

Bottom-Up Exposomics for Ensuring Chemical Safety

Jon R. Sobus, Ph.D.



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

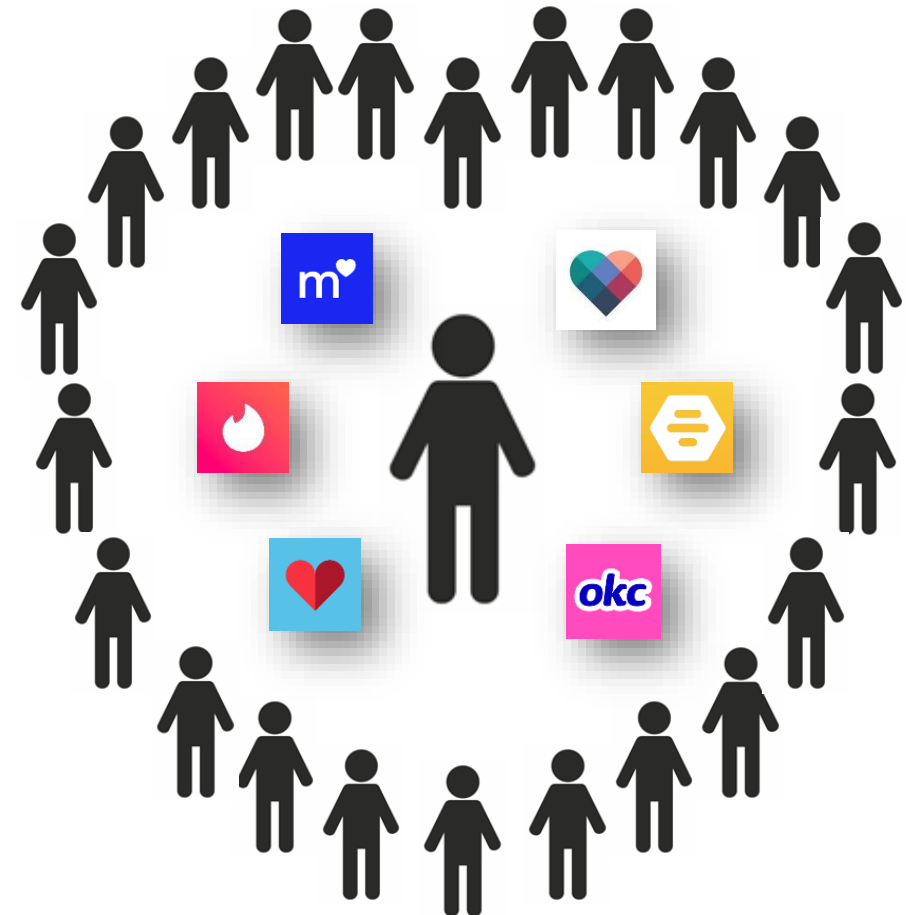
Exposomics: A Paradigm Shift for Exposure and Health Sciences

Traditional Research : Courtship

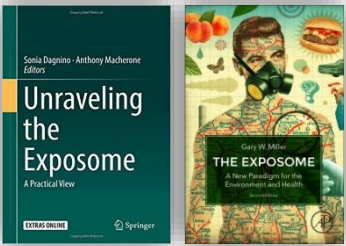


::

Exposomics : Online Dating



15 Years Later... How Far Have We Come?



Books



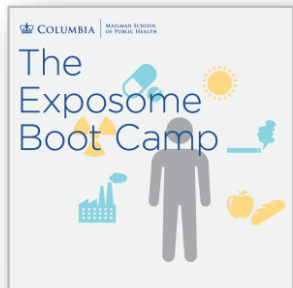
Centers

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

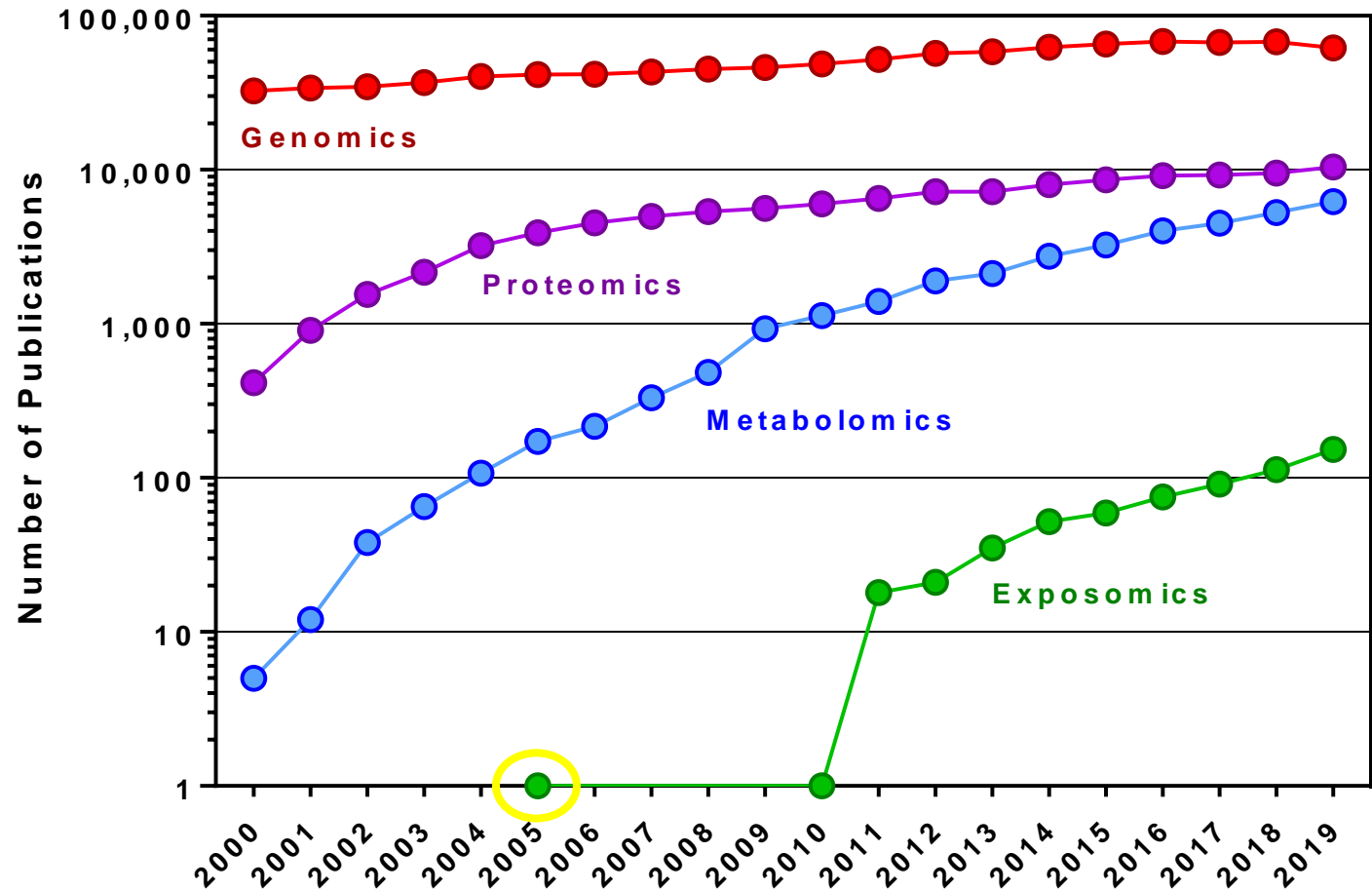
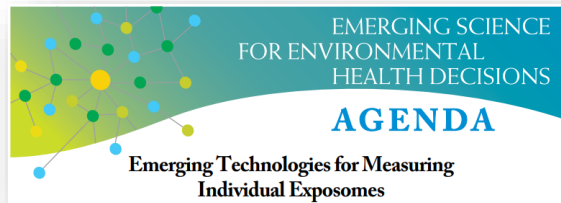
**Complementing the Genome with an "Exposome":
The Outstanding Challenge of Environmental
Exposure Measurement in Molecular Epidemiology**

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

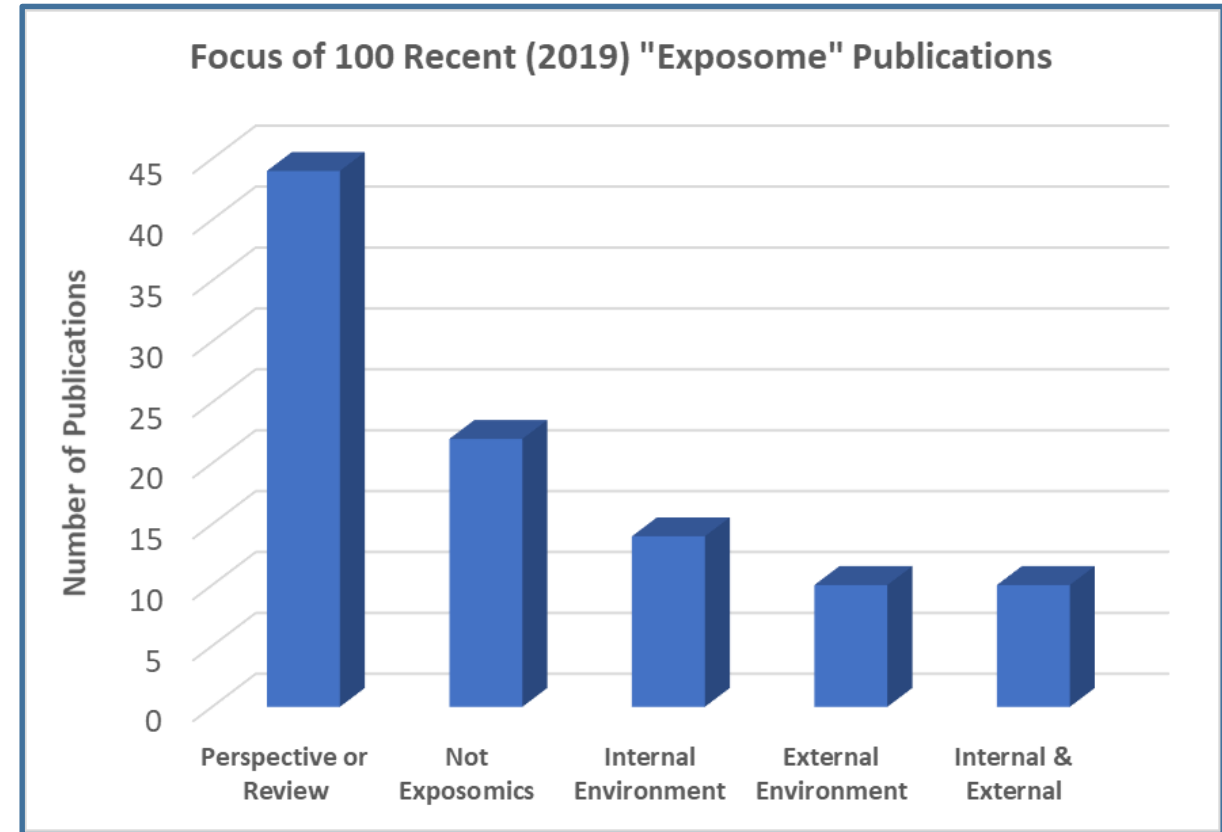
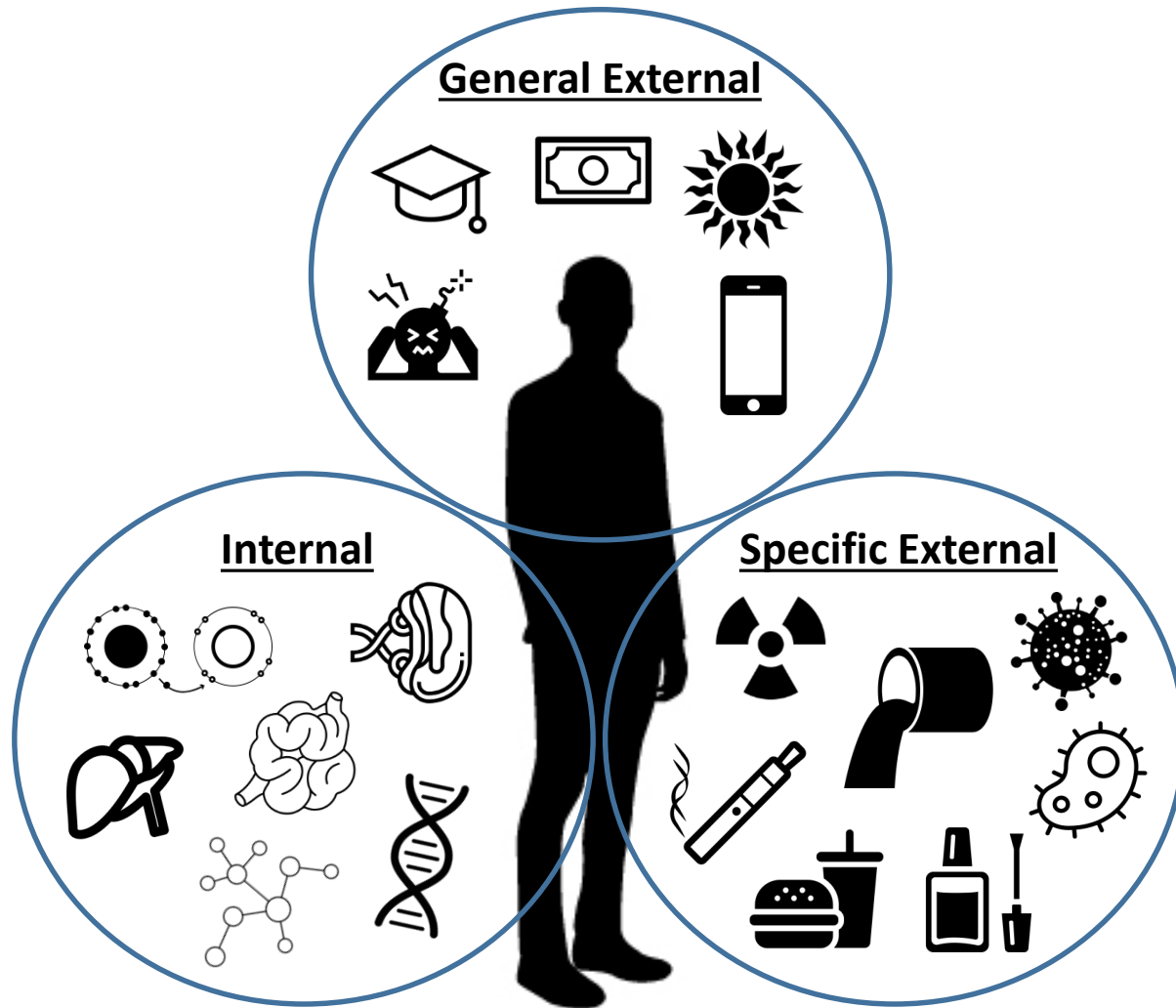
Training



