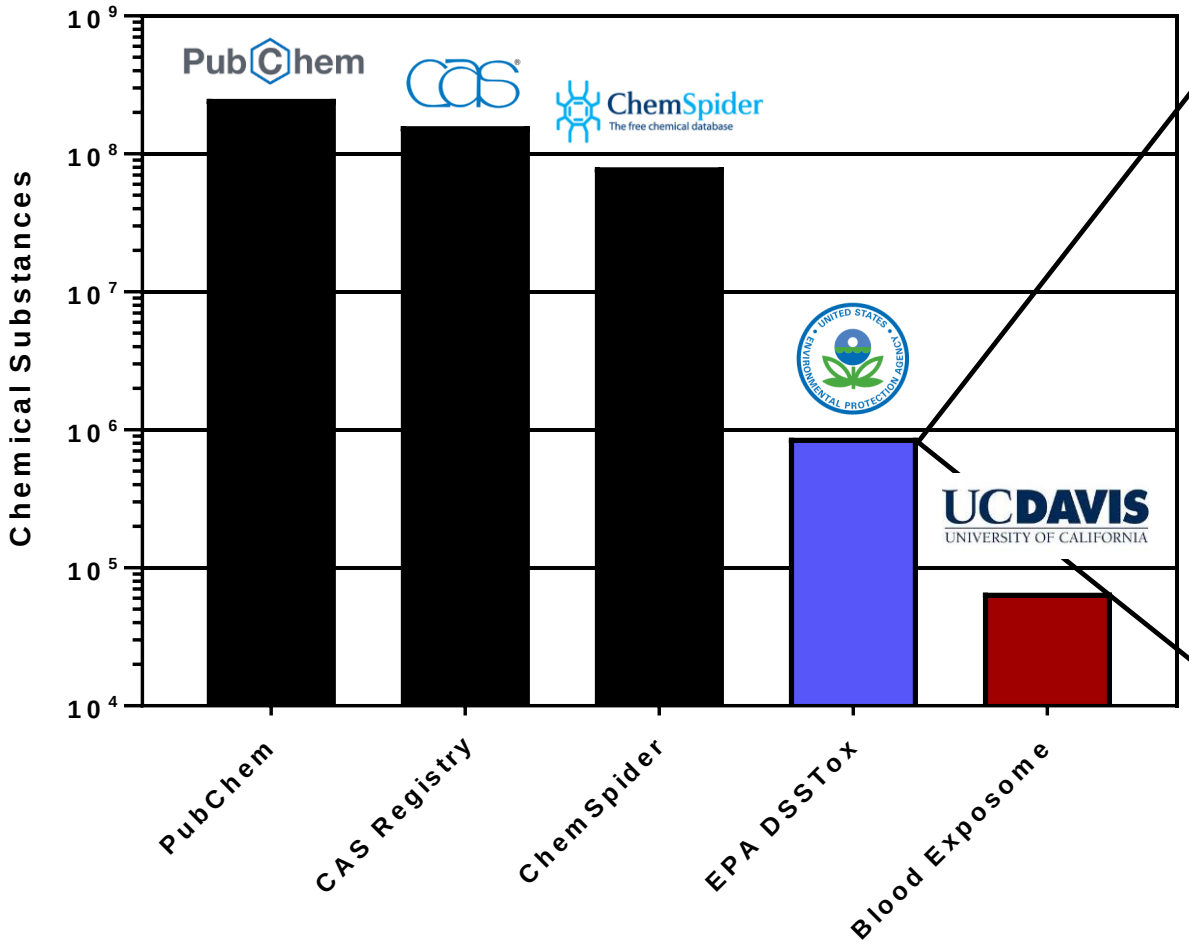

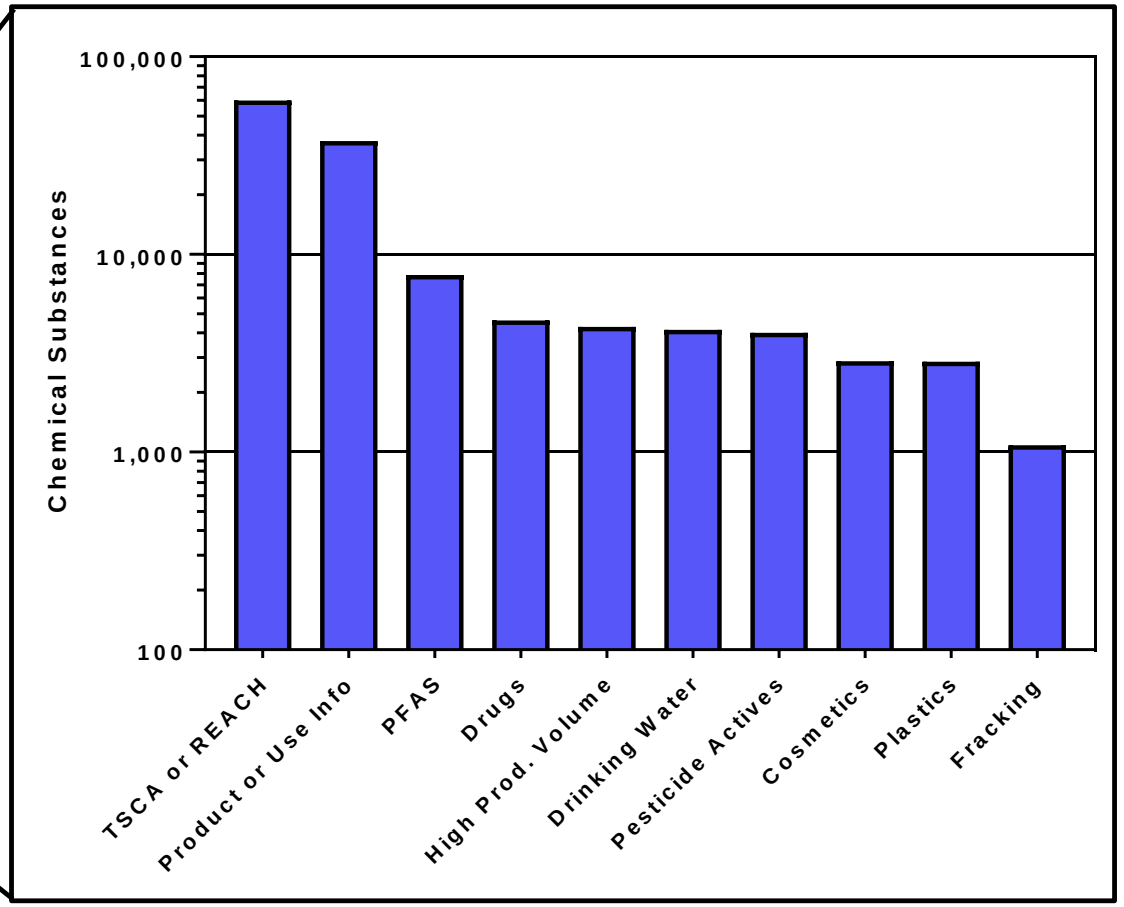

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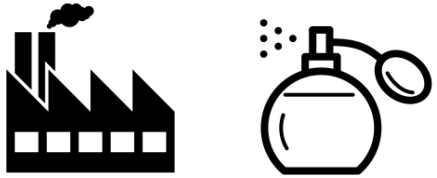
The Era of High-Throughput Assessments

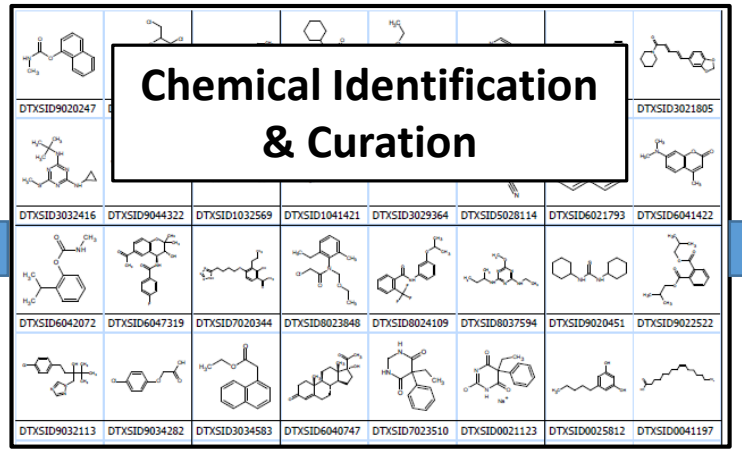
Exposure Forecasting (ExpoCast)

Toxicity Forecasting (ToxCast)

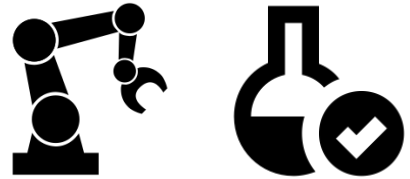
Production, Use & Scenario Data



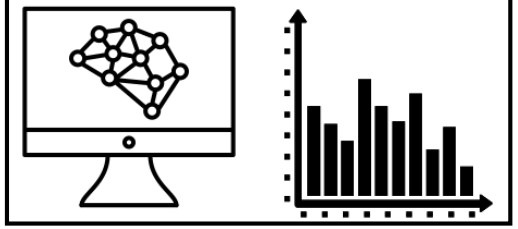
Chemical Identification & Curation



Procurement or Synthesis

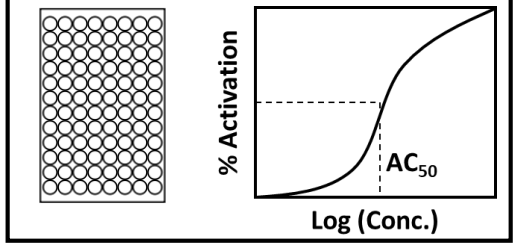


HT Exposure Prediction

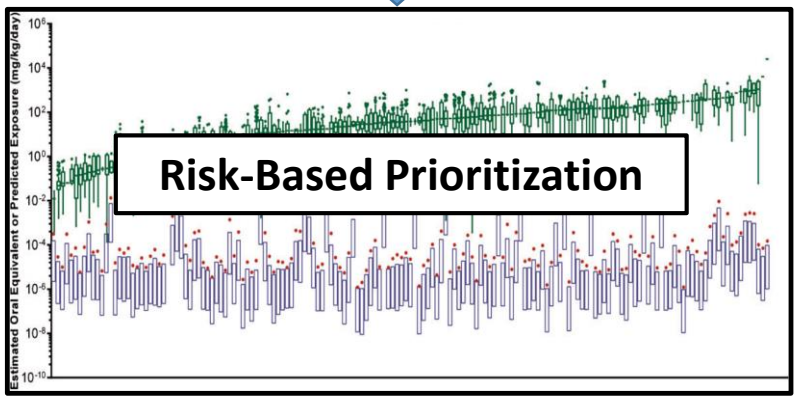


Extrapolation

HT Tox Assays



Risk-Based Prioritization



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The Era of High-Throughput Assessments

