EPA
United States
Environmental Protection
Agency

EPA's Non-Targeted Analysis Research Program: Building Tools to Enable Rapid Exposure Surveillance

Jon R. Sobus, Ph.D.

Center for Computational Toxicology and Exposure

Key Drivers for 21st Century Exposure Science

1) Understanding causes of disease

"…70-90% of disease risks are probably due to differences in environments"

EPIDEMIOLOGY

Environment and Disease Risks

A new paradigm is needed to assess how a lifetime of exposure to environmental factors affects the risk of developing chronic diseases.

Stephen M. Rappaport and Martyn T. Smith

Although the risks of developing chronic diseases are attributed to both genetic and environmental factors, 70 to 90% of disease risks are probably due to differences in environments (*1*–*3*). Yet, epidemiologists increasingly use genome-wide association studies (GWAS) to investigate diseases, while relying on questionnaires to characterize "environmental exposures." This is because GWAS represent the only approach for exploring the totality of any risk factor (genes, in this case) associated with disease prevalence. Moreover, the value of costly genetic information is diminished when inaccurate and imprecise environmental data lead to biased inferences regarding gene-environment interactions (*4*). A more comprehensive and quantitative view of environmental exposure is needed if epidemiologists are to discover the major causes of chronic diseases.

An obstacle to identifying the most important environmental exposures is the fragmentation of epidemiological research along lines defined by different factors. When epidemiologists investigate environmental risks, they tend to concentrate on a particular category of exposures involving air and water pollution, occupation, diet and obesity, stress and behavior, or types of infection. This slicing of the disease pie along parochial lines leads to scientific separation and confuses the definition of "environmental exposures." In fact, all of these exposure categories can contribute to chronic diseases and should be investigated collectively rather than separately.

To develop a more cohesive view of environmental exposure, it is important to recognize that toxic effects are mediated through chemicals that alter critical molecules, cells, and physiological processes inside the body. Thus, it would be reasonable to consider the "environment" as the body's internal chemical environment and "exposures" as the amounts of biologically active chemicals in this internal environment. Under this view, exposures are not restricted to chemicals (toxicants) entering the body from air, water, or food, for example, but also include chemicals produced by inflammation, oxidative stress, lipid peroxidation, infections, gut flora, and other natural processes (*5*, *6*) (see the figure). This internal chemical environment continually fluctuates during life due to changes in external and internal sources, aging, infections, life-style, stress, psychosocial factors, and preexisting diseases.

The term "exposome" refers to the totality of environmental exposures from conception onwards, and has been proposed to be a

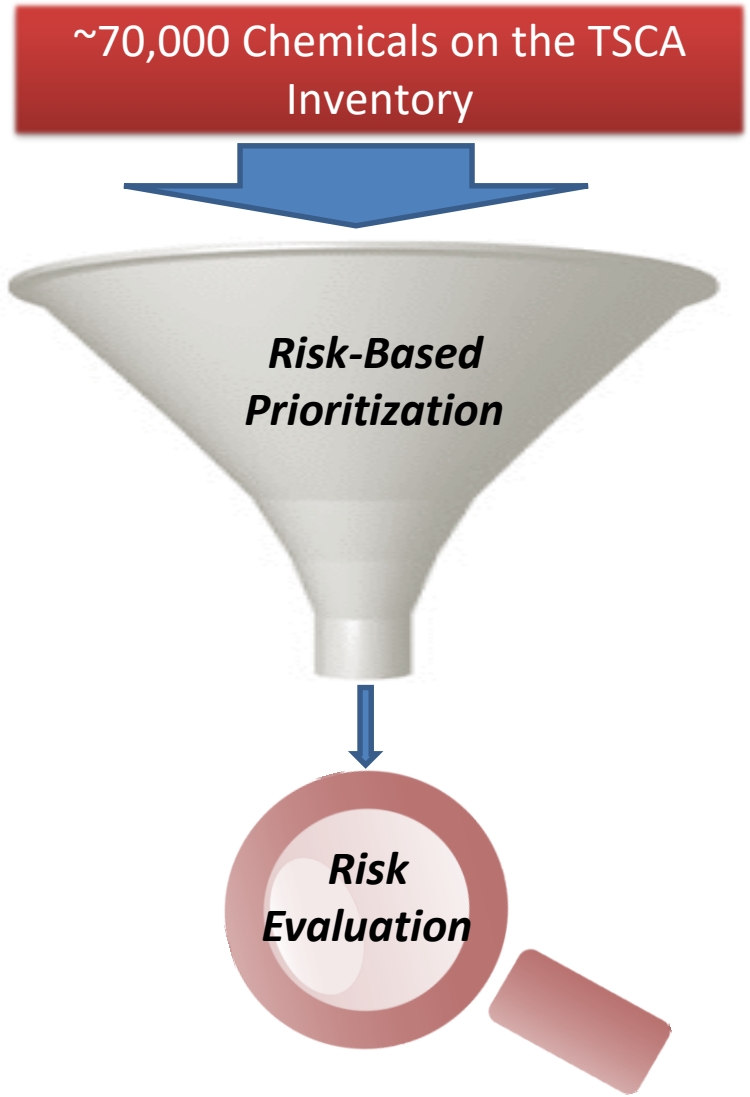
School of Public Health, University of California, Berkeley, CA 94720–7356, USA. E-mail: srappaport@berkeley.edu