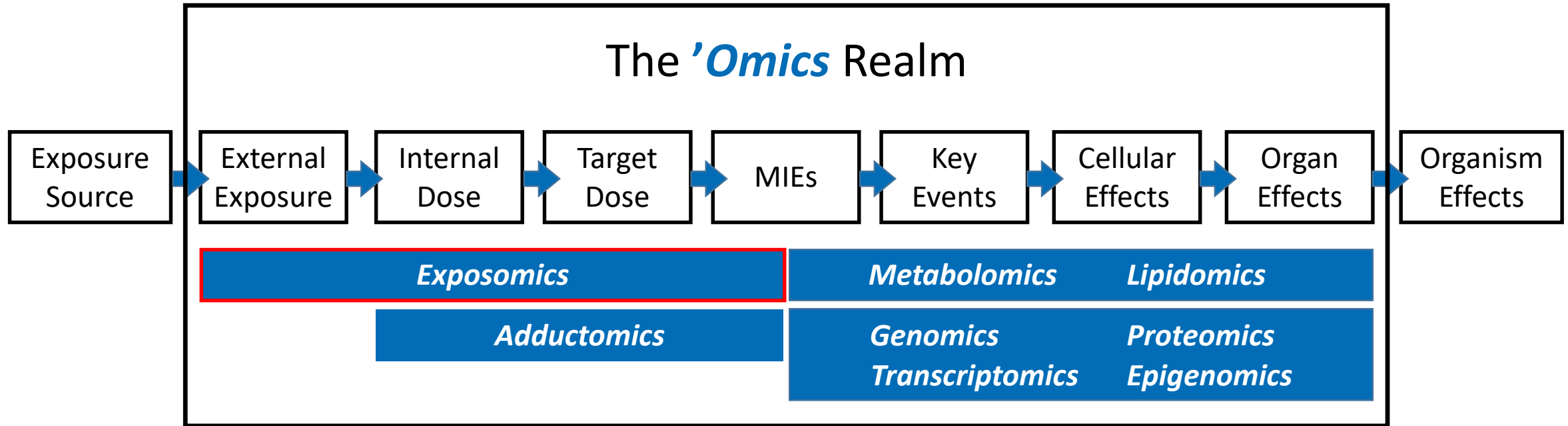
Conferences



What Are Researchers Studying?



What is Different About Exposomics?