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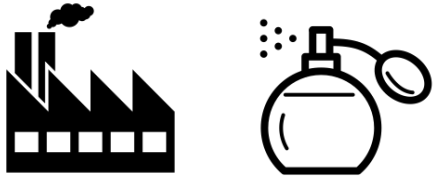
Toxicity Forecasting (ToxCast)

Production, Use & Scenario Data



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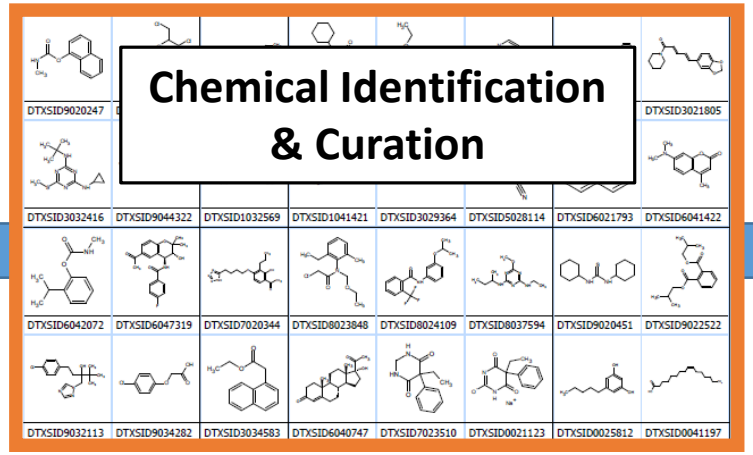
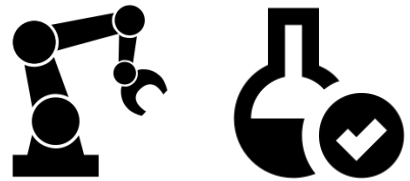


Procurement or Synthesis



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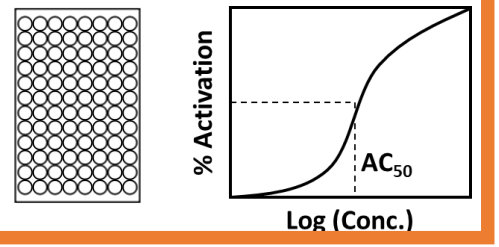

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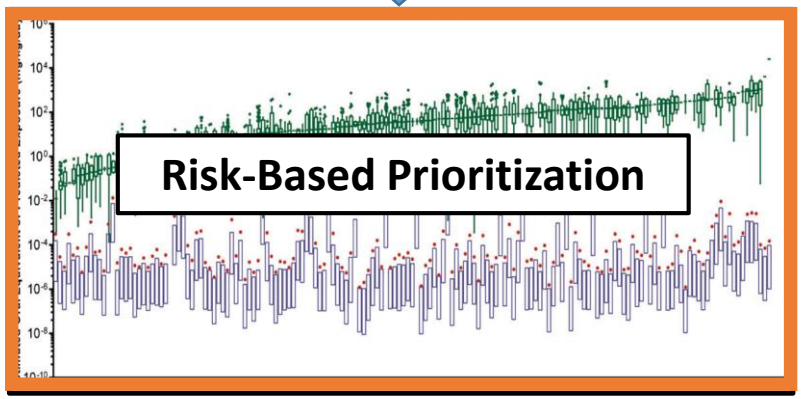
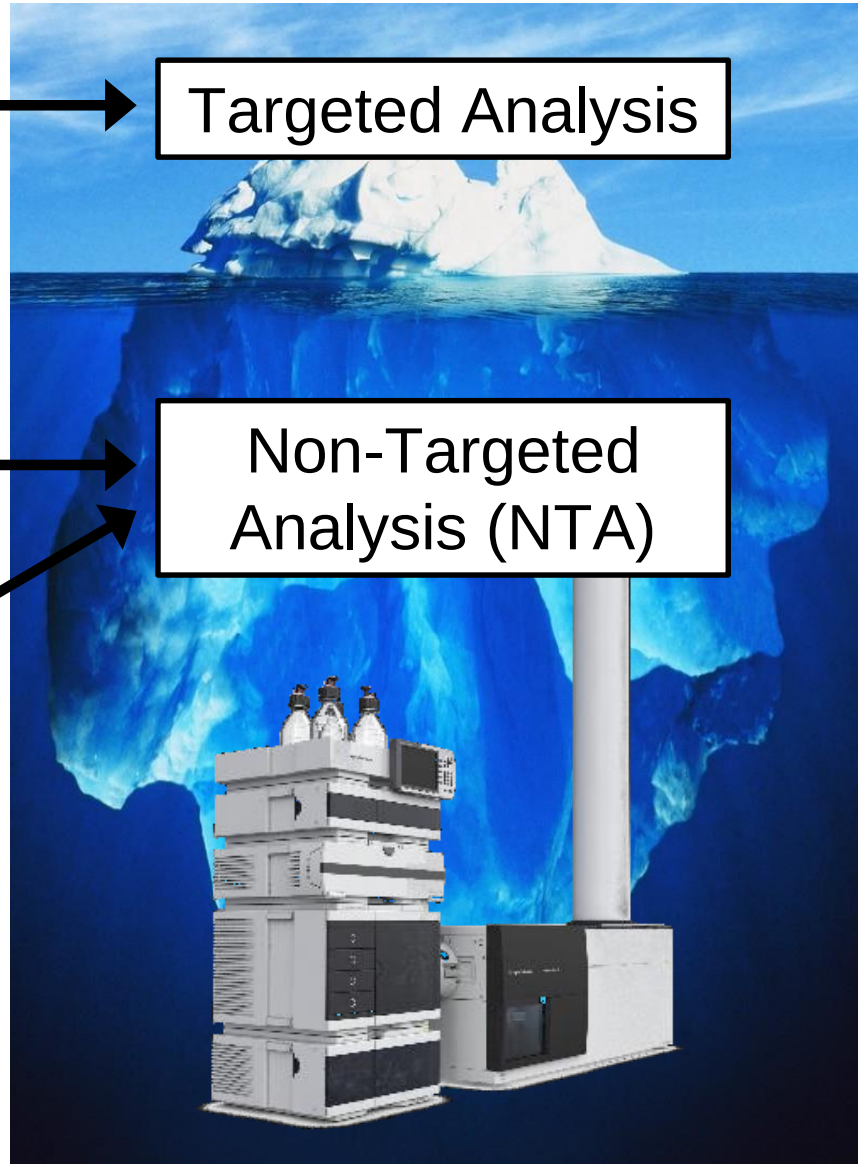


Guided and/or enhanced by Measurement Data!!

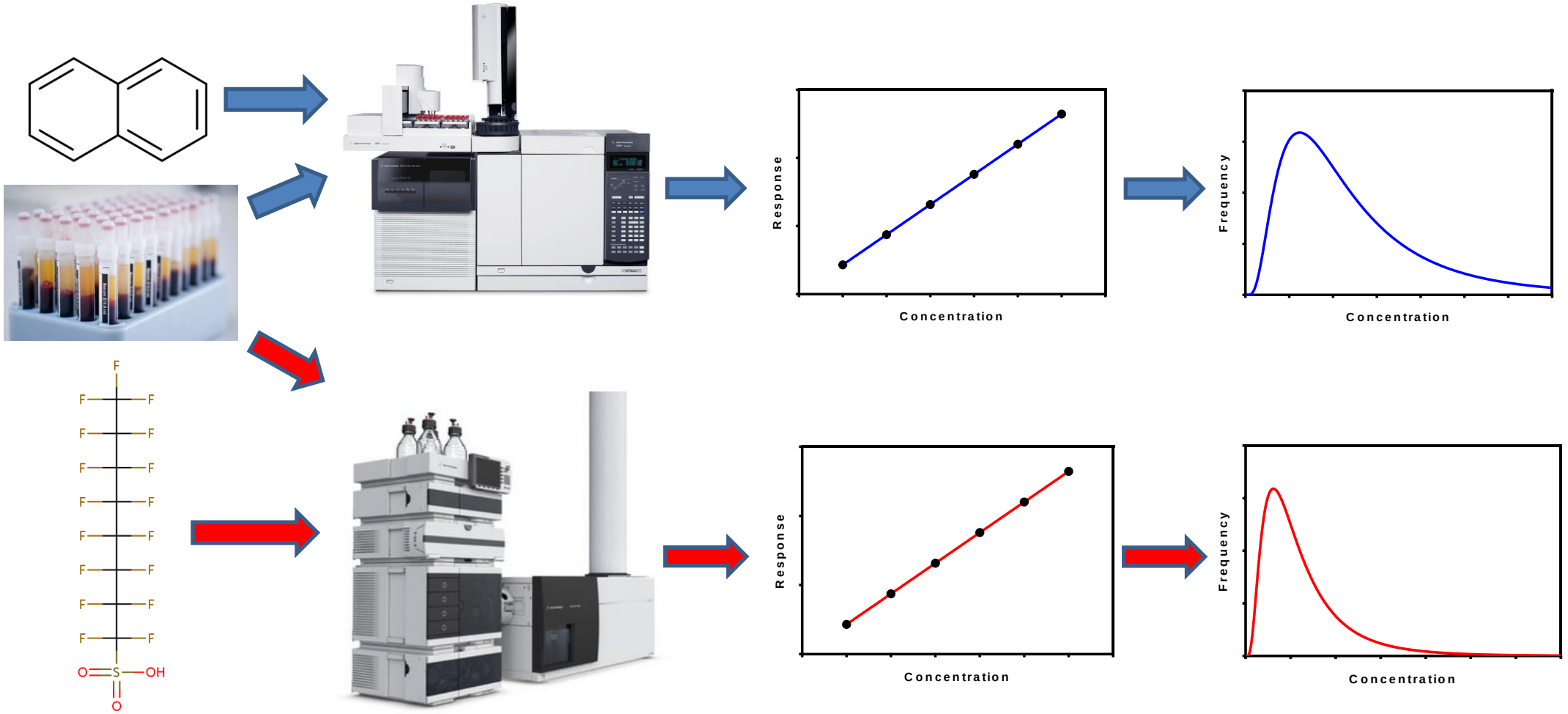
The Need for Chemical Measurement Data

- **Well-known chemicals**
  - 100s - 1,000s (e.g., NHANES)
  - Quality exposure data
- **Known but data-poor chemicals**
  - 1,000s - 1,000,000s (e.g., TSCA)
  - Limited exposure data
- **Chemicals not yet known to exist**
  - Unknown #
  - No exposure data