Published by AAAS

2) Ensuring chemical safety

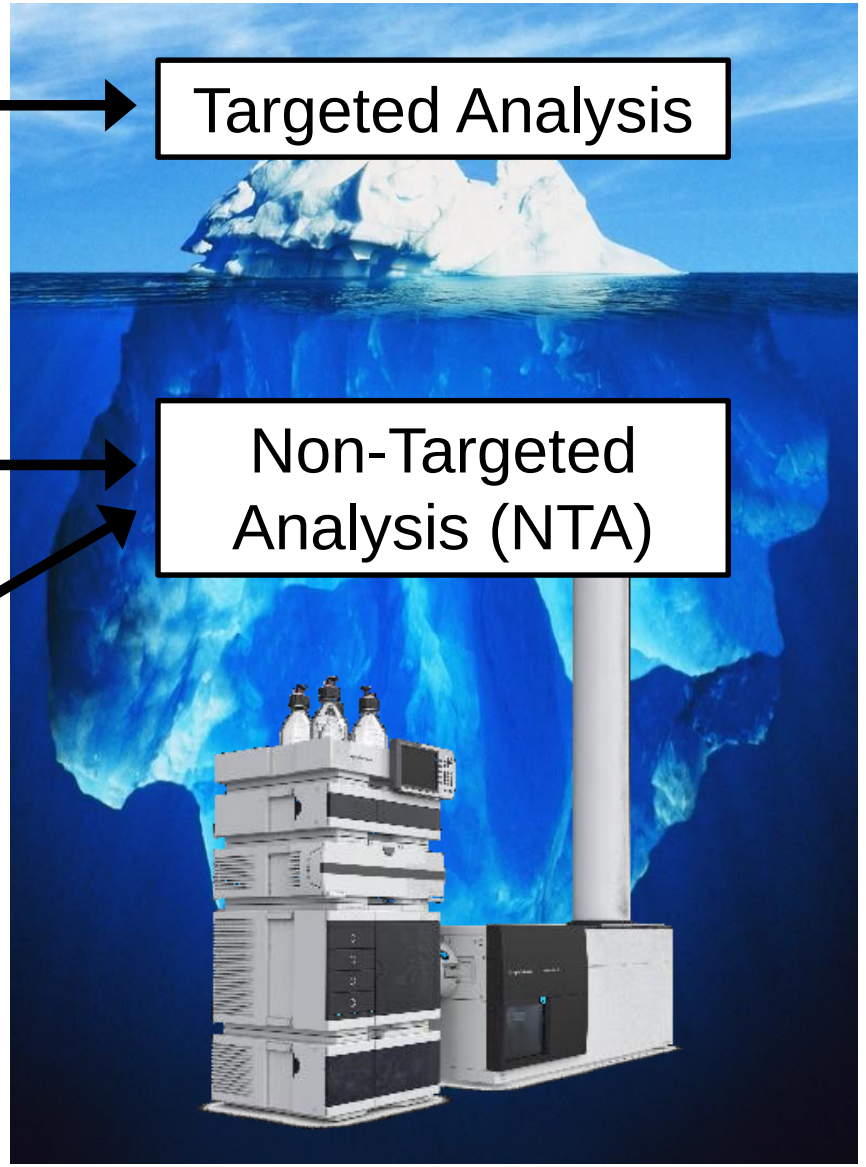
High-Throughput Risk Characterization

- Many industrial & commercial chemicals are covered by the Toxic Substances Control Act (TSCA), which is administered by EPA.
- TSCA updated in June 2016 to allow *risk-based* evaluation of existing and new chemicals.
- Characterization of risk requires exposure and hazard data.
- EPA's Office of Research and Development (ORD) is developing new approach methodologies (NAMs) for rapid risk characterization.
- <u>NTA is a promising NAM, but requires careful evaluation and implementation</u>



For Which Chemicals Must We Assess Exposure?