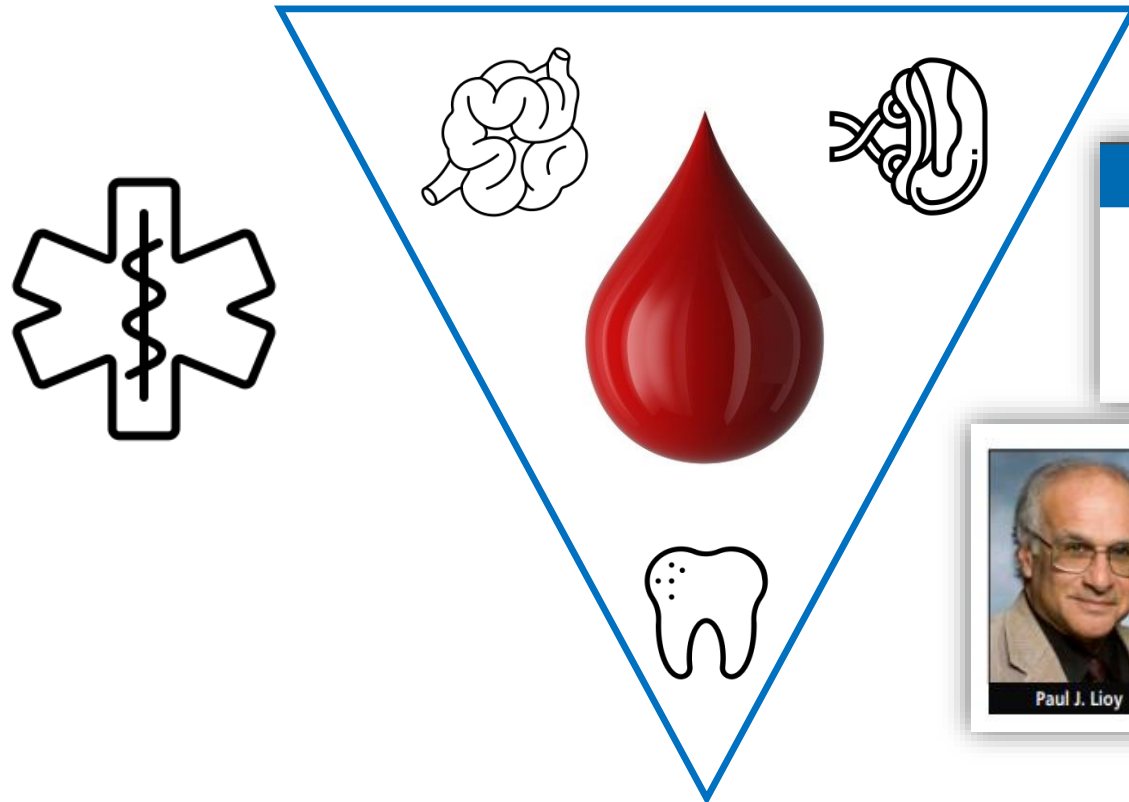


Exposomics is the one 'omics discipline that puts focus on external exposure

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

Exposomics Approaches

Top-Down Exposomics



Measure Important Exposures
Within the Receptor

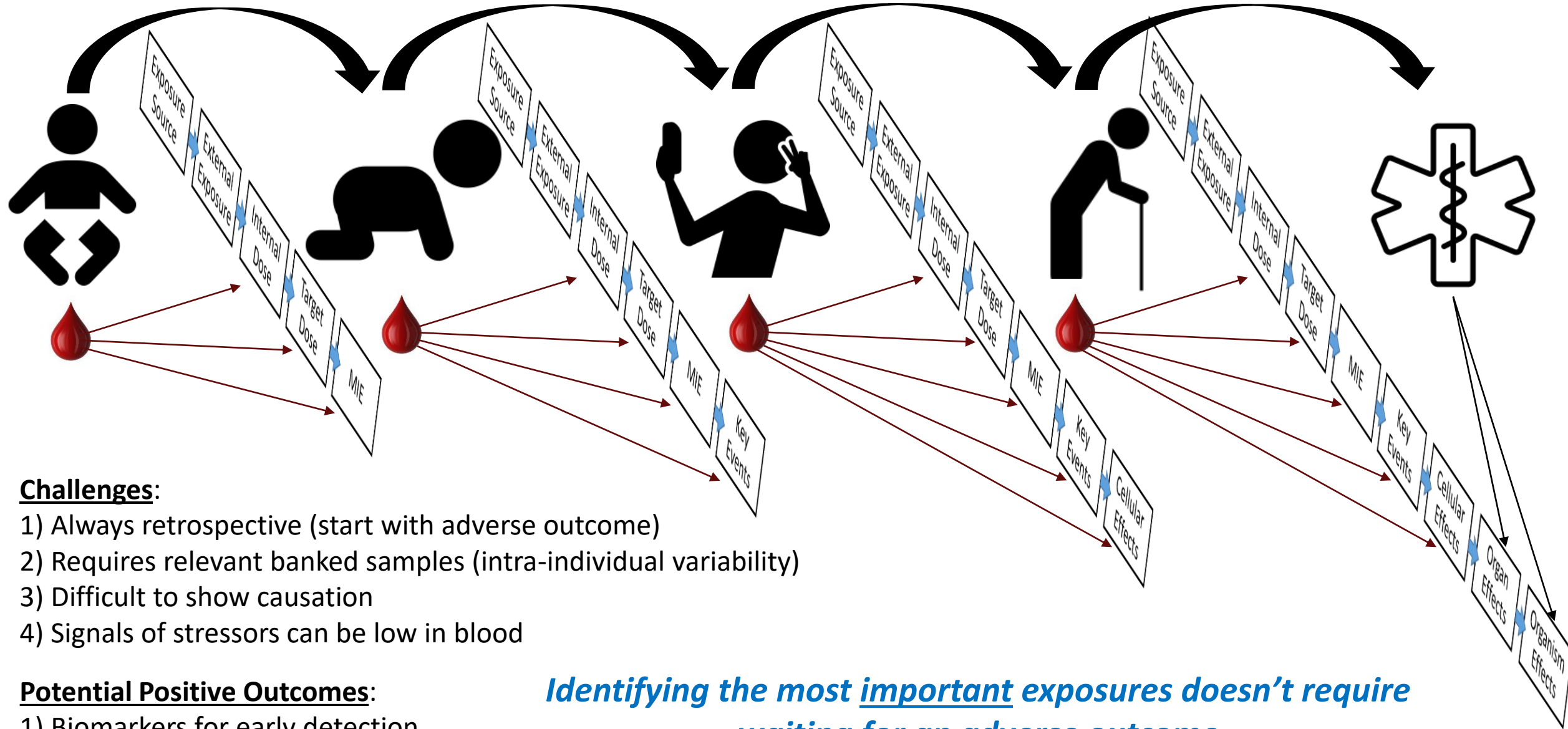
Bottom-Up Exposomics



Measure Important Exposures in All
Relevant Media



Challenges with Top-Down Efforts

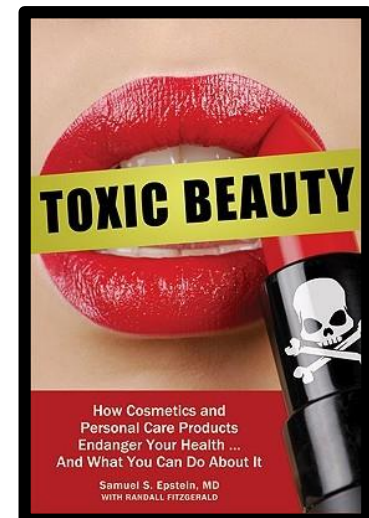
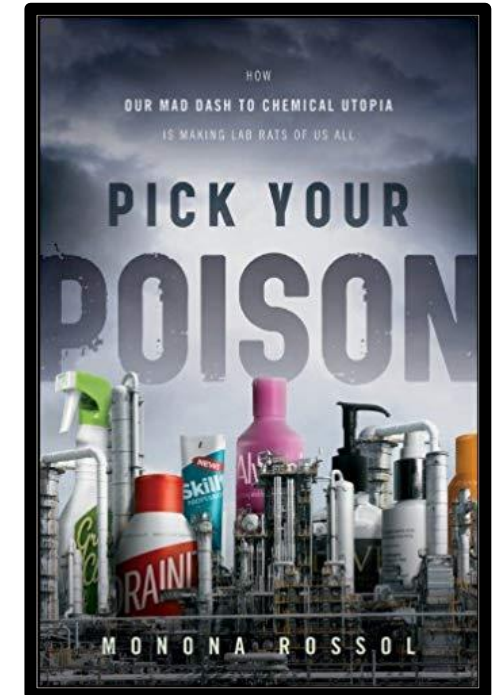
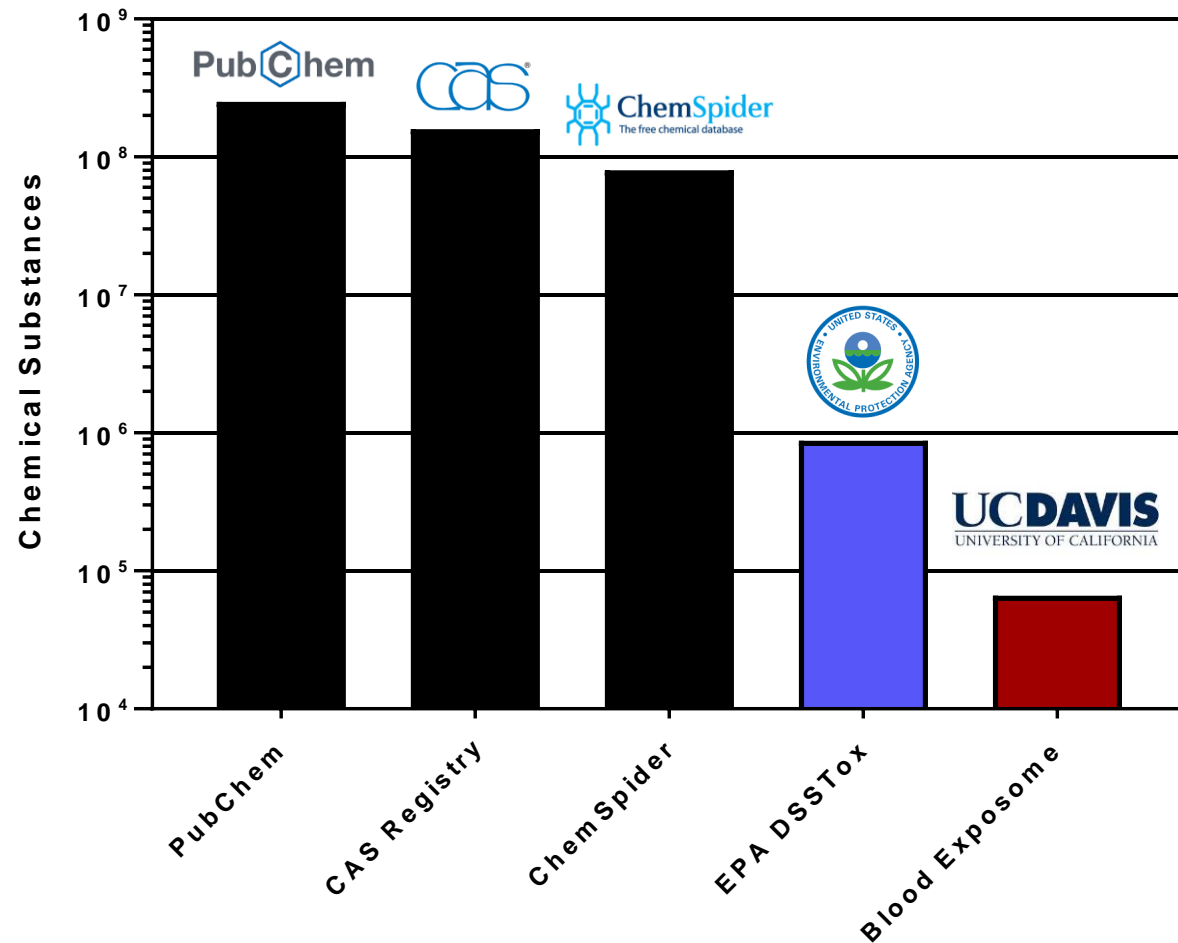


Potential Positive Outcomes:

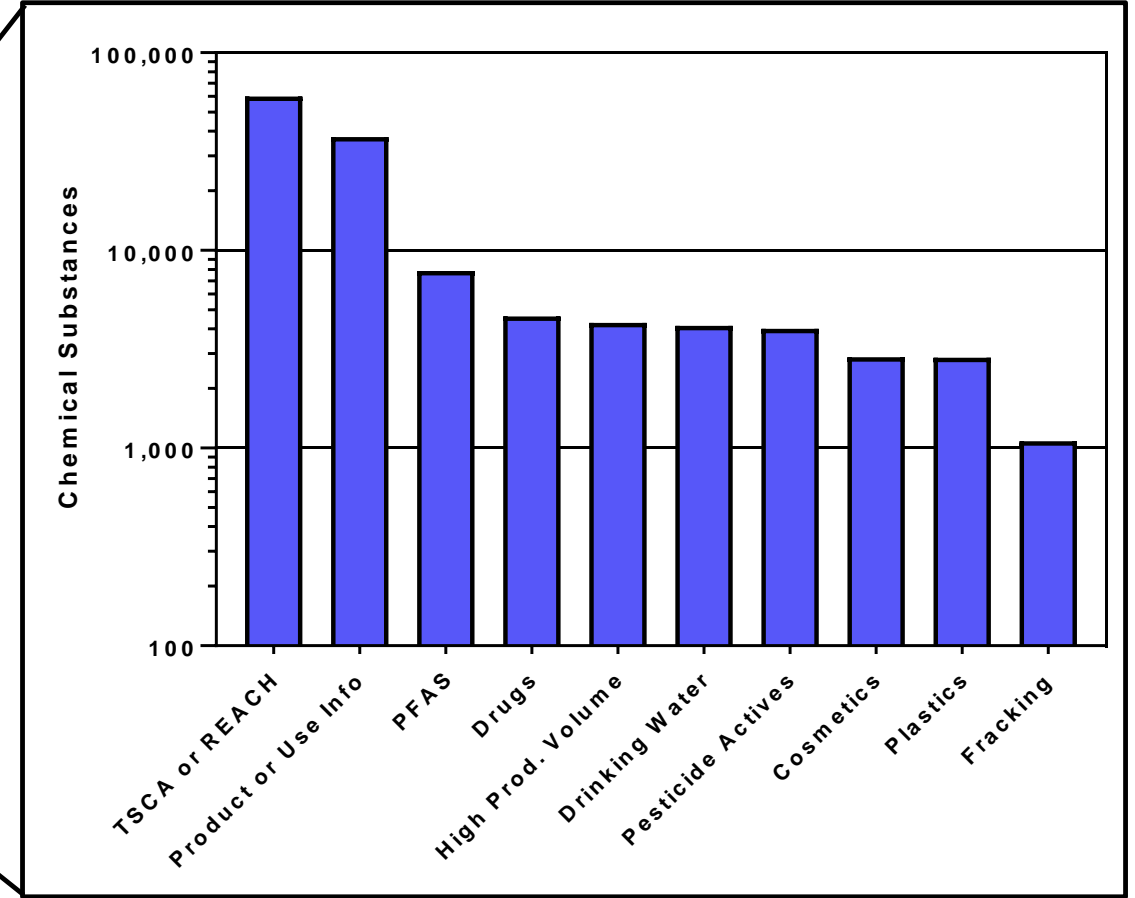
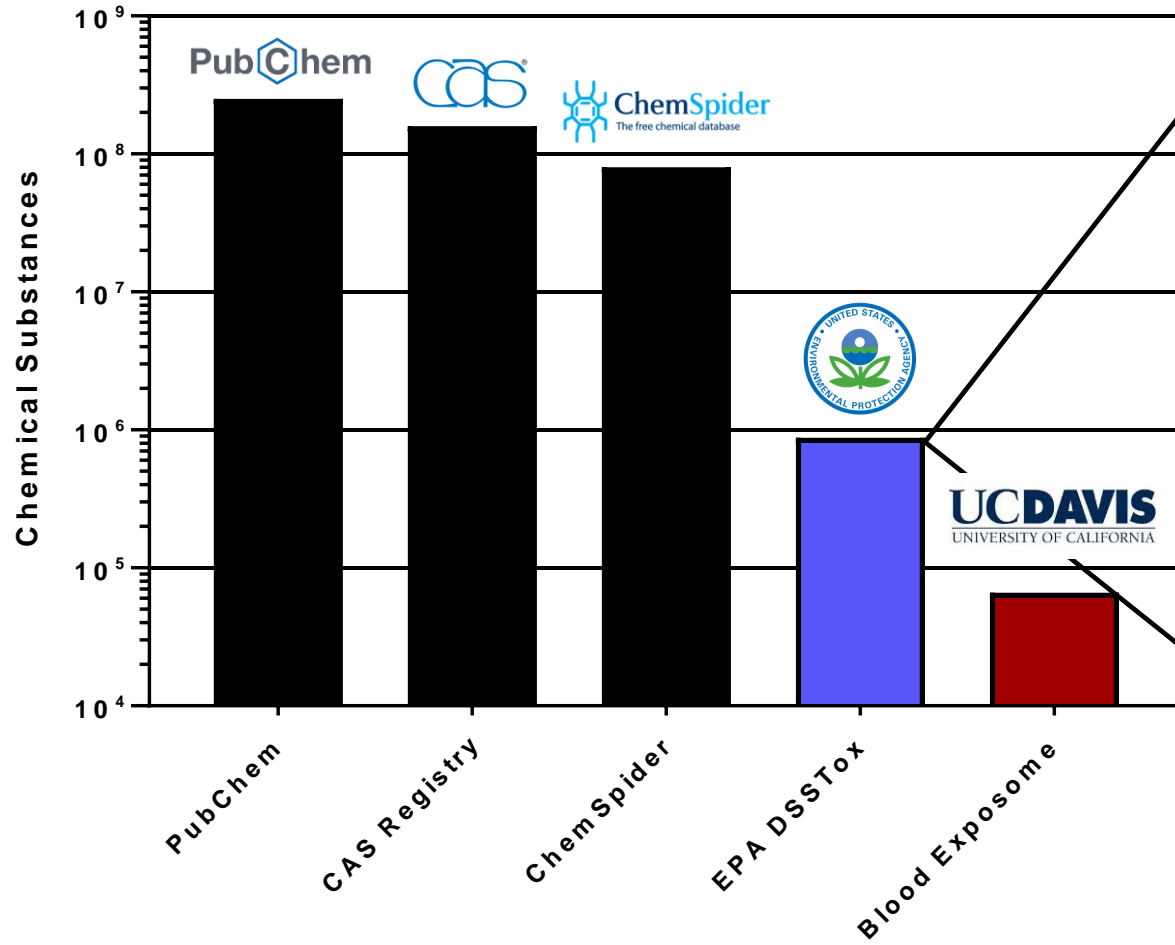
- 1) Biomarkers for early detection
- 2) Drugs for early treatment

Identifying the most important exposures doesn't require waiting for an adverse outcome

Rationale for Bottom-Up Efforts



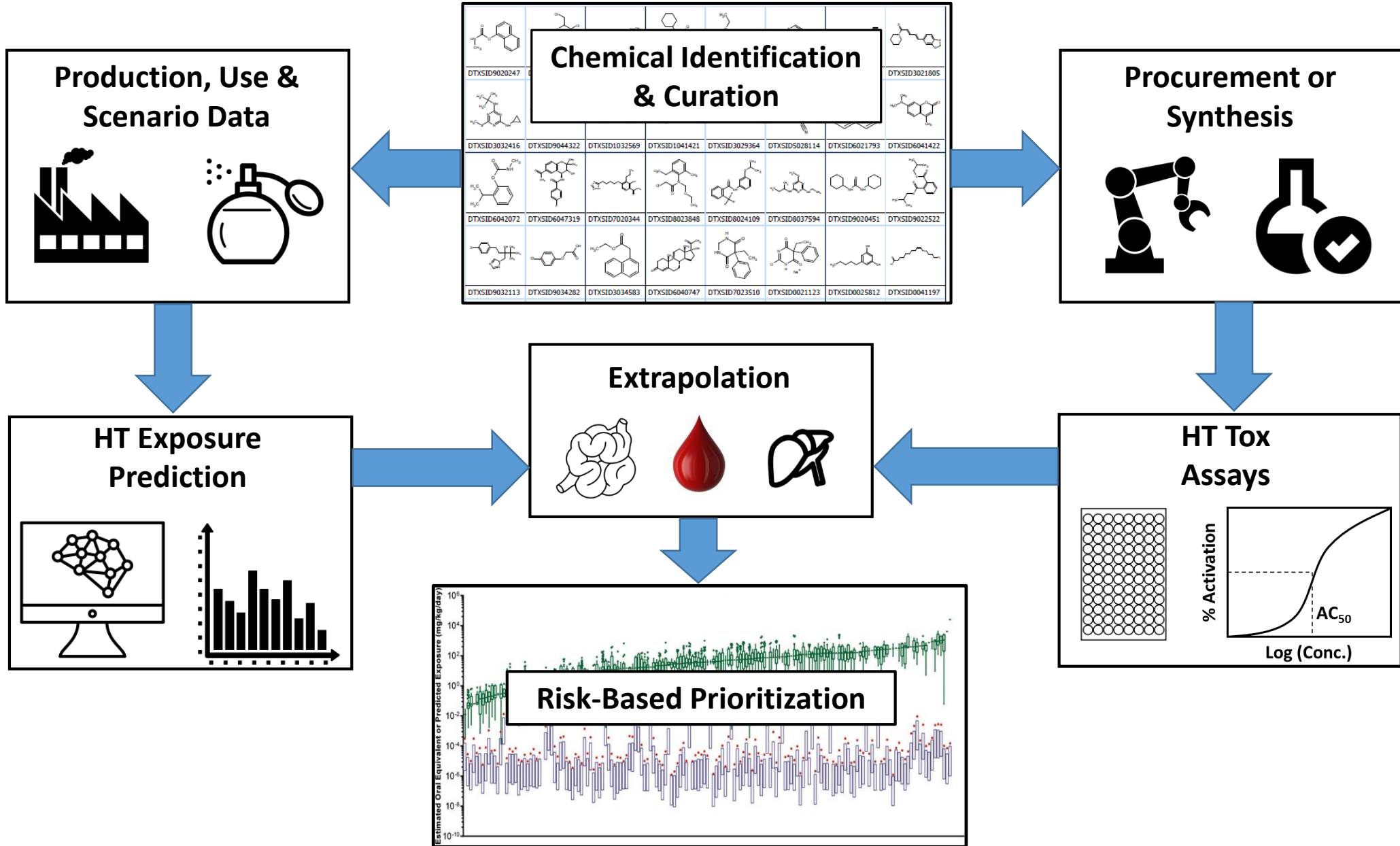
Rationale for Bottom-Up Efforts



The Era of High-Throughput Assessments

Exposure Forecasting
(ExpoCast)