Typical Targeted Analysis Workflow

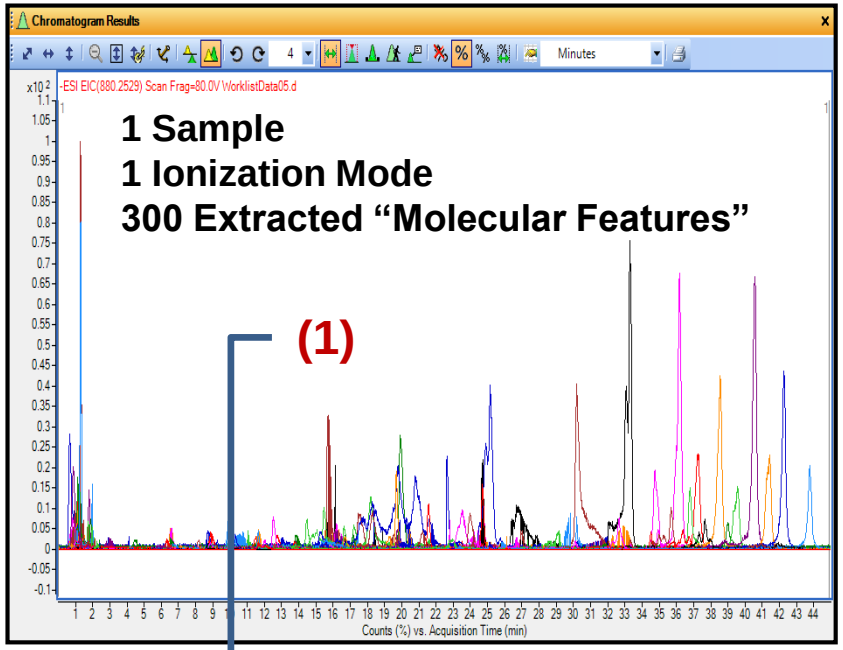
Standards/Samples | **Lab Analysis** | **Calibration** | **Quantitation**



Simplified NTA Workflow

Samples

High-Resolution MS

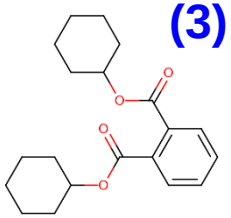


1) Prioritize "molecular features"
2) Correctly assign formulas
3) Correctly assign structures
4) Predict chemical concentrations
5) Determine chemical sources

(2)

$C_{20}H_{26}O_4$

(3)



(4)

12 µg/mL

(5)

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Integration Framework

Journal of Exposure Science & Environmental Epidemiology (2018) 28:411–426
https://doi.org/10.1038/s41370-017-0012-y

REVIEW ARTICLE

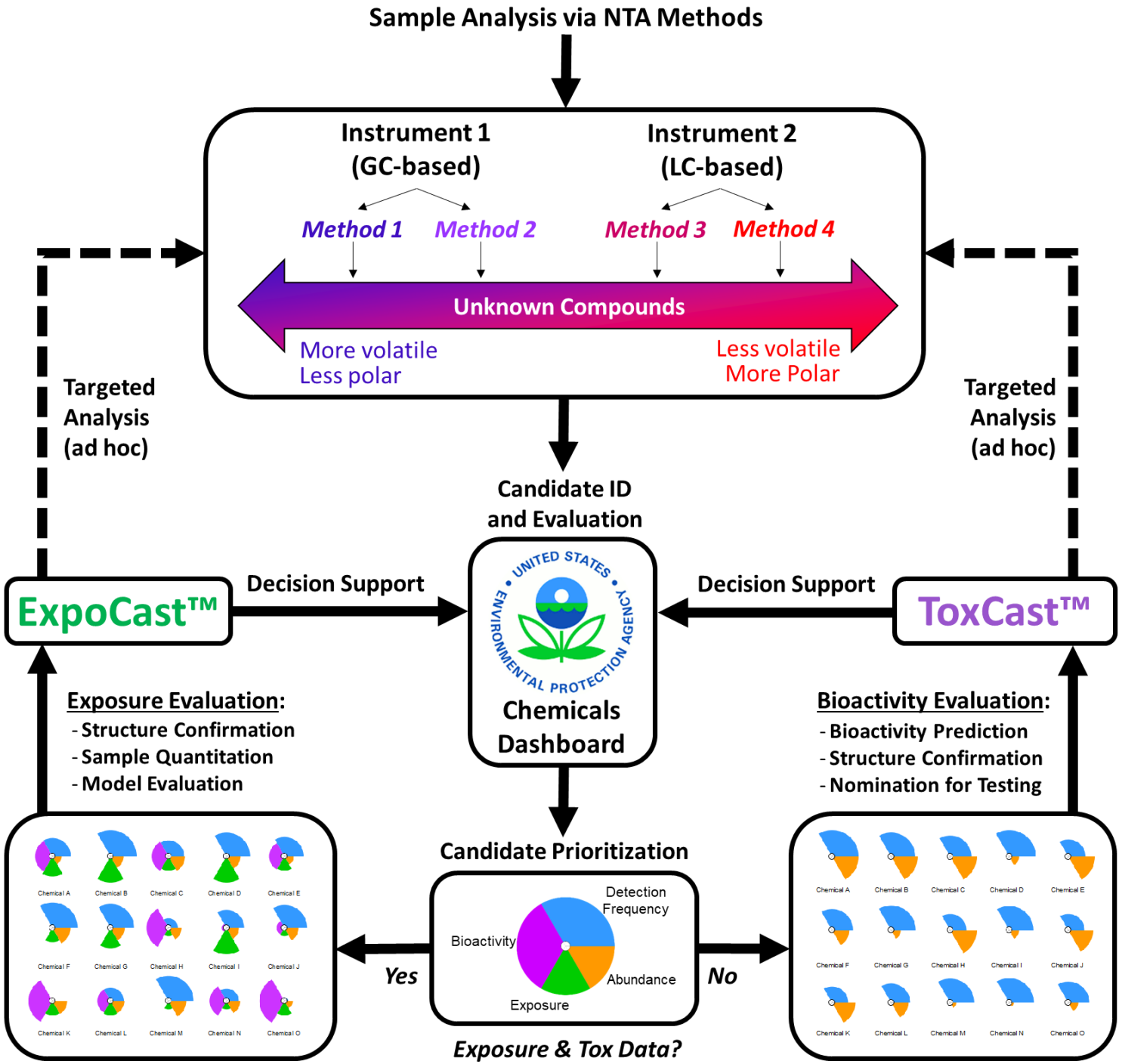
Integrating tools for non-targeted analysis research and chemical safety evaluations at the US EPA

Jon R. Sobus[1] · John F. Wambaugh[2] · Kristin K. Isaacs[1] · Antony J. Williams[2] · Andrew D. McEachran[3] · Ann M. Richard[2] · Christopher M. Grulke[2] · Elin M. Ulrich[1] · Julia E. Rager[3,4] · Mark J. Strynar[1] · Seth R. Newton[1]

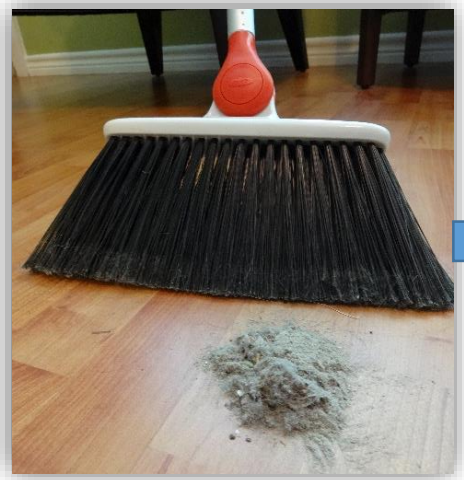
Received: 27 May 2017 / Revised: 4 August 2017 / Accepted: 25 August 2017 / Published online: 29 December 2017

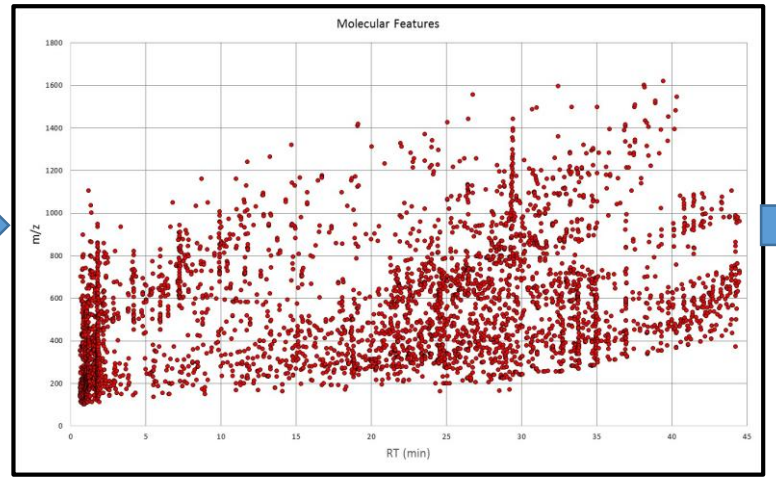
Abstract
Tens-of-thousands of chemicals are registered in the U.S. for use in countless processes and products. Recent evidence suggests that many of these chemicals are measureable in environmental and/or biological systems, indicating the potential for widespread exposures. Traditional public health research tools, including in vivo studies and targeted analytical chemistry methods, have been unable to meet the needs of screening programs designed to evaluate chemical safety. As such, new tools have been developed to enable rapid assessment of potentially harmful chemical exposures and their attendant biological responses. One group of tools, known as "non-targeted analysis" (NTA) methods, allows the rapid characterization of thousands of never-before-studied compounds in a wide variety of environmental, residential, and biological media. This article discusses current applications of NTA methods, challenges to their effective use in chemical screening studies, and ways in which shared resources (e.g., chemical standards, databases, model predictions, and media measurements) can advance their use in risk-based chemical prioritization. A brief review is provided of resources and projects within EPA's Office of Research and Development (ORD) that provide benefit to, and receive benefits from, NTA research endeavors. A summary of EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) is also given, which makes direct use of ORD resources to benefit the global NTA research community. Finally, a research framework is described that shows how NTA methods will bridge chemical prioritization efforts within ORD. This framework exists as a guide for institutions seeking to understand the complexity of chemical exposures, and the impact of these exposures on living systems.