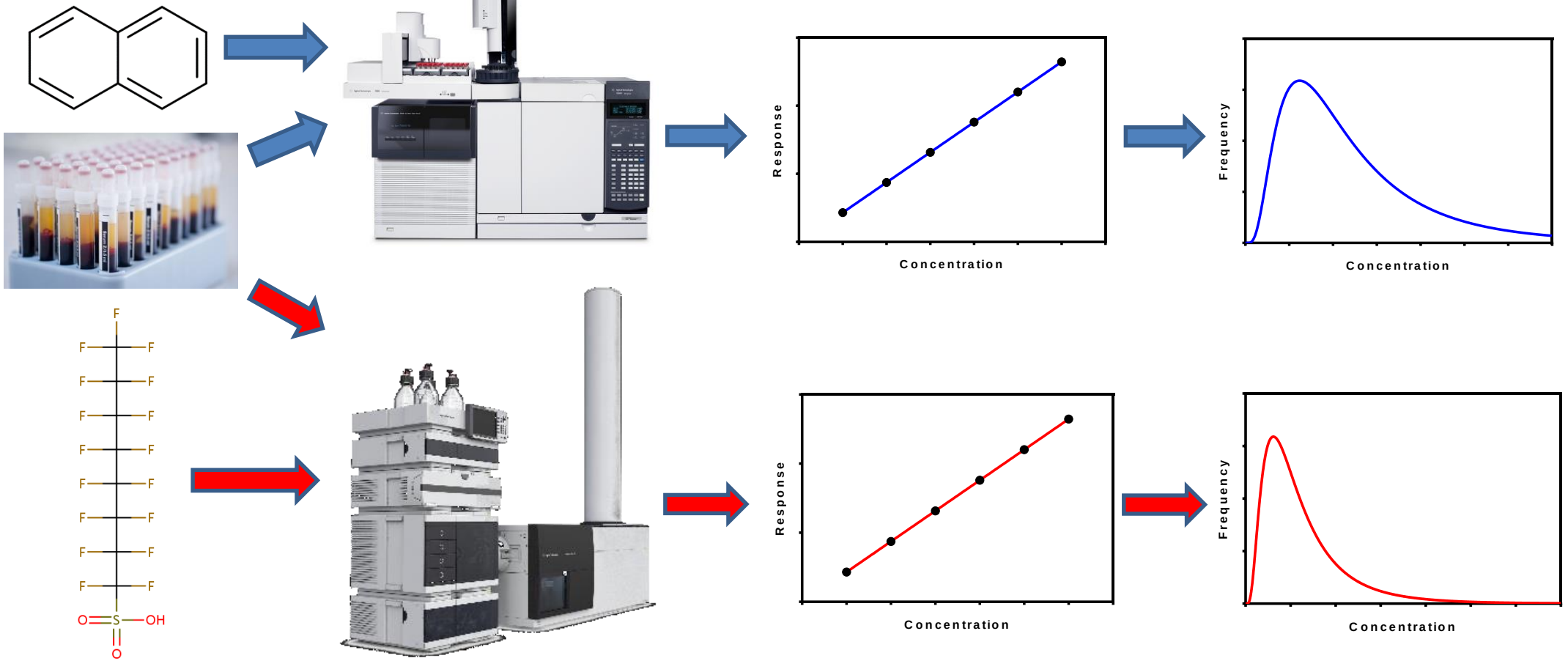
- **Well-known chemicals**
  - 100s - 1,000s (e.g., NHANES)
  - Quality exposure data
- **Known but data-poor chemicals**
  - 1,000s - 1,000,000s (e.g., TSCA)
  - Limited exposure data
- **Chemicals not yet known to exist**
  - Unknown #
  - No exposure data