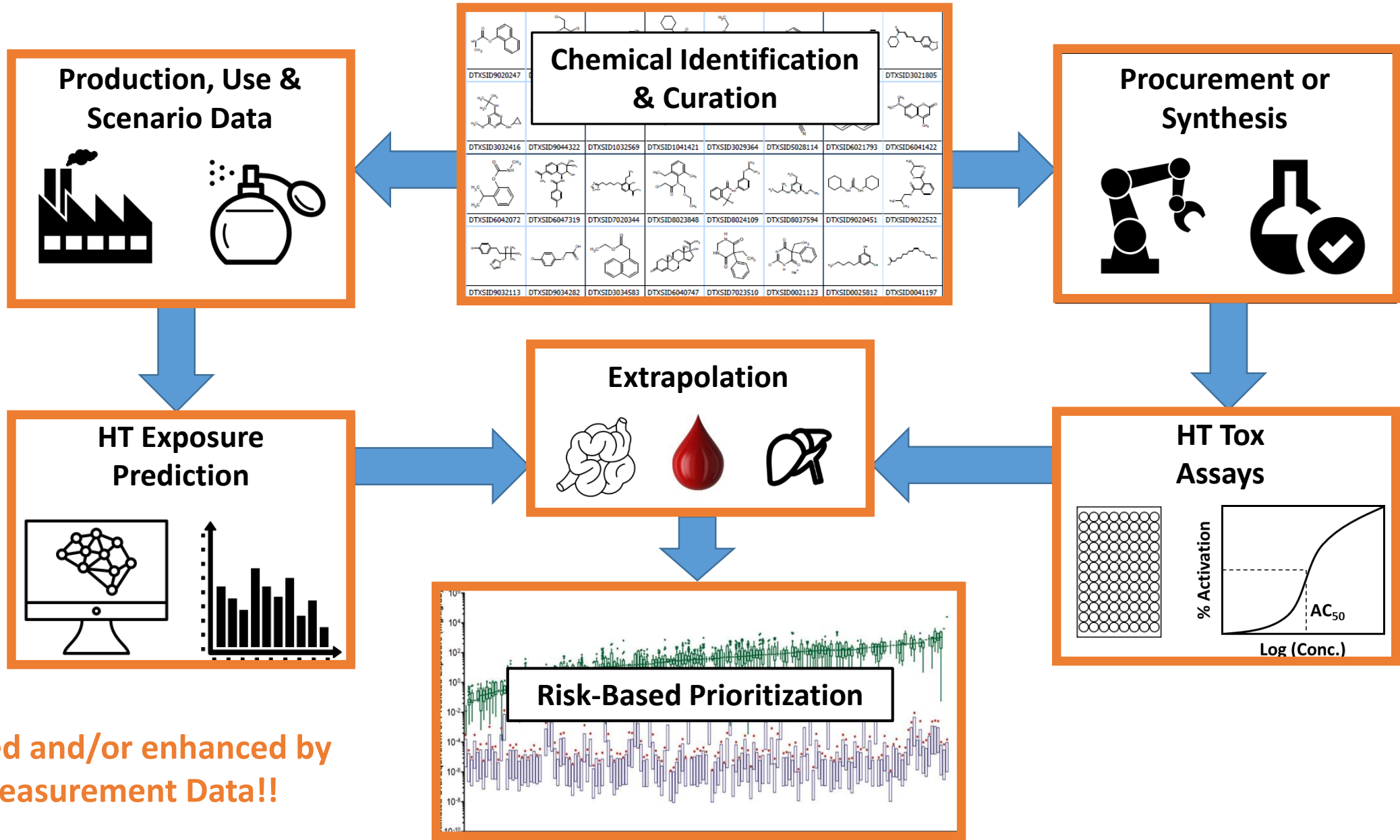
Toxicity Forecasting
(ToxCast)



The Era of High-Throughput Assessments

Exposure Forecasting
(ExpoCast)

Toxicity Forecasting
(ToxCast)



The Need for Chemical Measurement Data

- **Well-known chemicals**

- 100s - 1,000s (e.g., NHANES)
- Quality exposure data

- **Known but data-poor chemicals**

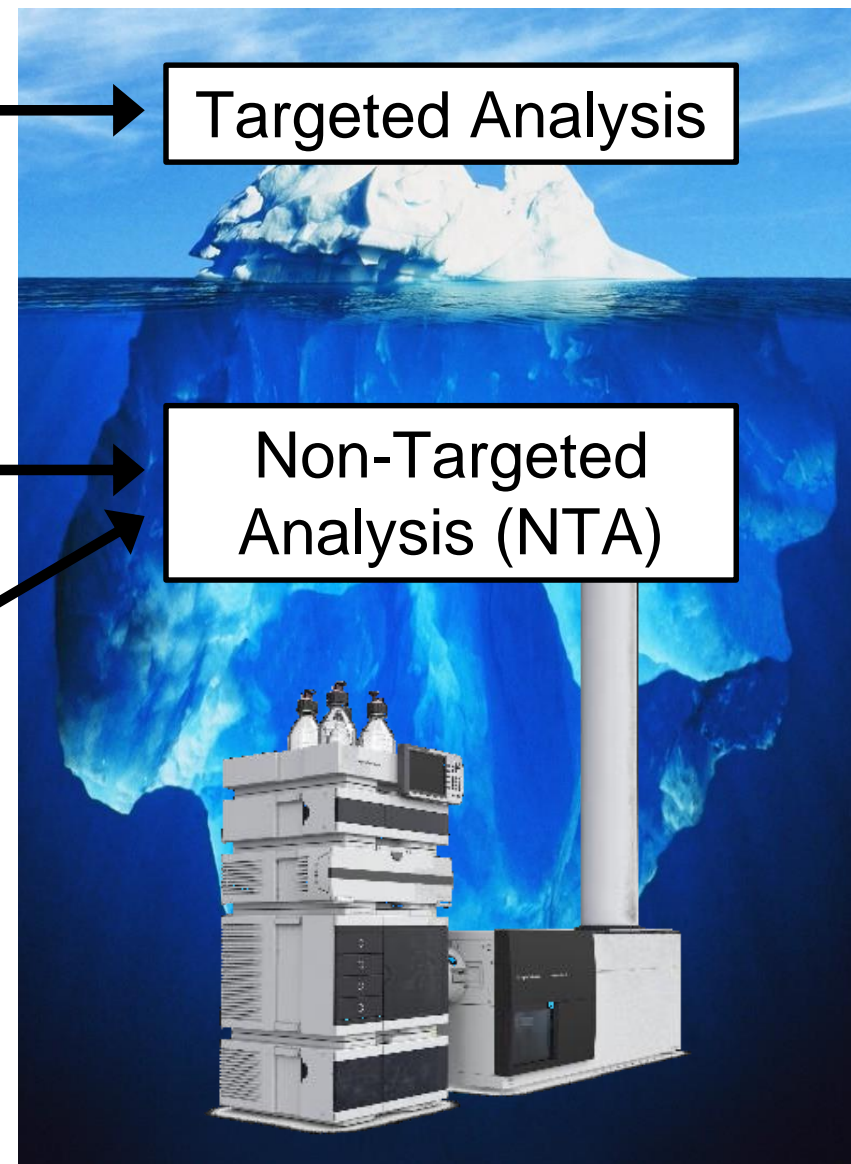
- 1,000s - 1,000,000s (e.g., TSCA)
- Limited exposure data

- **Chemicals not yet known to exist**

- Unknown #
- No exposure data

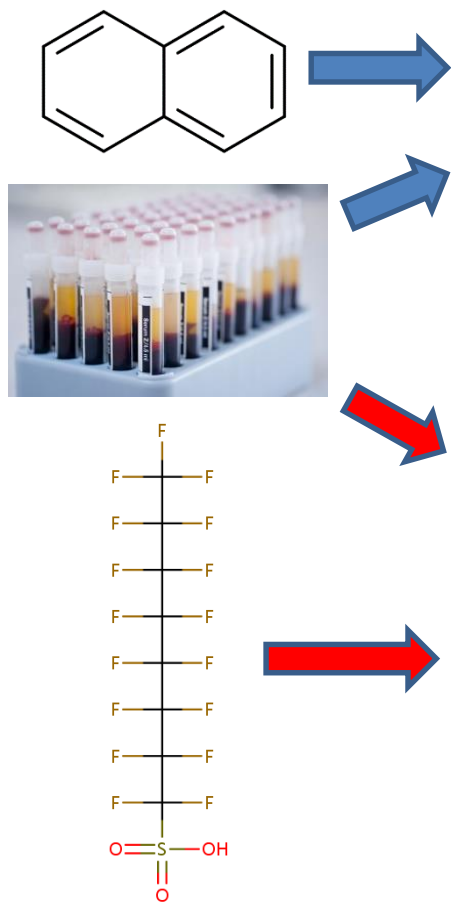
Targeted Analysis

Non-Targeted
Analysis (NTA)



Typical Targeted Analysis Workflow

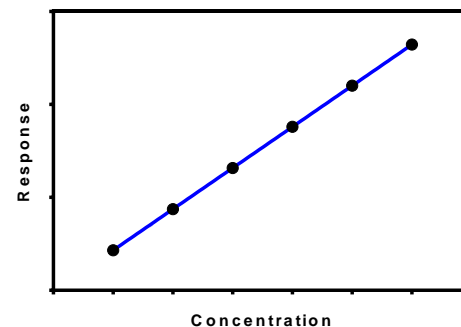
Standards/Samples



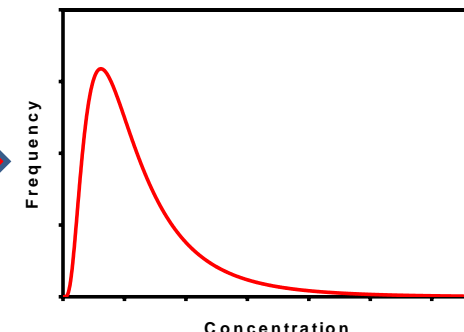
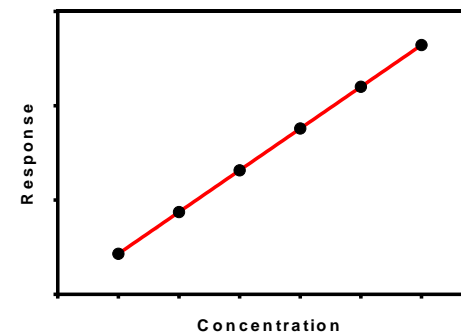
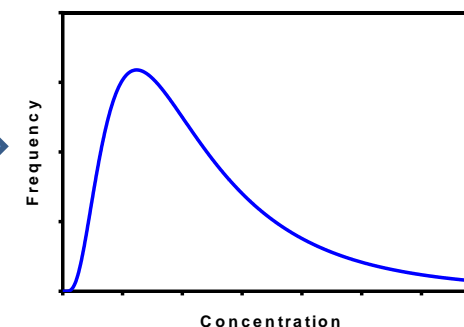
Lab Analysis



Calibration



Quantitation



Simplified NTA Workflow

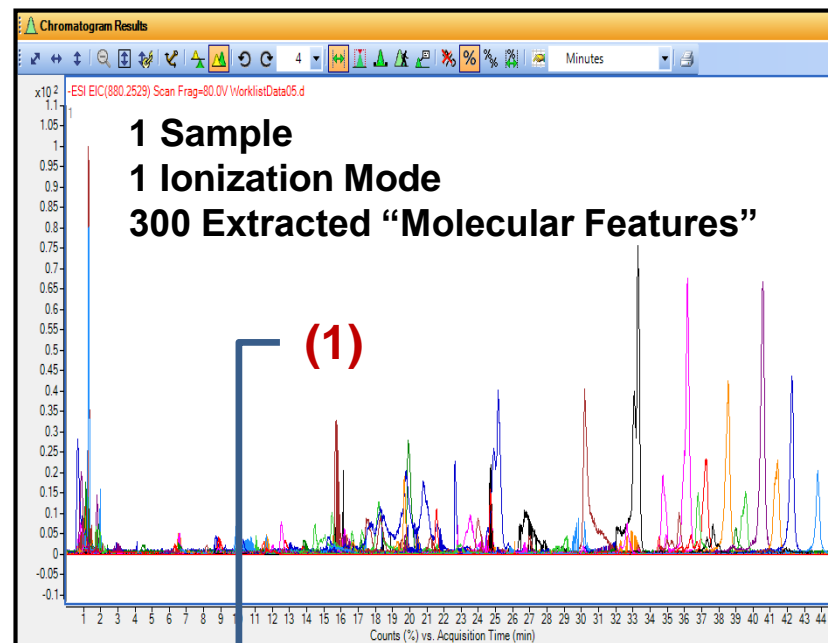
Samples



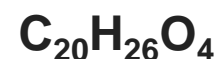
High-Resolution MS



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



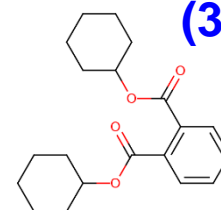
(2)