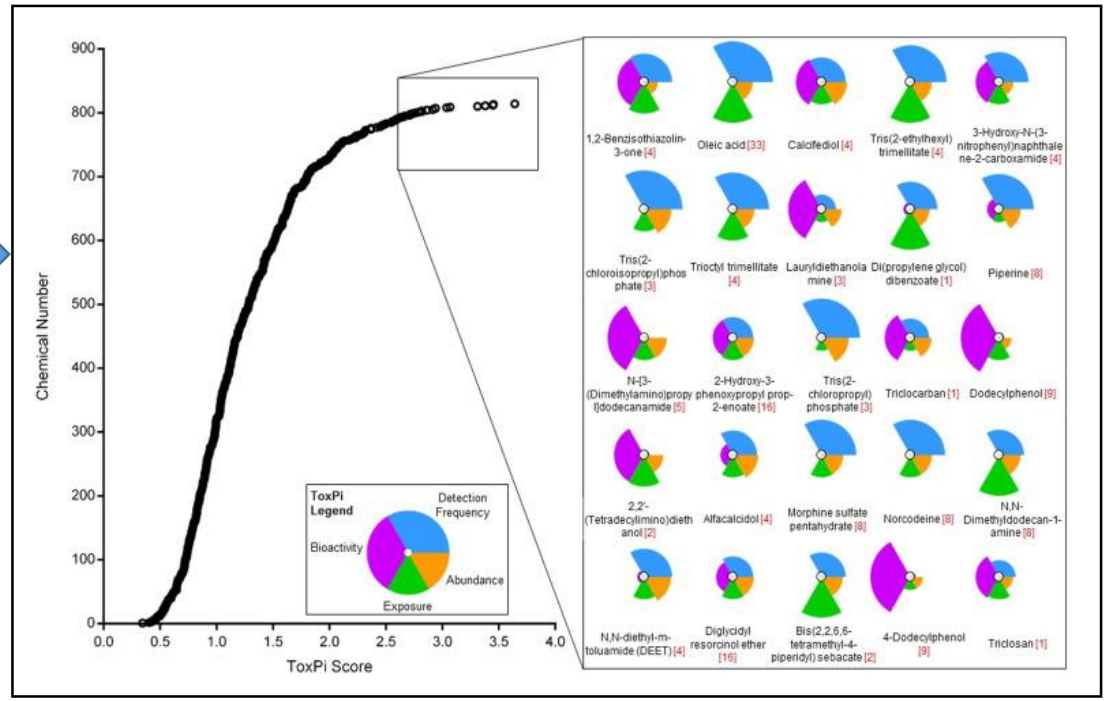
Keywords Non-targeted analysis · Suspect screening · Exposome · ENTACT

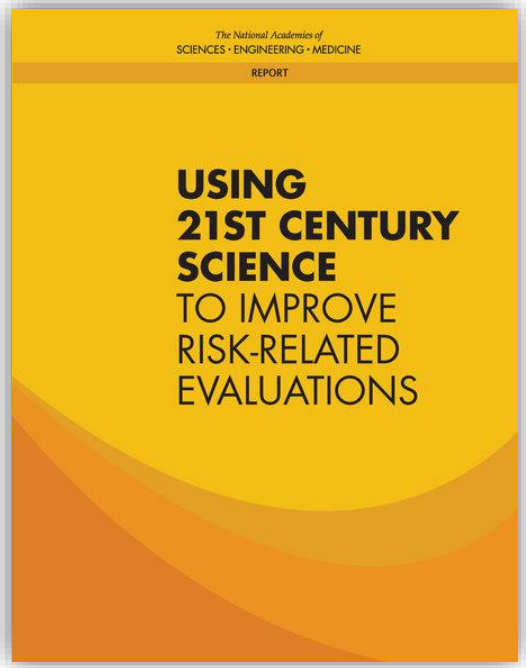


Initial Application of Framework





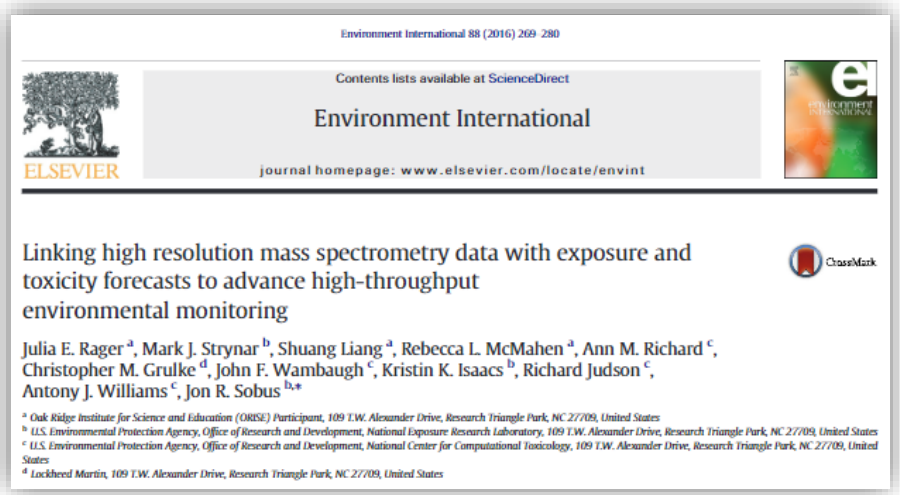




Research Highlights:

- Dust from 56 Households
- 3,228 Chemical Candidates
- Prioritization via ExpoCast & Tox21
- Standards acquired for 100 priority candidates
- 33 compounds confirmed
- 45% never before associated with house dust

Bottom-Up Methods Identified Most Important Exposures!


Environment International 88 (2016) 269–280
Contents lists available at ScienceDirect
Environment International
journal homepage: www.elsevier.com/locate/envint

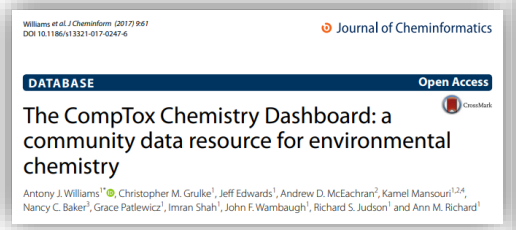
Linking high resolution mass spectrometry data with exposure and toxicity forecasts to advance high-throughput environmental monitoring

Julia E. Rager [a], Mark J. Strynar [b], Shuang Liang [a], Rebecca L. McMahen [a], Ann M. Richard [c], Christopher M. Grulke [d], John F. Wambaugh [c], Kristin K. Isaacs [b], Richard Judson [c], Antony J. Williams [c], Jon R. Sobus [b,*]

[a] Oak Ridge Institute for Science and Education (ORISE) Participant, 109 T.W. Alexander Drive, Research Triangle Park, NC 27709, United States
[b] U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, 109 T.W. Alexander Drive, Research Triangle Park, NC 27709, United States
[c] U.S. Environmental Protection Agency, Office of Research and Development, National Center for Computational Toxicology, 109 T.W. Alexander Drive, Research Triangle Park, NC 27709, United States
[d] Lockheed Martin, 109 T.W. Alexander Drive, Research Triangle Park, NC 27709, United States

Building an NTA Research Program

Data, Tools & Informatics

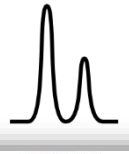

Journal of Cheminformatics

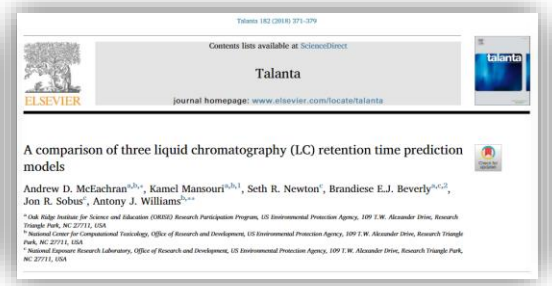
DATABASE Open Access

The CompTox Chemistry Dashboard: a community data resource for environmental chemistry

Antony J. Williams, Christopher M. Grulke, Jeff Edwards, Andrew D. McEachran, Kamel Mansouri, Nancy C. Baker, Grace Patlewicz, Imran Shah, John F. Wambaugh, Richard S. Judson and Ann M. Richard

Chemicals Dashboard

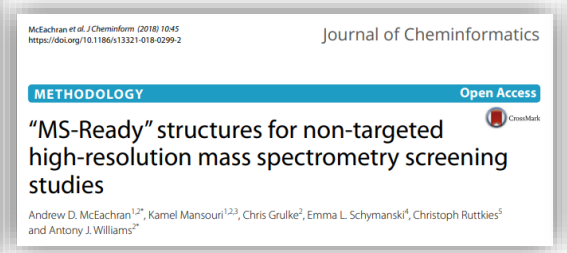



Talanta

A comparison of three liquid chromatography (LC) retention time prediction models

Andrew D. McEachran, Kamel Mansouri, Seth R. Newton, Brandiese E.J. Beverly, Jon R. Sobus, Antony J. Williams

RT Models

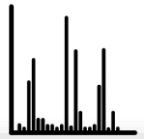

Journal of Cheminformatics

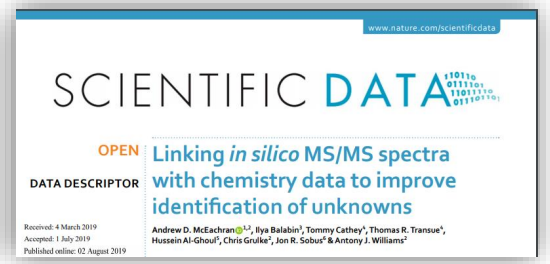
METHODOLOGY Open Access

"MS-Ready" structures for non-targeted high-resolution mass spectrometry screening studies

Andrew D. McEachran, Kamel Mansouri, Chris Grulke, Emma L. Schymanski, Christoph Ruttkies and Antony J. Williams

"MS Ready" Structures




SCIENTIFIC DATA

OPEN DATA DESCRIPTOR

Linking *in silico* MS/MS spectra with chemistry data to improve identification of unknowns

In Silico Spectra

Exposure Networks

Semi Quant. Methods

EPA NTA WebApp

2016… 2017… 2019… 2020…

NTA Applications

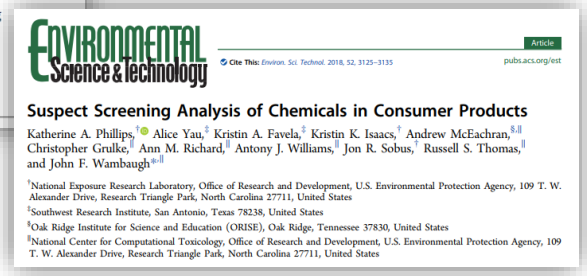
Brita Filters


Environmental Pollution

Suspect screening and non-targeted analysis of drinking water using point-of-use filters

Seth R. Newton, Rebecca L. McMahen, Jon R. Sobus, Kamel Mansouri, Antony J. Williams, Andrew D. McEachran, Mark J. Strynar

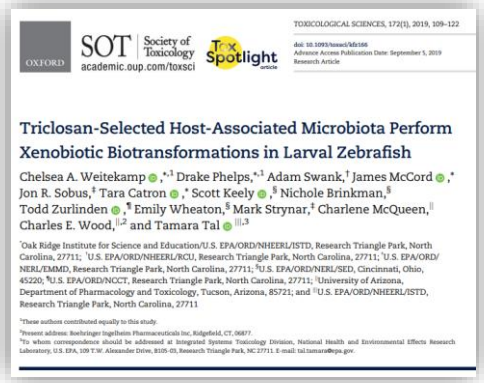
Consumer Products


Environmental Science & Technology

Suspect Screening Analysis of Chemicals in Consumer Products

Katherine A. Phillips, Alice Yau, Kristin A. Favela, Kristin K. Isaacs, Andrew McEachran, Christopher Grulke, Ann M. Richard, Antony J. Williams, Jon R. Sobus, Russell S. Thomas, and John F. Wambaugh

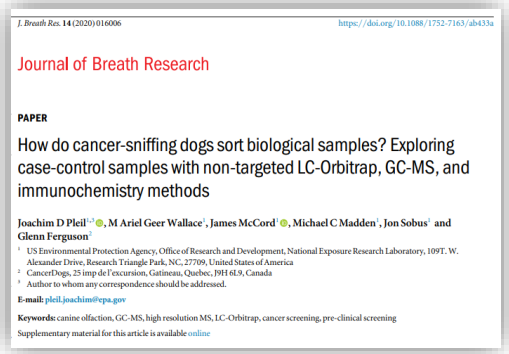
Model Organisms


SOT Society of Toxicology academic.oup.com/toxsci

Triclosan-Selected Host-Associated Microbiota Perform Xenobiotic Biotransformations in Larval Zebrafish