Targeted Analysis for Quantitation of Knowns

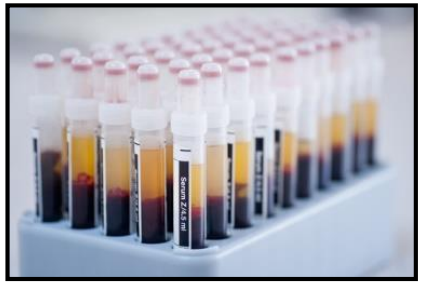
United States Environmental Protection Agency

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

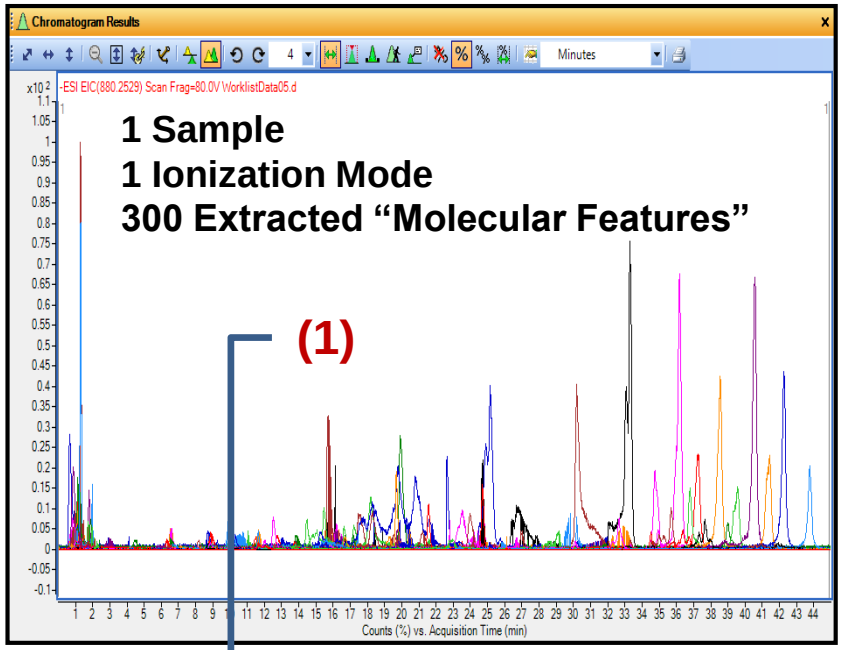


NTA for Chemical Discovery

Samples



High-Resolution MS

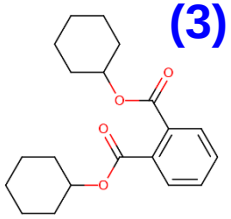


1) Prioritize "molecular features"
2) Correctly assign formulas
3) Correctly assign structures
4) Predict chemical concentrations
5) Determine chemical sources

(2)

$C_{20}H_{26}O_4$

(3)



(4)

12 µg/mL

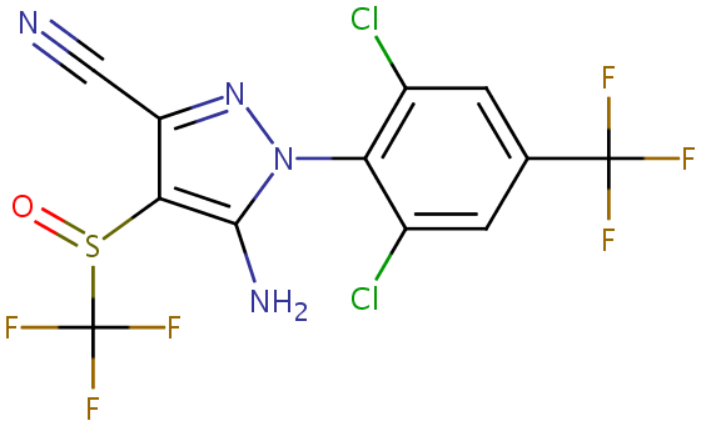
(5)