(5)



(3)



(4)

12 $\mu\text{g/mL}$

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

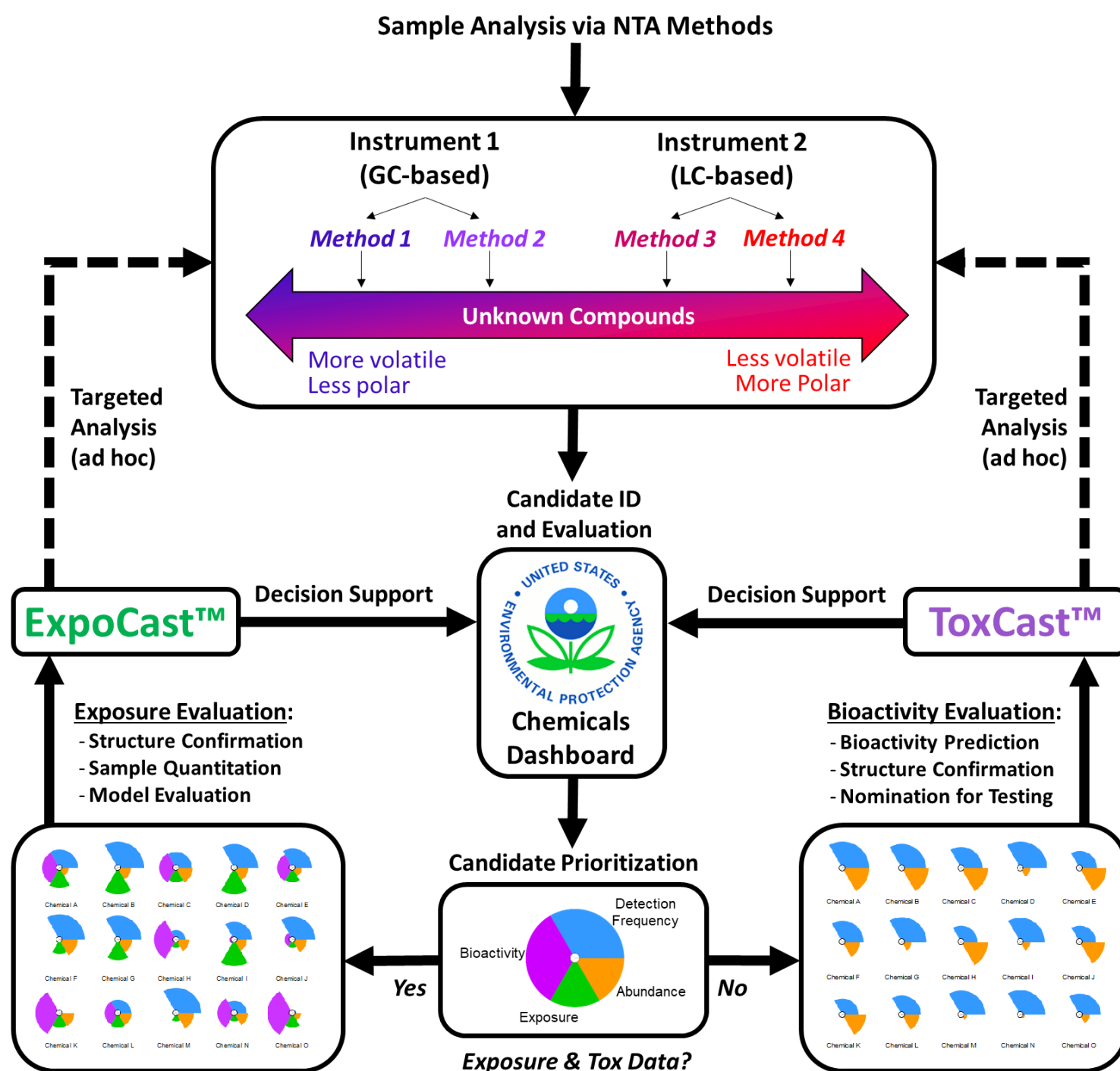
Jon R. Sobus¹ · John F. Wambaugh² · Kristin K. Isaacs¹ · Antony J. Williams² · Andrew D. McEachran³ · Ann M. Richard² · Christopher M. Grulke² · Elin M. Ulrich¹ · Julia E. Rager^{3,4} · Mark J. Strynar¹ · Seth R. Newton¹

Received: 27 May 2017 / Revised: 4 August 2017 / Accepted: 25 August 2017 / Published online: 29 December 2017
© The Author(s) 2017. This article is published with open access

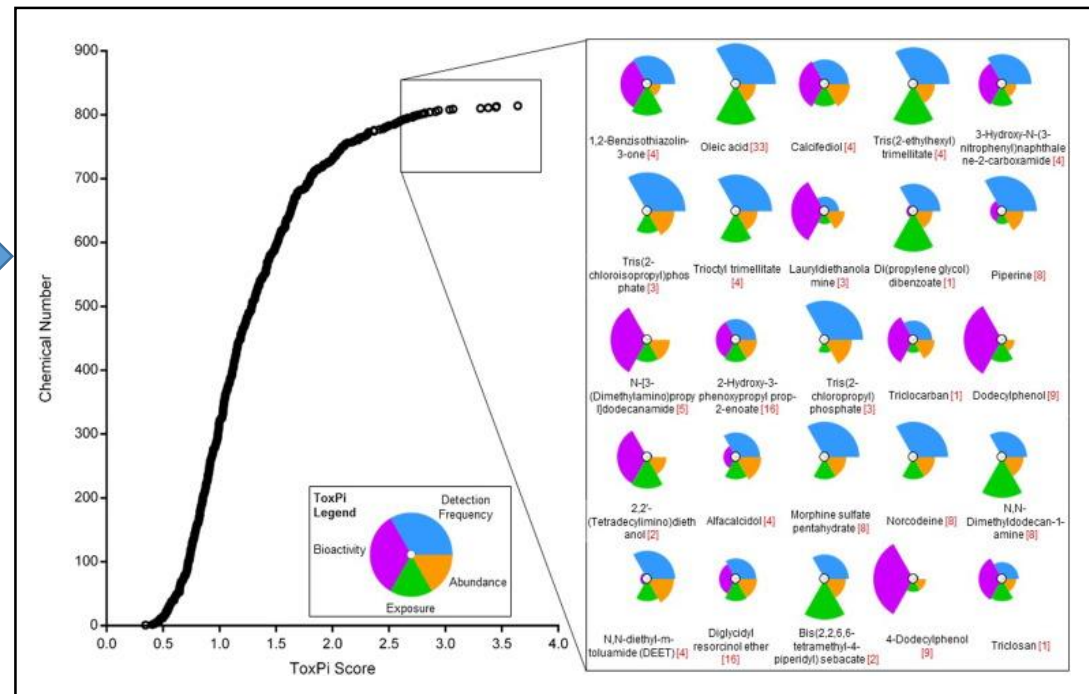
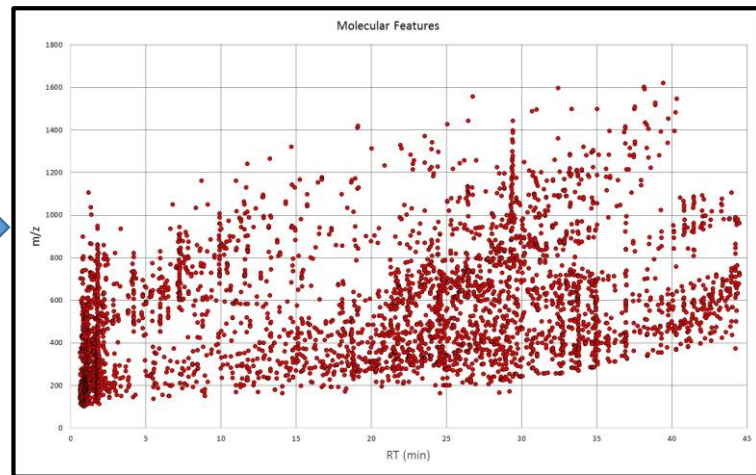
Abstract

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 measurable 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



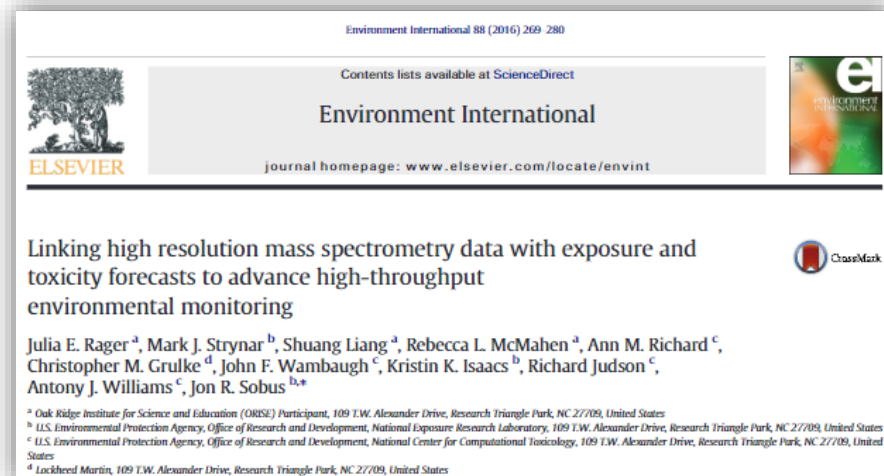
Research Highlights:

- 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!

The National Academies of
SCIENCES • ENGINEERING • MEDICINE
REPORT

**USING
21ST CENTURY
SCIENCE
TO IMPROVE
RISK-RELATED
EVALUATIONS**



Building an NTA Research Program

Exposure
Networks

Semi Quant.
Methods

EPA NTA
WebApp

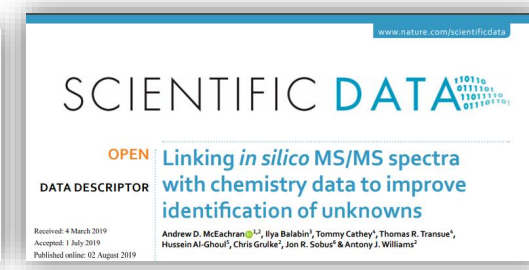
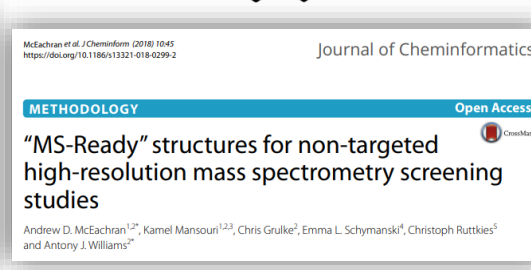
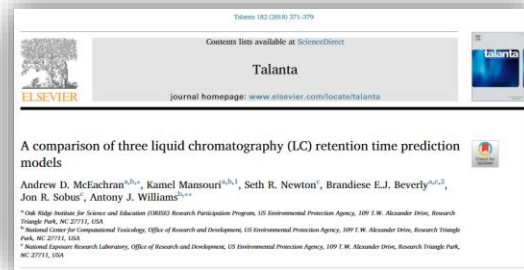
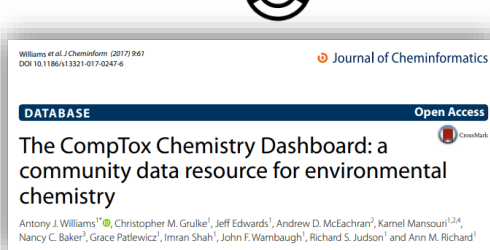
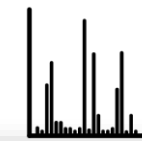
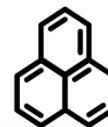
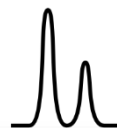
Recycled
Products

Pooled
Blood

Human
Placentas

Data, Tools & Informatics

NTA Applications



Chemicals Dashboard

RT Models

"MS Ready" Structures

In Silico Spectra

2016...

2017...

2019...