Chelsea A. Weitekamp, Drake Phelps, Adam Swank, James McCord, Jon R. Sobus, Tara Catron, Scott Keely, Nichole Brinkman, Todd Zurlinden, Emily Wheaton, Mark Strynar, Charlene McQueen, Charles E. Wood, and Tamara Tal

Hospital Masks


Journal of Breath Research

PAPER

How do cancer-sniffing dogs sort biological samples? Exploring case-control samples with non-targeted LC-Orbitrap, GC-MS, and immunochemistry methods

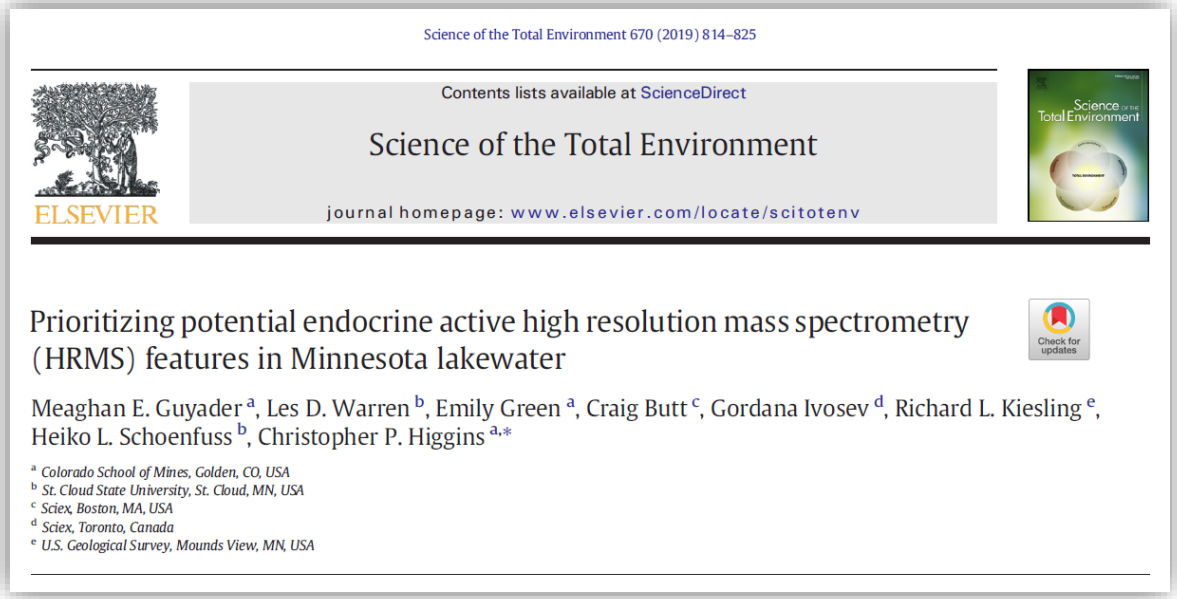
Joachim D Pleil, M Ariel Geer Wallace, James McCord, Michael C Madden, Jon Sobus and Glenn Ferguson

Recycled Products

Pooled Blood

Human Placentas

NTA State-of-the-Science



Science of the Total Environment 670 (2019) 814–825

Contents lists available at ScienceDirect

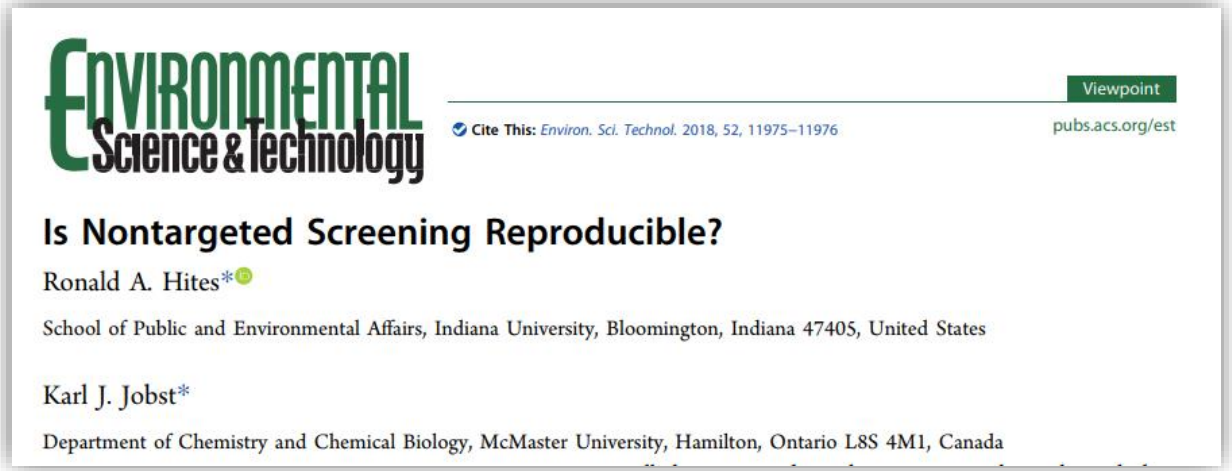
Science of the Total Environment

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

Prioritizing potential endocrine active high resolution mass spectrometry (HRMS) features in Minnesota lakewater

Meaghan E. Guyader [a], Les D. Warren [b], Emily Green [a], Craig Butt [c], Gordana Ivosev [d], Richard L. Kiesling [e], Heiko L. Schoenfuss [b], Christopher P. Higgins [a,*]

[a] Colorado School of Mines, Golden, CO, USA
[b] St. Cloud State University, St. Cloud, MN, USA
[c] Sciex, Boston, MA, USA
[d] Sciex, Toronto, Canada
[e] U.S. Geological Survey, Mounds View, MN, USA



Environmental Science & Technology

Viewpoint

Cite This: Environ. Sci. Technol. 2018, 52, 11975–11976

pubs.acs.org/est

Is Nontargeted Screening Reproducible?

Ronald A. Hites*

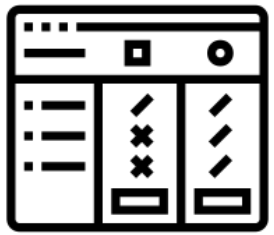
School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana 47405, United States

Karl J. Jobst*

Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Ontario L8S 4M1, Canada

"*The novelty of nontarget analysis, particularly its current lack of implementation by regulatory agencies, has prevented the establishment of streamlined quality assurance and quality control (QA/QC) procedures.*"

"*No single analytical technique is suitable for the analysis of all compounds, and successful nontargeted screening will require the development of multiplatform approaches, facilitated and validated through interlaboratory collaborations*."



EPA Takes a Leadership Role

2015…

Non-Targeted Analysis Workshop

Home | Agenda | Registration | Abstract Submission | Logistics

The U.S. Environmental Protection Agency (EPA) will host the Non-Targeted Analysis Workshop August 18-19, 2015 at EPA's Research Triangle Park Campus.

2016…

EPA United States Environmental Protection Agency

Environmental Topics | Laws & Regulations | About EPA | Search EPA.gov

Related Topics: Science Matters | CONTACT US | SHARE

EPA's ENTACT Study Breaks New Ground with Non-Targeted Research

Published July 30, 2018

EPA scientists are leading a multi-phase project to evaluate the ability of non-targeted analysis laboratory methods to consistently and correctly identify unknown chemicals in samples. EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) was formed in late 2015 and includes nearly 30 academic, government, and industry groups. Non-targeted analysis involves analyzing water, soil and other types of samples to identify unknown chemicals that may be present, without having a preconceived idea of what chemicals may be in the samples.

"One of our main goals is to figure out what scientists are doing with non-targeted analysis as a group at large, particularly which chemicals we correctly identify and why," says Elin Ulrich, an EPA scientist who co-leads ENTACT with EPA's Jon Sobus.

2018…

Environmental Protection Agency (EPA) 2018

The U.S. Environmental Protection Agency (EPA) hosted a workshop focused on EPA's Non-Targeted Analysis Collaborative Trial (ENTACT). ENTACT was designed to assess the characteristics and performance of cutting-edge non-targeted analysis (NTA) methods using a set of highly controlled synthetic mixtures and reference samples. This workshop brought together ENTACT participants, NTA experts, and key stakeholders to discuss findings from ENTACT, as well as next steps for the NTA research community.

PAST

August 13-15, 2018

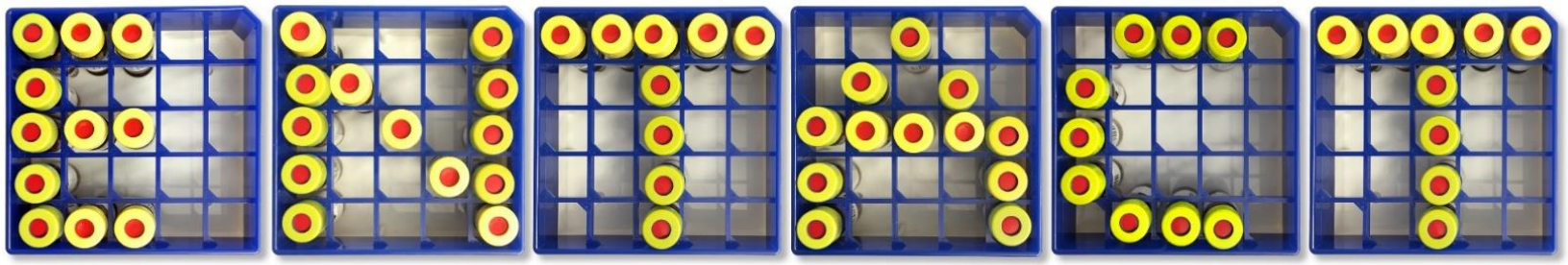
EPA 2018

www.eventbrite.com/e/us-epa-2018-non-targeted-analysis-collaborative-research-trial-entact-workshop-tickets-34838702497