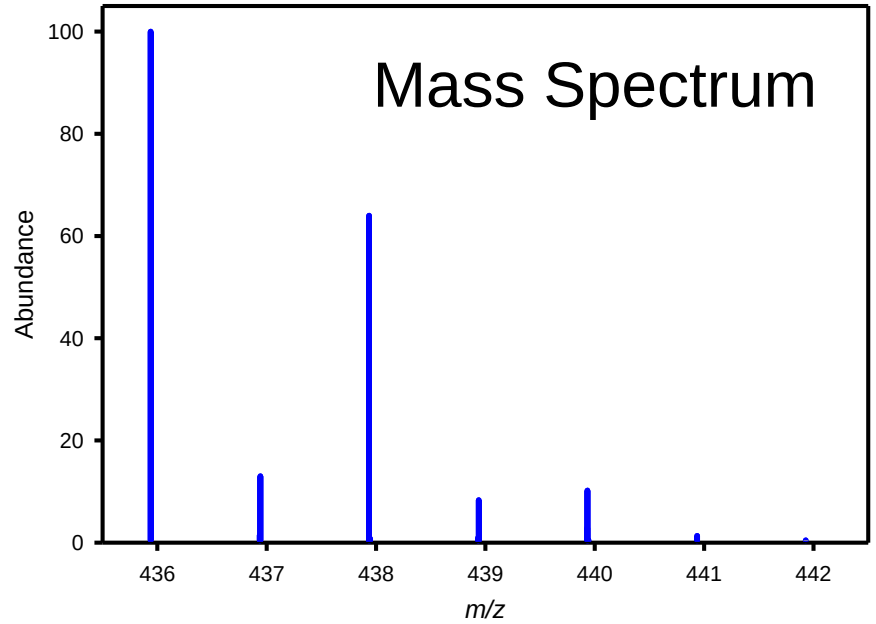
How does High Resolution MS Work?

Atom	Natural Abundance	Exact Mass
^{1}H	99.9885%	1.007825
^{2}H	0.0115%	2.014102
^{12}C	98.93%	12.000000
^{13}C	1.07%	13.003355
^{14}N	99.632%	14.003074
^{15}N	0.368%	15.000109
^{16}O	99.757%	15.994915
^{17}O	0.038%	16.999131
^{18}O	0.205%	17.999159
^{19}F	100%	18.998403
^{32}S	94.93%	31.972072
^{33}S	0.76%	32.971459
^{34}S	4.29%	33.967868
^{36}S	0.02%	35.967079
^{35}Cl	75.78%	34.968853
^{37}Cl	24.22%	36.965903

Example: **Fipronil**
Molecular Formula: **$C_{12}H_4Cl_2F_6N_4OS$**
Monoisotopic Mass: **435.938706**

= (12.0000*12 Carbon) + (1.007825*4 Hydrogen) + (34.968853*2 Chlorine) + (18.998403*6 Fluorine) + (14.003074*4 Nitrogen) + (15.994915*1 Oxygen) + (31.972072*1 Sulfur)





NTA Applications at EPA

- **Exposure surveillance**
  - What chemicals are in water, products, dust, blood, etc.?
- **Chemical prioritization**
  - What are relevant chemicals & mixtures?
- **Exposure forensics**
  - What are chemical signatures of exposure sources?
- **Biomarker discovery**
  - What chemicals are associated with health impairment?

Exposure Surveillance for Consumer Products

Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2018, 52, 3125−3135

pubs.acs.org/est

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*[,∥]