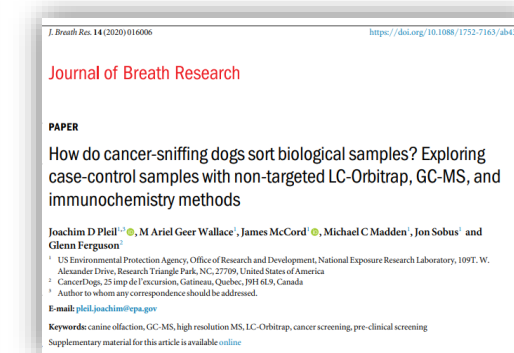
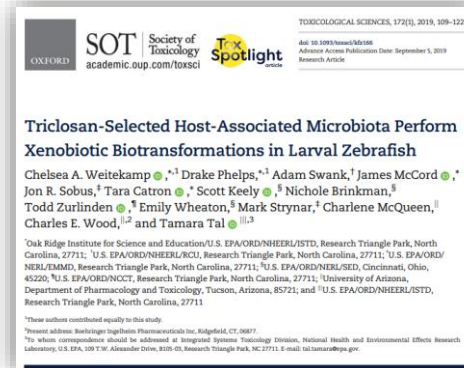
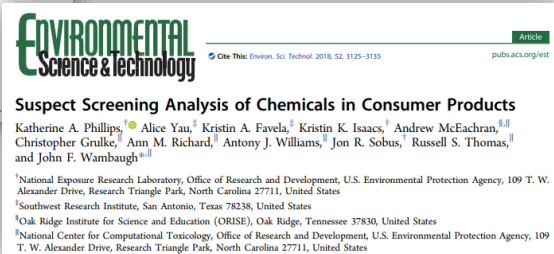
2020...

Brita Filters

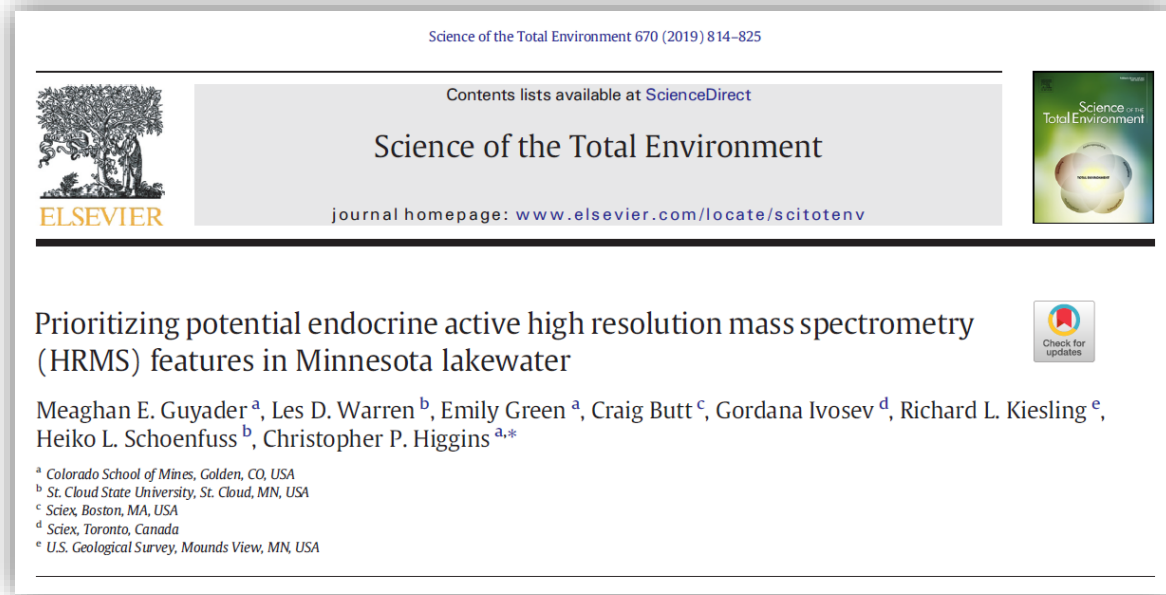
Consumer Products

Model Organisms

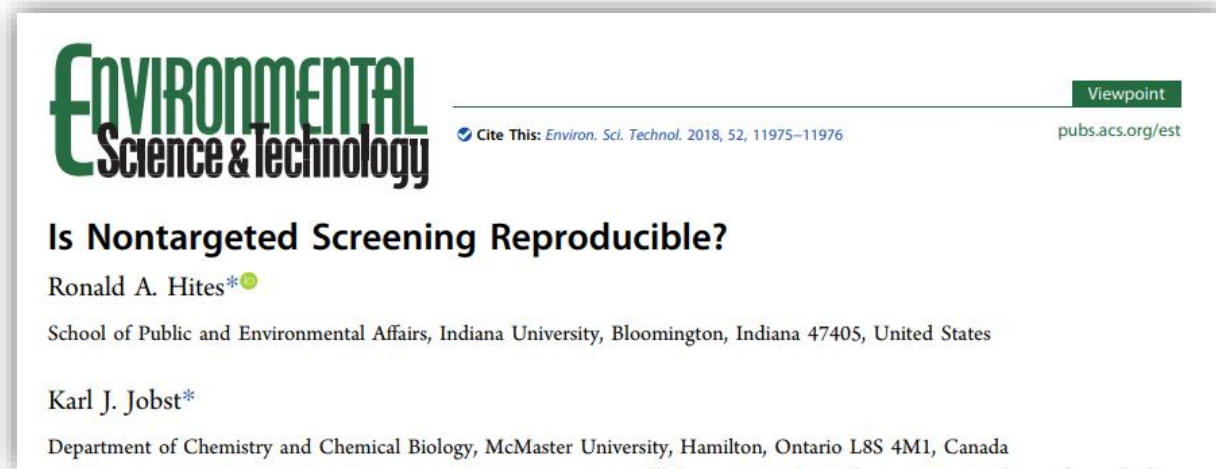
Hospital Masks



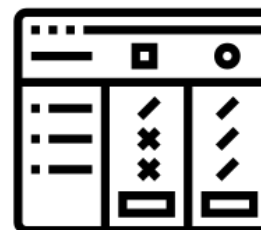
NTA State-of-the-Science



“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...

[Environmental Topics](#) [Laws & Regulations](#) [About EPA](#)

Search EPA.gov

Related Topics: [Science Matters](#)

[CONTACT US](#) [SHARE](#) [f](#) [t](#) [p](#) [e](#)

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.

 August 13-15, 2018

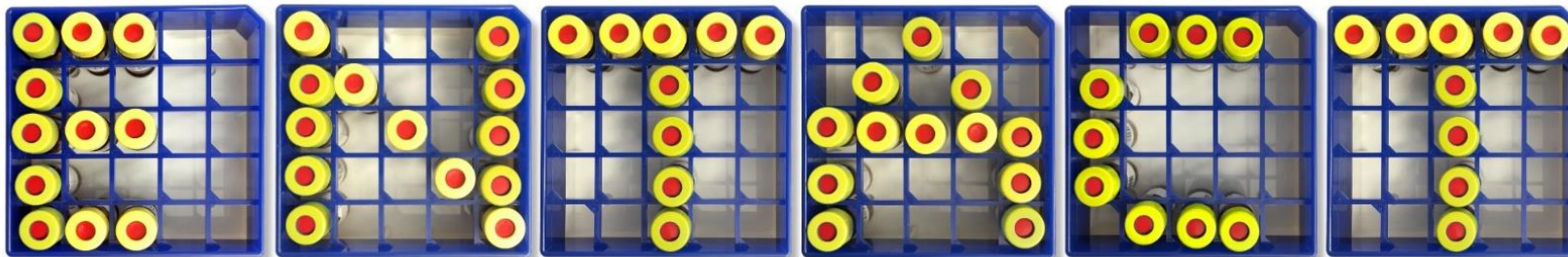
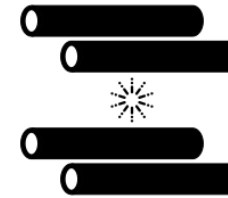
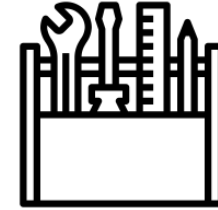
 **EPA 2018**
www.eventbrite.com/e/us-epa-2018-non-targeted-analysis-collaborative-research-trial-entact-workshop-tickets-34838702497

 Durham, NC, USA

PAST

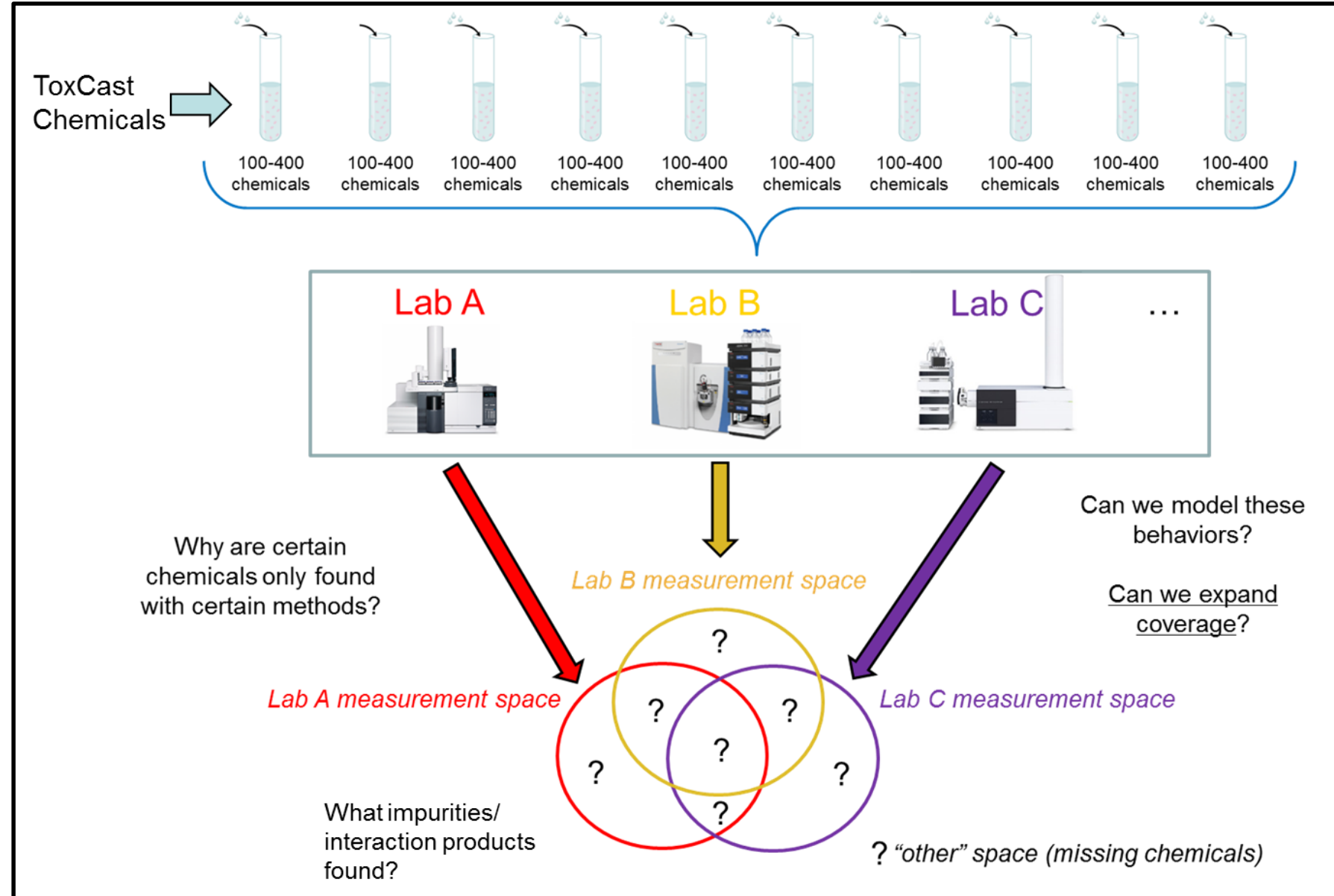
Science Questions for the Research Community

- 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?