Durham, NC, USA

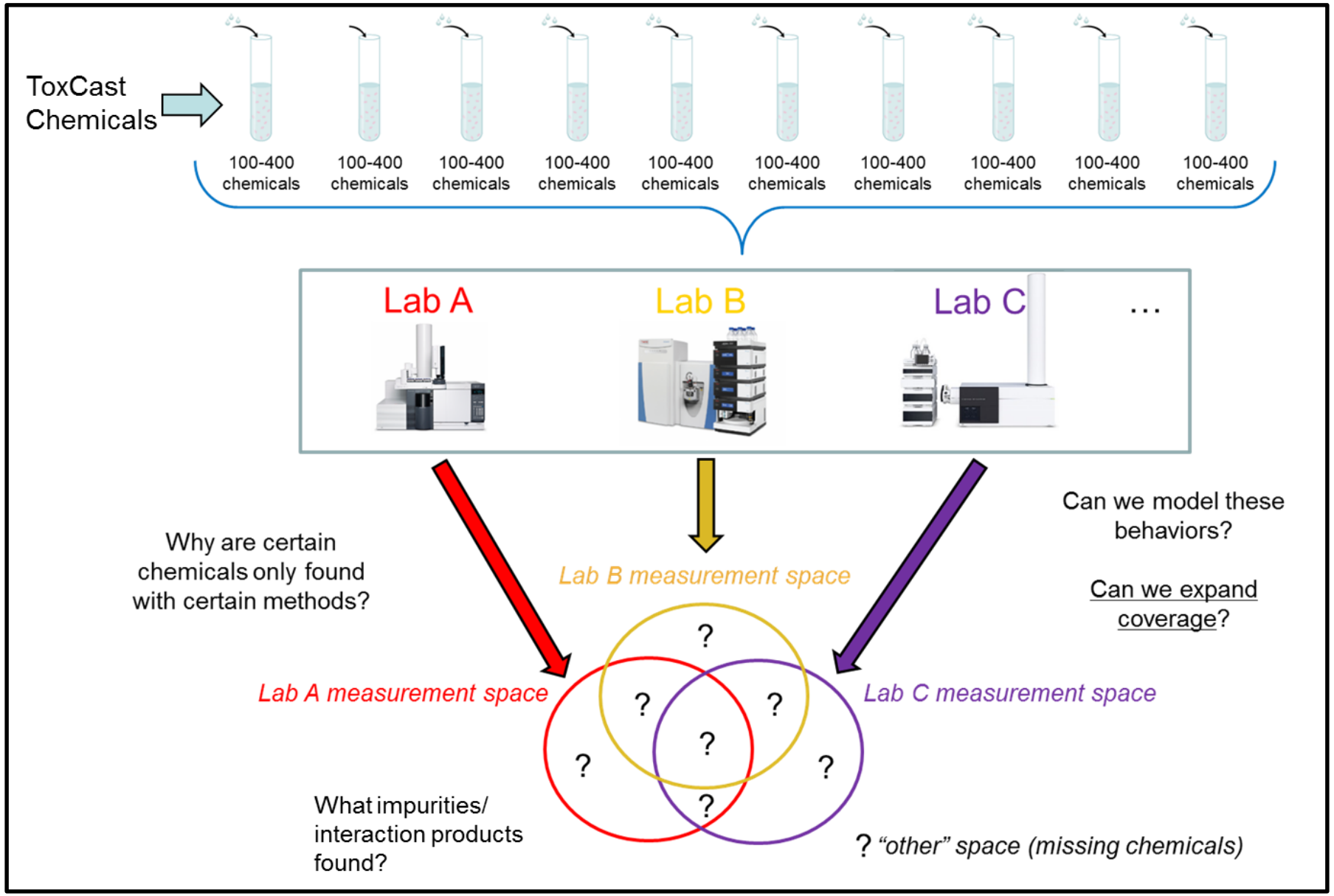
Science Questions for the Research Community

- How variable are tools and results from lab to lab?
- Are some methods/tools better than others?
- How does sample complexity affect performance?
- What chemical space does a given method cover?
- How sensitive are specific instruments/methods?



EPA's Non-Targeted Analysis Collaborative Trial

Original ENTACT Concept



EPA United States Environmental Protection Agency

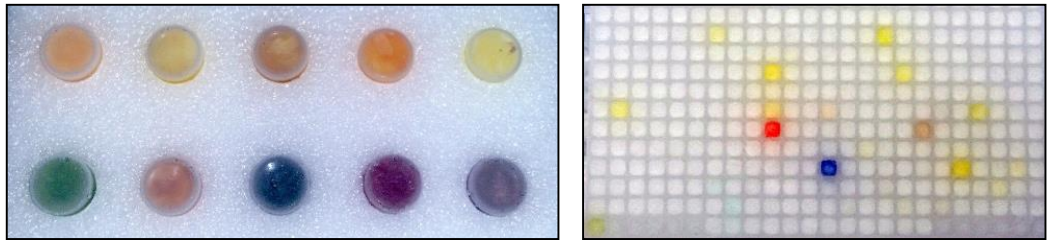
ENTACT Part 1

Chemicals from ToxCast Library

~1200 ToxCast Chemicals (highest quality)

10 Mixtures (100-400 chemicals each)

*Multi-Well Plates**



~25 Collaborators & 5 Contractors*:

1st: Blinded analysis

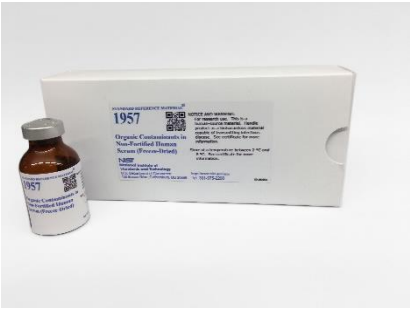
2nd: Unveiling of chemicals

3rd: Unblinded evaluation

ENTACT Part 2

Reference & Fortified House Dust

Reference & Fortified Human Serum



Reference & Fortified Silicone Wristbands

Who is Working on ENTACT?

Contractors:

University of Alberta
Emory University
Duke University
Oregon State University
San Diego State University
Pacific Northwest National Laboratory

19 Blind submissions

15 Unblinded submissions

Vendors:

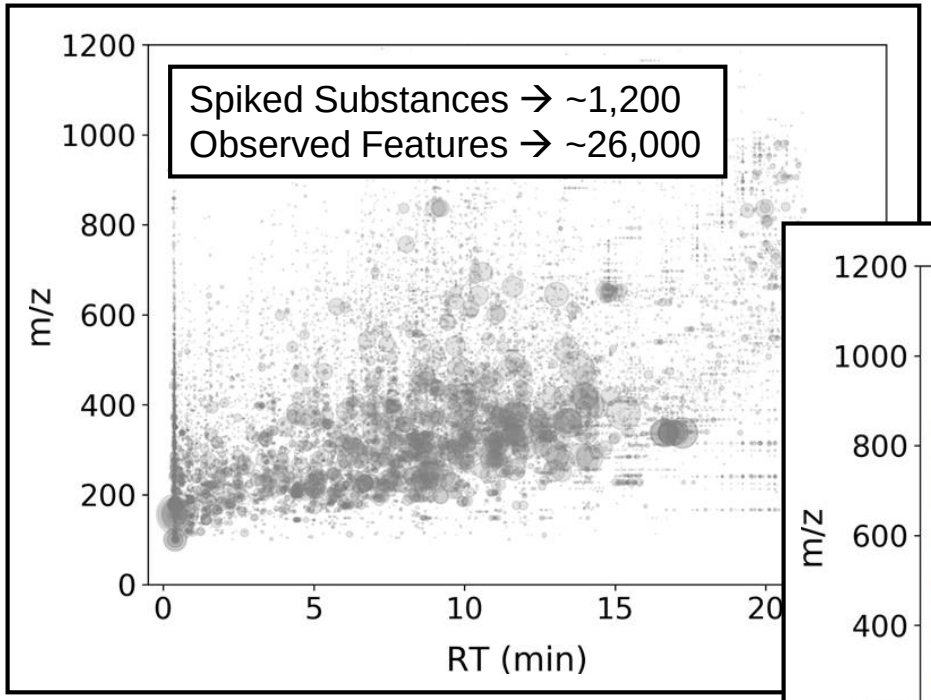
The Scripps Research Institute
Agilent Technologies
Thermo Scientific
Leco
AB SCIEX
Waters — The Science of What's Possible.

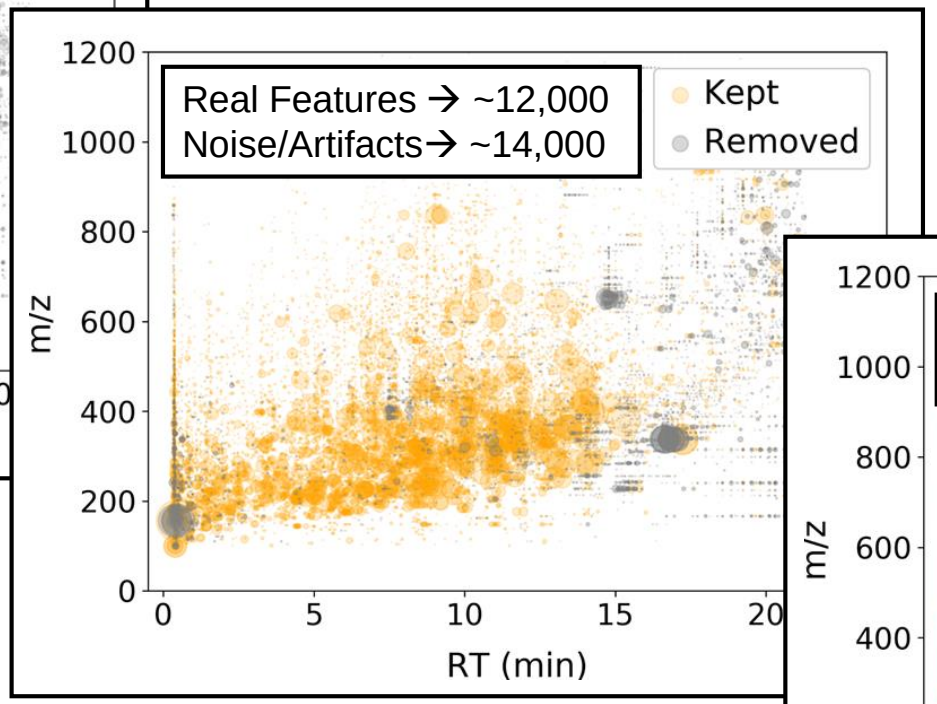
General Participants:

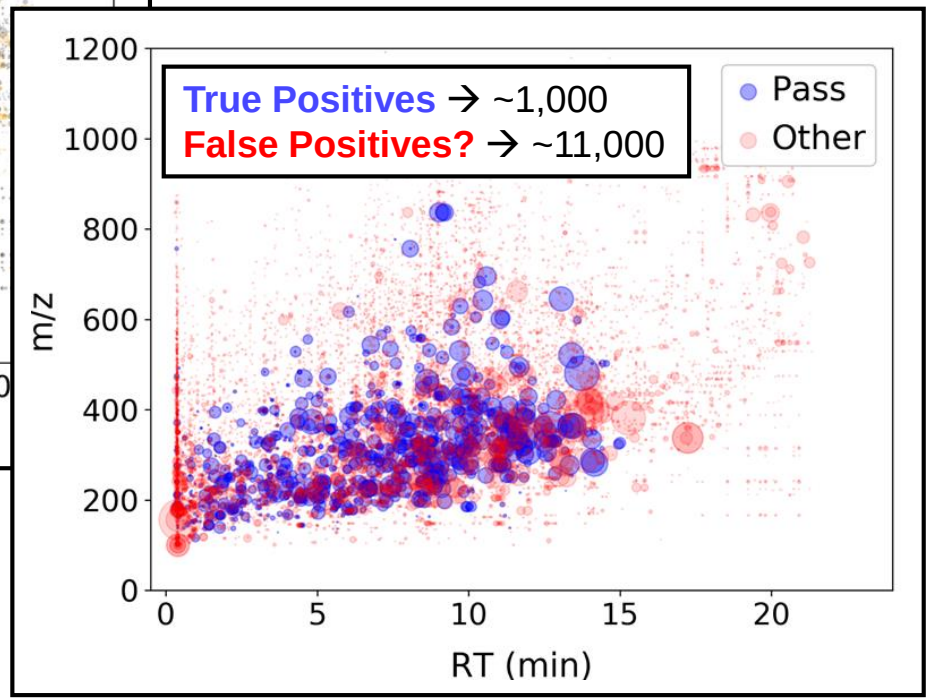
Université du Luxembourg
University of Florida
Wisconsin State Laboratory of Hygiene, University of Wisconsin–Madison
NIST
NC State University
Recetox
Cornell University
UW Tacoma
Mount Sinai
University of Birmingham
CalEPA California Environmental Protection Agency
eawag aquatic research
United States Environmental Protection Agency
FIU

EPA Lab Results

LC-QTOF HRMS (ESI+ and ESI-)







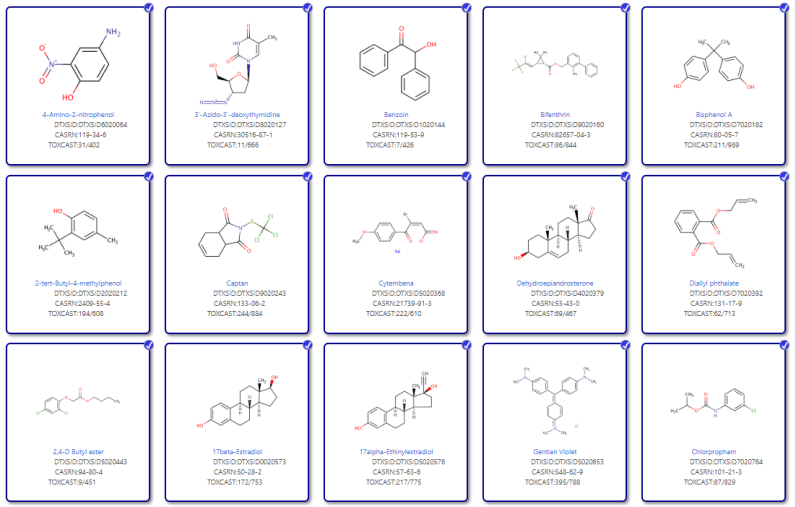
Substance Observed?	Substance Spiked? Yes	Substance Spiked? No
Yes	True Positives (≤ 65%)	False Positives?
No	False Negatives (≥ 35%)	True Negatives?

Sobus et al. 2019. https://doi.org/10.1007/s00216-018-1526-4

Cross-Lab Comparison

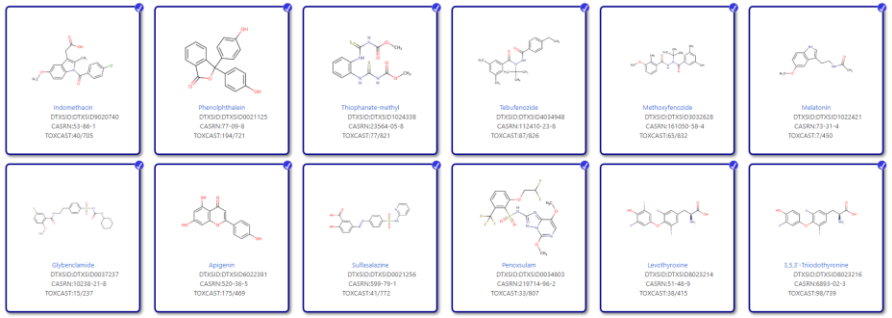
3 Labs, 6 Methods, All LC-HRMS

12% Not Observed by Any Method



...

3% Observed by All 6 Methods



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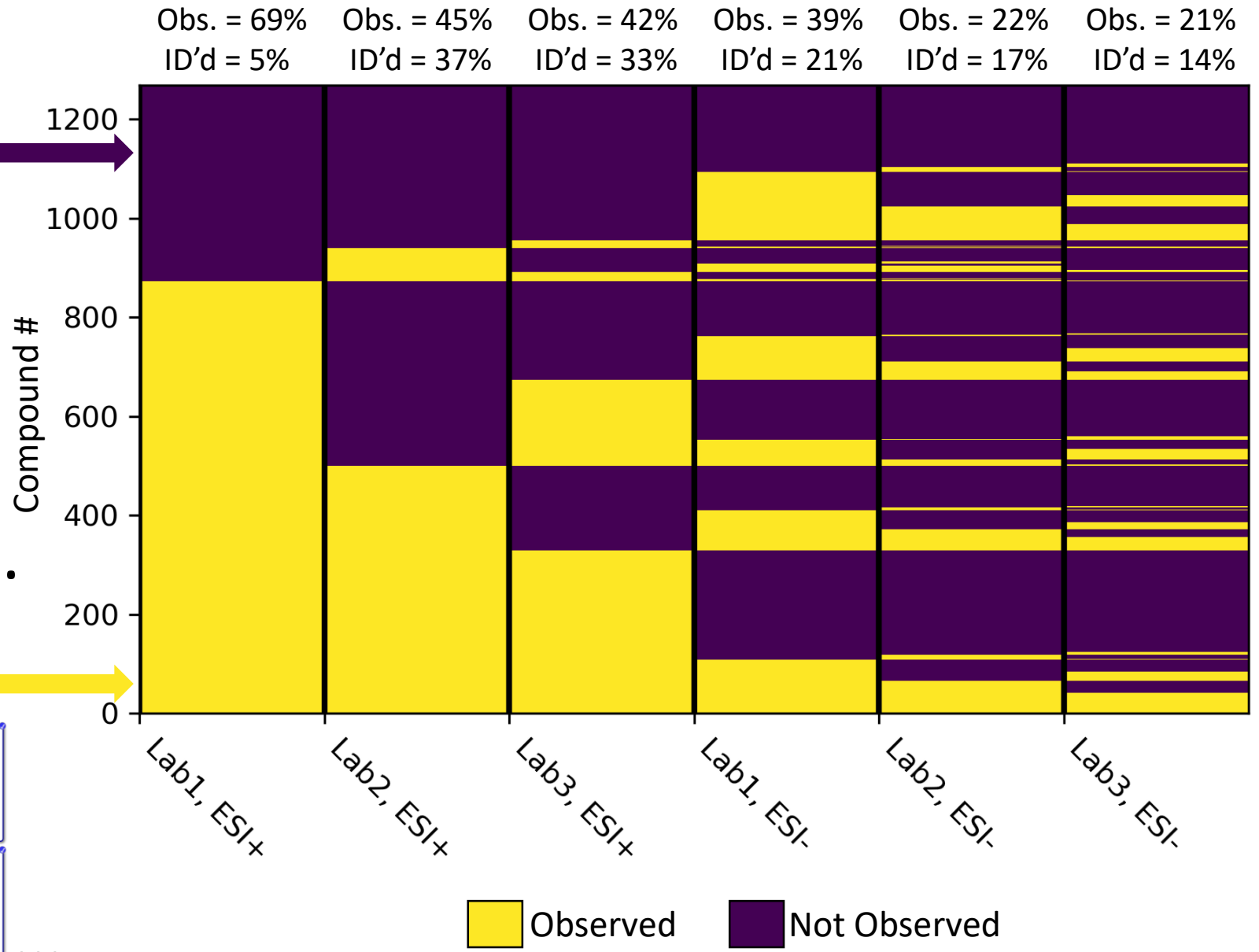


Evaluating *In Silico* Spectra with ENTACT Data

Metabolomics (2015) 11:98–110
DOI 10.1007/s11306-014-0676-4

ORIGINAL ARTICLE

Competitive fragmentation modeling of ESI-MS/MS spectra for putative metabolite identification

Felicity Allen · Russ Greiner · David Wishart

Training Set

Fragmentation Prediction Model

DSSTox structures

DSSTox MS2 spectra

SCIENTIFIC DATA

OPEN

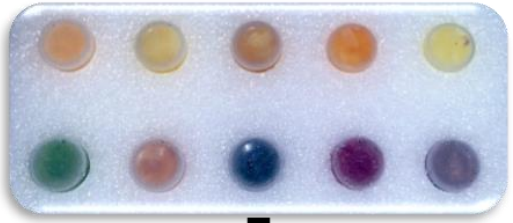
DATA DESCRIPTOR

Linking *in silico* MS/MS spectra with chemistry data to improve identification of unknowns

Received: 4 March 2019
Accepted: 1 July 2019
Published online: 02 August 2019

Andrew D. McEachran[1,2], Ilya Balabin[3], Tommy Cathey[4], Thomas R. Transue[4], Hussein Al-Ghoul[5], Chris Grulke[2], Jon R. Sobus[6] & Antony J. Williams[2]

10 Synthetic Mixtures:
1,269 Unique ToxCast Substances



LC-QTOF HRMS:
Data Dependent Acquisition

MS2 Reference Library

No Library Matches

Probable Structures

MS2 *in silico* Library
(~765,000 DSSTox Substances)

Tentative Structures

Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-019-02351-7

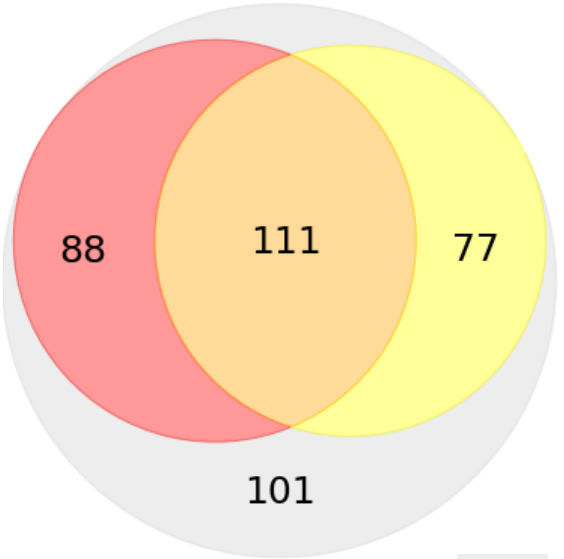
RESEARCH PAPER

In silico MS/MS spectra for identifying unknowns: a critical examination using CFM-ID algorithms and ENTACT mixture samples

Alex Chao[1,2] · Hussein Al-Ghoul[1,2] · Andrew D. McEachran[1,3] · Ilya Balabin[4] · Tom Transue[4] · Tommy Cathey[4] · Jarod N. Grossman[2,3] · Randolph Singh[1,5] · Elin M. Ulrich[2] · Antony J. Williams[6] · Jon R. Sobus[2]

377 ENTACT Compounds with MS2 Spectra

Top Reference Library Match



Top *in silico* Library Match

Not Top Match

ENTACT Publications to Date

Analytical and Bioanalytical Chemistry (2019) 411:853–866
https://doi.org/10.1007/s00216-018-1435-6

RESEARCH PAPER

EPA's non-targeted analysis collaborative trial (ENTACT): genesis, design, and initial findings