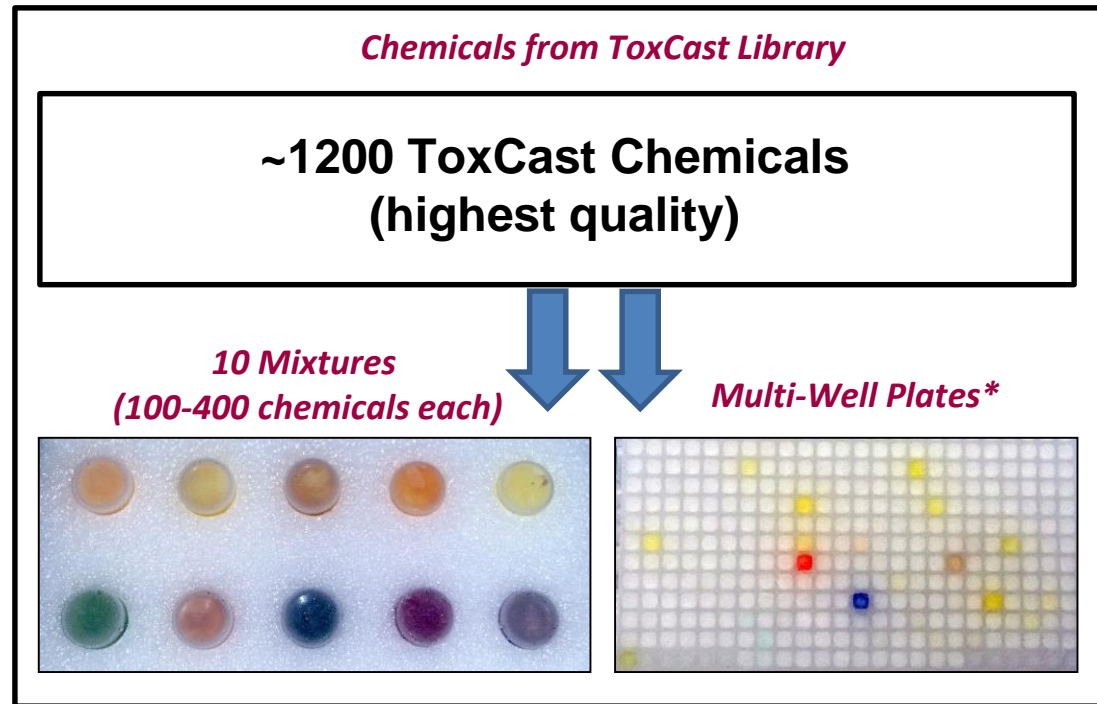


EPA's Non-Targeted Analysis Collaborative Trial

Original ENTACT Concept



ENTACT Part 1



~25 Collaborators & 5 Contractors*:

1st: Blinded analysis

2nd: Unveiling of chemicals

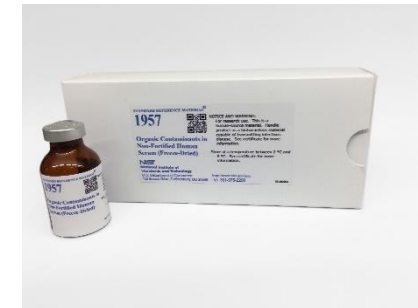
3rd: Unblinded evaluation

ENTACT Part 2

Reference & Fortified House Dust



Reference & Fortified Human Serum



Reference & Fortified Silicone Wristbands



Who is Working on ENTACT?

Contractors:



**19 Blind
submissions**

**15 Unblinded
submissions**

Vendors:



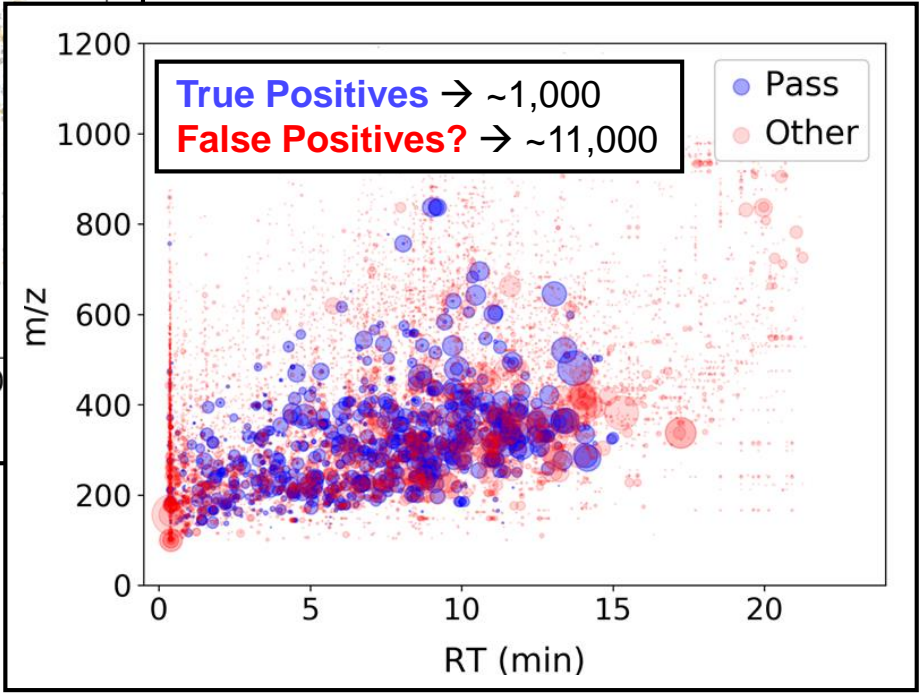
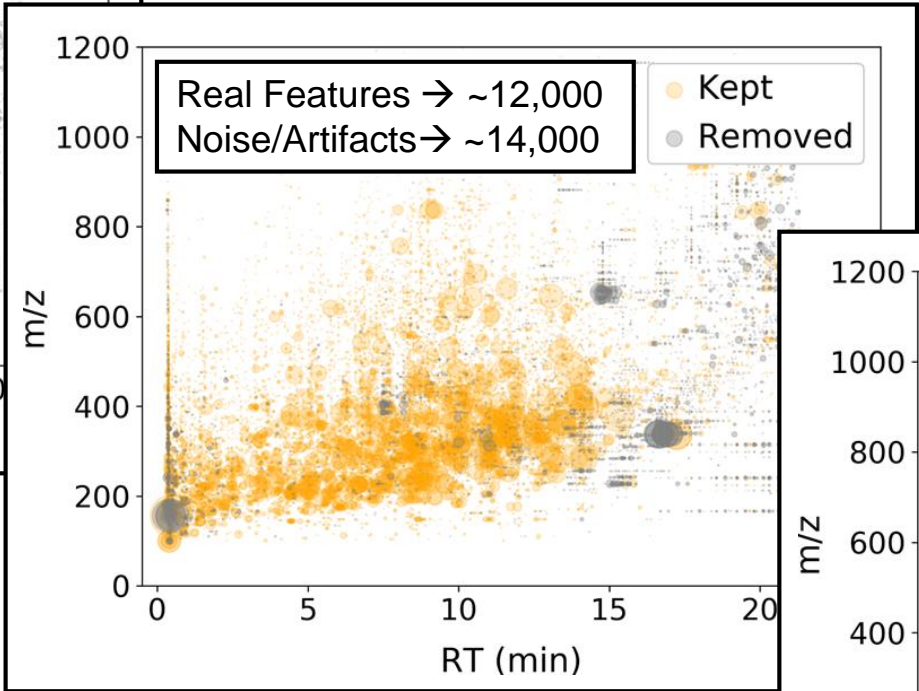
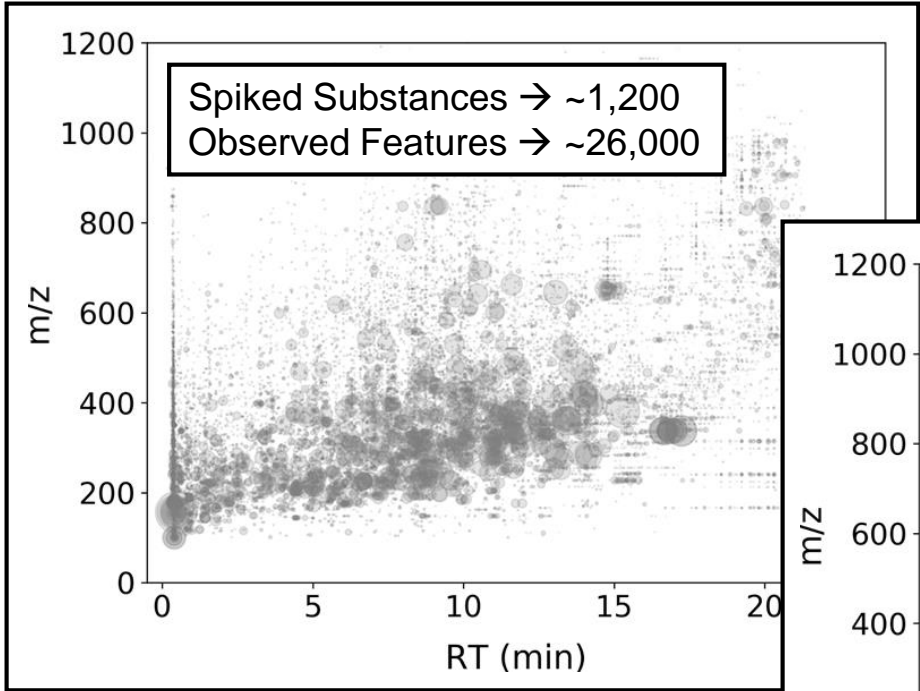
General Participants:



EPA Lab Results



LC-QTOF HRMS (ESI+ and ESI-)



Substance Spiked?

Yes

No

Substance
Observed?

Yes

No

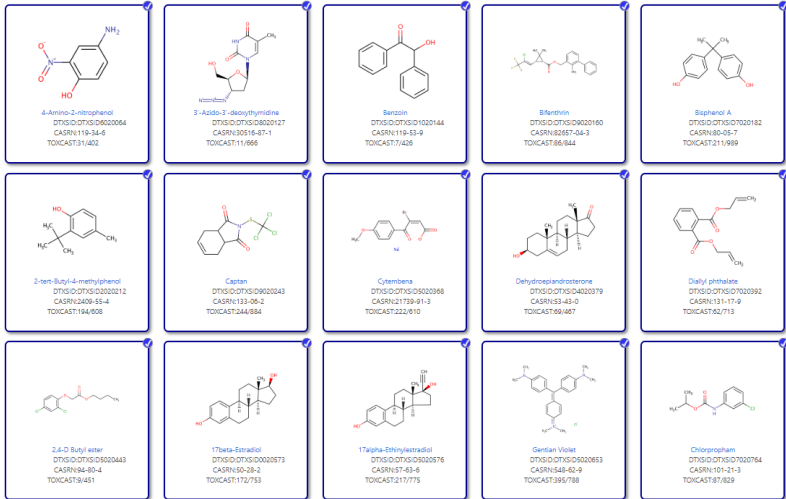
	Yes	True Positives (≤ 65%)	False Positives?
	No	False Negatives (≥ 35%)	True Negatives?

Cross-Lab Comparison

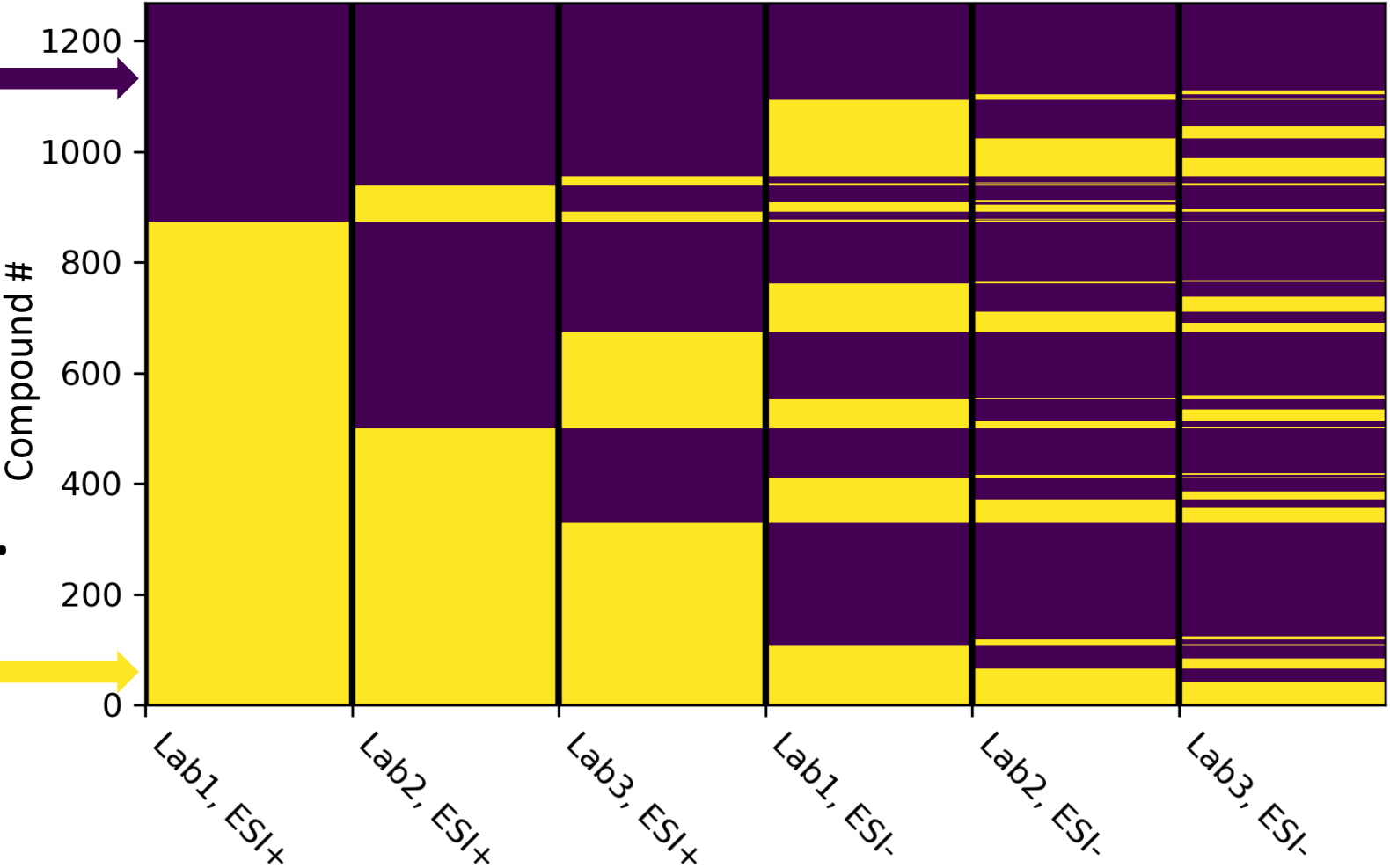
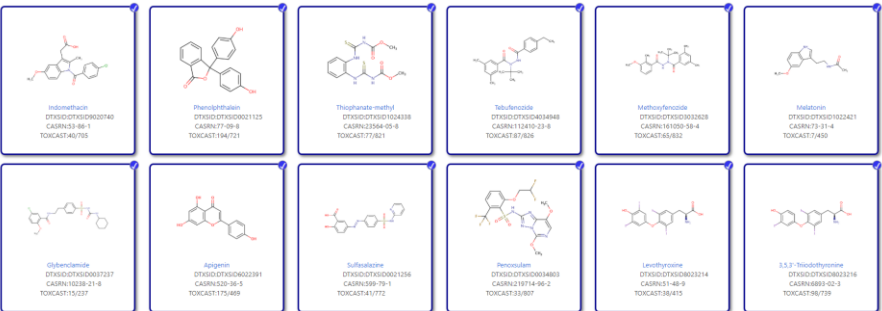
3 Labs, 6 Methods, All LC-HRMS