Elin M. Ulrich[1] · Jon R. Sobus[1] · Christopher M. Grulke[2] · Ann M. Richard[2] · Seth R. Newton[1] · Mark J. Strynar[1] · Kamel Mansouri[3,4] · Antony J. Williams[2]

Received: 30 July 2018 / Revised: 14 September 2018 / Accepted: 17 October 2018 / Published online: 6 December 2018

Analytical and Bioanalytical Chemistry (2019) 411:835–851
https://doi.org/10.1007/s00216-018-1526-4

RESEARCH PAPER

Using prepared mixtures of ToxCast chemicals to evaluate non-targeted analysis (NTA) method performance

Jon R. Sobus[1] · Jarod N. Grossman[2,3] · Alex Chao[2] · Randolph Singh[4] · Antony J. Williams[5] · Christopher M. Grulke[5] · Ann M. Richard[5] · Seth R. Newton[1] · Andrew D. McEachran[4] · Elin M. Ulrich[1]

Received: 19 September 2018 / Revised: 14 November 2018 / Accepted: 27 November 2018 / Published online: 5 January 2019

20 CHROMATOGRAPHY TODAY February / March 2018

Comprehensive, Non-Target Characterisation of Blinded Environmental Exposome Standards Using GCxGC and High Resolution Time-of-Flight Mass Spectrometry

by Lorne Fell*, Todd Richards and Joe Binkley
LECO, Saint Joseph, Michigan, USA
*Corresponding Author: lorne_fell@leco.com

JCIM JOURNAL OF CHEMICAL INFORMATION AND MODELING
Article
Cite This: J. Chem. Inf. Model. 2019, 59, 4052–4060
pubs.acs.org/jcim

Evaluation of *In Silico* Multifeature Libraries for Providing Evidence for the Presence of Small Molecules in Synthetic Blinded Samples

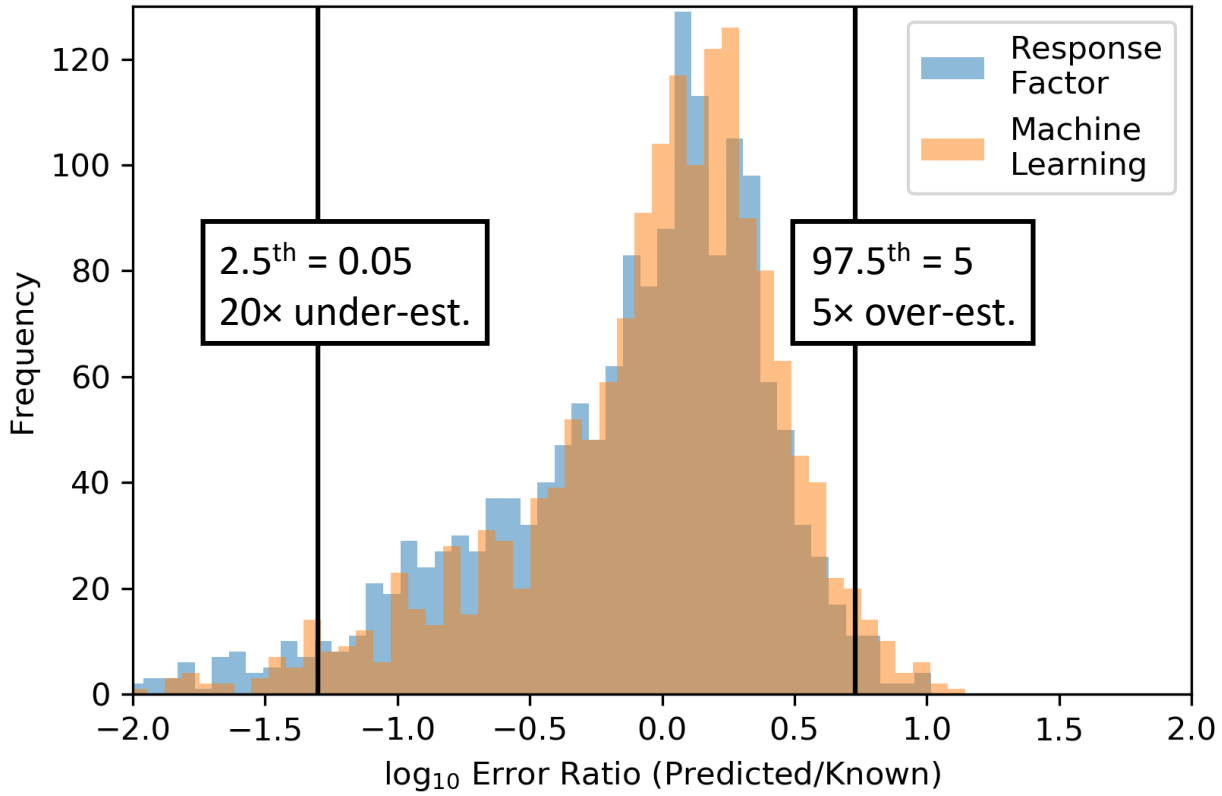
Jamie R. Nuñez,[†] Sean M. Colby,[†] Dennis G. Thomas,[†] Malak M. Tfaily,[†,⊥] Nikola Tolic,[†] Elin M. Ulrich,[‡] Jon R. Sobus,[‡] Thomas O. Metz,[*,†] Justin G. Teeguarden,[*,†,§] and Ryan S. Renslow[*,†]

[†]Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, Washington 99354, United States
[‡]U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Research Triangle Park, North Carolina 27711, United States
[§]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon 97331, United States
[⊥]Department of Environmental Science, University of Arizona, Tucson 85712, United States

Future Focus Areas Within EPA/ORD

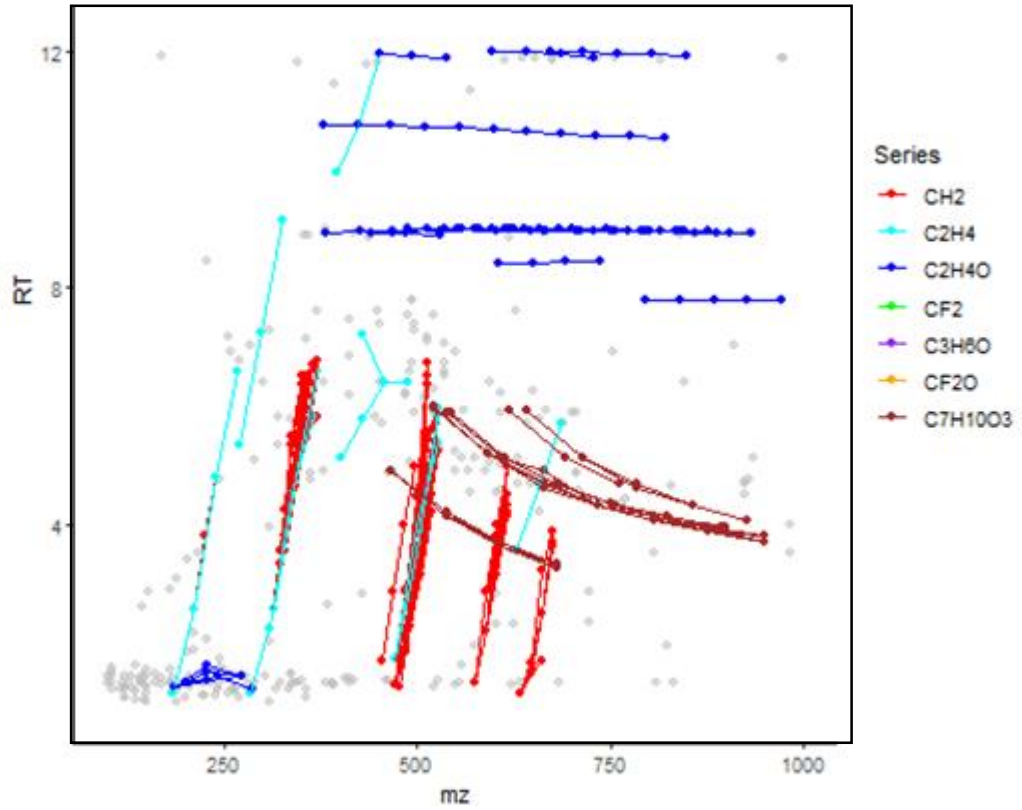
Semi-Quant. NTA:

ENTACT Data (ESI+); n= 544 compounds



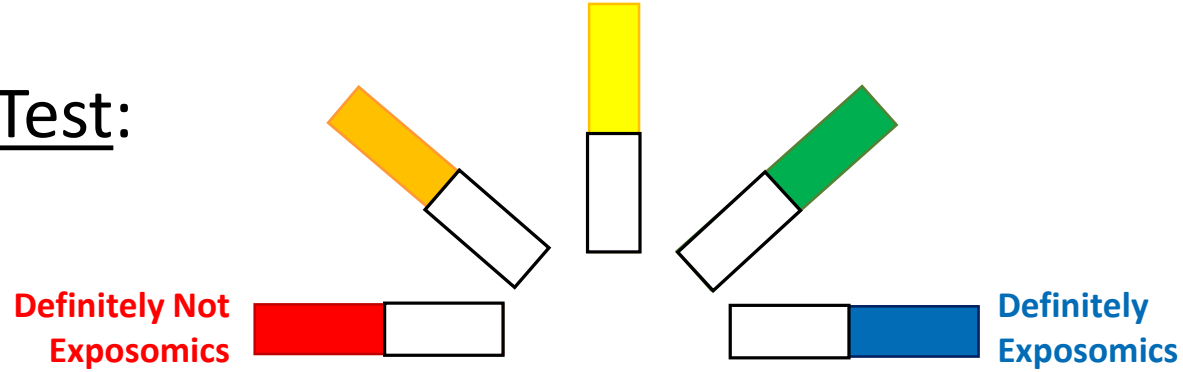
NTA for UVCBs:

Complex Mixture Data (ESI+)



NTA and Exposomics Moving Forward…

- Exposomics Litmus Test:



  - Does it advance knowledge of the totality of exposures?
    - If focused on measurements, is it non-targeted (or “untargeted”)?
    - If external, can measures be quantitatively linked to a receptor?
    - If internal, can measures be linked to a source?

- Good examples of exposomics research should be featured
- Integrated studies (external and internal) should be encouraged
- Explicit curricula should be developed and disseminated

Contributing Researchers

This work was supported, in part, by ORD's Pathfinder Innovation Program (PIP) and an ORD EMVL award

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Hannah Liberatore
Charles Lowe
James McCord
Kelsey Miller
Jeff Minucci
Seth Newton
Katherine Phillips
Allison Phillips*
Tom Purucker
Randolph Singh*
Mark Strynar
Elin Ulrich
Nelson Yeung*

EPA ORD (cont.)
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Kamel Mansouri*
Andrew McEachran*
Ann Richard
Adam Swank
John Wambaugh
Antony Williams

Agilent
Jarod Grossman
Andrew McEachran

GDIT
Ilya Balabin
Tom Transue
Tommy Cathey

* = ORISE/ORAU

Questions?

sobus.jon@epa.gov

The views expressed in this presentation are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.