Obs. = 69%	Obs. = 45%	Obs. = 42%	Obs. = 39%	Obs. = 22%	Obs. = 21%
ID'd = 5%	ID'd = 37%	ID'd = 33%	ID'd = 21%	ID'd = 17%	ID'd = 14%

12% Not Observed by Any Method



3% Observed by All 6 Methods



Observed Not Observed

Evaluating *In Silico* Spectra with ENTACT Data

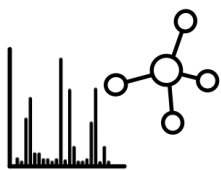
Metabolomics (2015) 11:98–110
DOI 10.1007/s11306-014-0676-4

ORIGINAL ARTICLE

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

Felicity Allen · Russ Greiner · David Wishart

Training Set

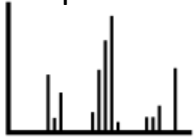


Fragmentation
Prediction Model

DSSTox structures



DSSTox MS2
spectra



SCIENTIFIC DATA

OPEN

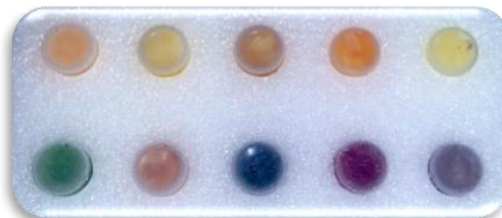
DATA DESCRIPTOR

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 Balabin³, Tommy Cathey⁴, Thomas R. Transue⁴,
Hussein Al-Ghoul⁵, Chris Grulke⁶, Jon R. Sobus⁶ & Antony J. Williams²

10 Synthetic Mixtures:
1,269 Unique ToxCast Substances



LC-QTOF HRMS:
Data Dependent Acquisition

MS2
Reference
Library

Probable
Structures

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

Tentative
Structures

No Library
Matches

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

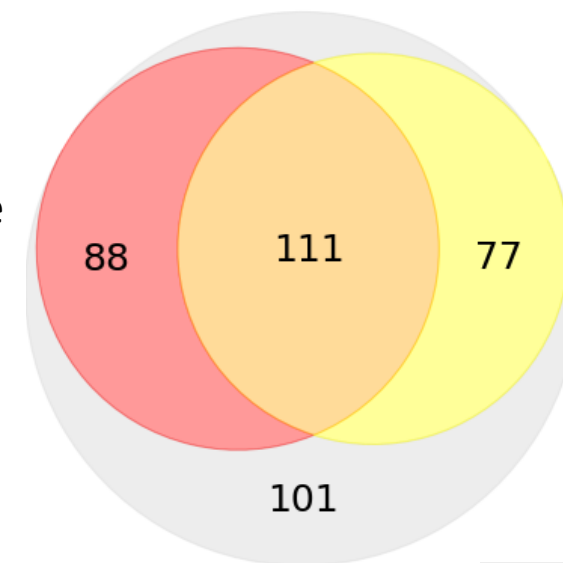
Alex Chao^{1,2} · Hussein Al-Ghoul^{1,2} · Andrew D. McEachran^{1,3} · Ilya Balabin⁴ · Tom Transue⁴ · Tommy Cathey⁴ · Jarod N. Grossman^{2,3} · Randolph Singh^{1,5} · Elin M. Ulrich² · Antony J. Williams⁶ · Jon R. Sobus²

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. Ulrich¹ • Jon R. Sobus¹ • Christopher M. Grulke² • Ann M. Richard² • Seth R. Newton¹ • Mark J. Strynar¹ • Kamel Mansouri^{3,4} • Antony J. Williams²


Received: 30 July 2018 / Revised: 14 September 2018 / Accepted: 17 October 2018 / Published online: 6 December 2018
© This is a U.S. Government work and not under copyright protection in the US; foreign copyright protection may apply 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. Sobus¹  • Jarod N. Grossman^{2,3} • Alex Chao² • Randolph Singh⁴ • Antony J. Williams⁵ • Christopher M. Grulke⁵ • Ann M. Richard⁵ • Seth R. Newton¹ • Andrew D. McEachran⁴ • Elin M. Ulrich¹

Received: 19 September 2018 / Revised: 14 November 2018 / Accepted: 27 November 2018 / Published online: 5 January 2019
© This is a U.S. Government work and not under copyright protection in the US; foreign copyright protection may apply 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

Cite This: *J. Chem. Inf. Model.* 2019, 59, 4052–4060

Article

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

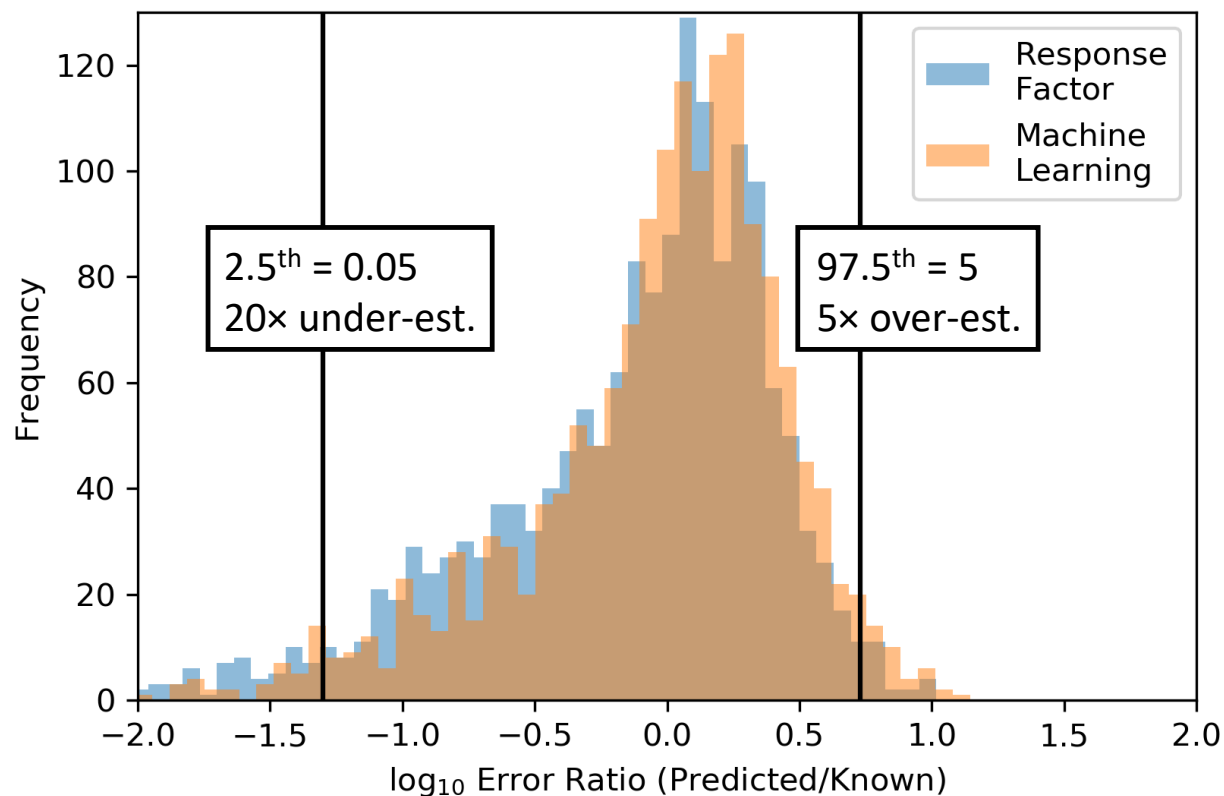
[§]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon 97331, United States

^{*}Department of Environmental Science, University of Arizona, Tucson 85712, United States

Future Focus Areas Within EPA/ORD

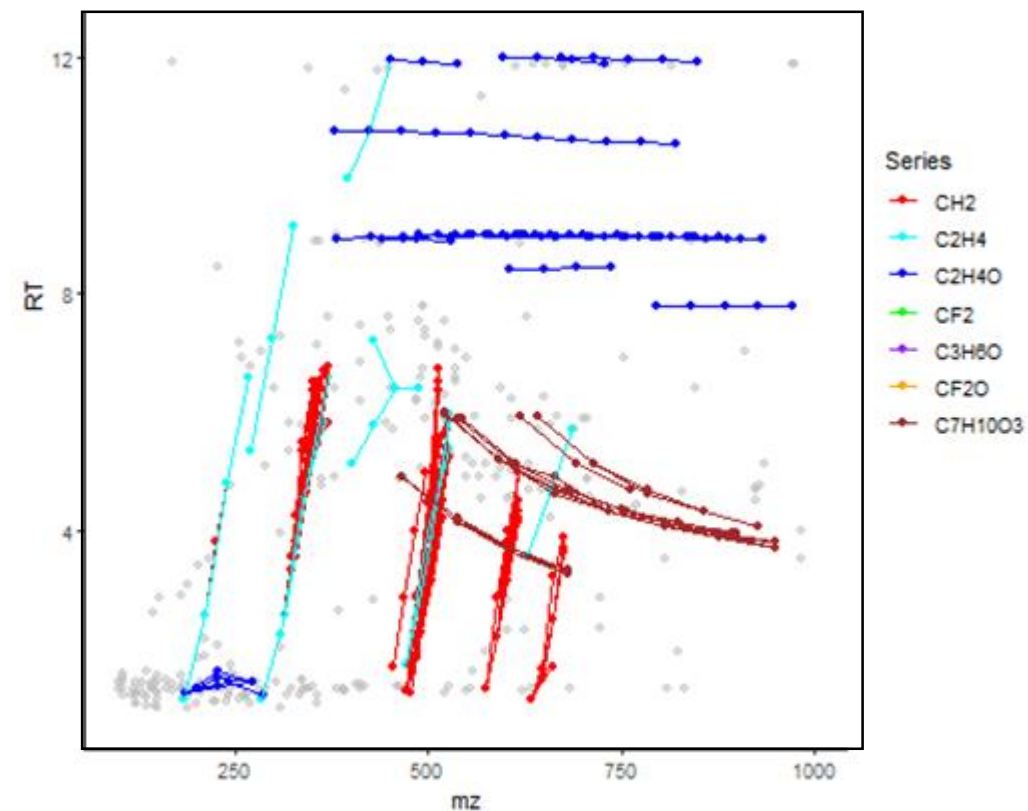
Semi-Quant. NTA:

ENTACT Data (ESI+); n= 544 compounds



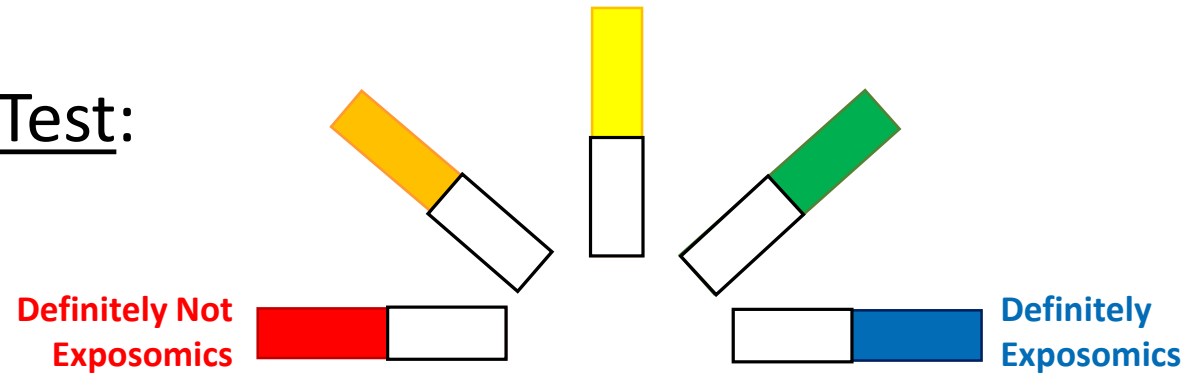
NTA for UVCBs:

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



Credit: the Research Triangle Foundation

EPA ORD

Hussein Al-Ghoul*
Alex Chao*
Louis Groff*
Jarod Grossman*
Kristin Isaacs
Sarah Laughlin*
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.)

Chris Grulke
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.