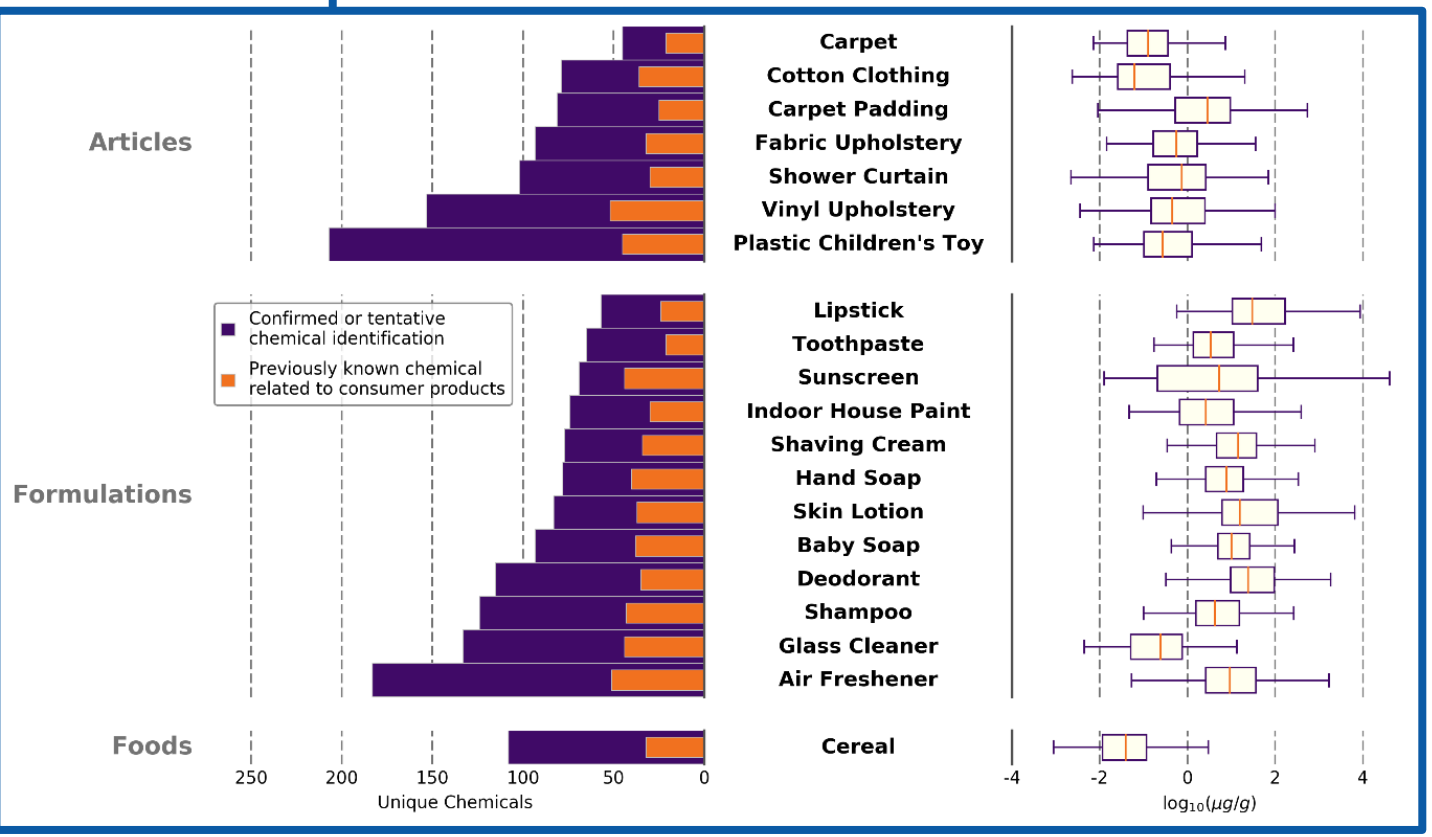
[†]National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Alexander Drive, Research Triangle Park, North Carolina 27711, United States

[‡]Southwest Research Institute, San Antonio, Texas 78238, United States

[§]Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, Tennessee 37830, United Stat

[∥]National Center for Computational Toxicology, Office of Research and Development, U.S. Environn T. W. Alexander Drive, Research Triangle Park, North Carolina 27711, United States

19% of chemicals identified by NTA are on consumer product chemical lists



Chemical Prioritization for Drinking Water

Environmental Pollution 234 (2018) 297–306

Contents lists available at ScienceDirect

Environmental Pollution

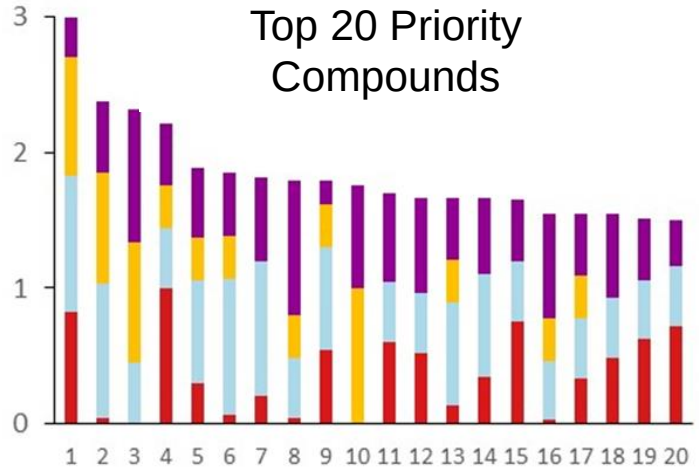
journal homepage: www.elsevier.com/locate/envpol

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

Seth R. Newton [a, *], Rebecca L. McMahen [a, b], Jon R. Sobus [a], Kamel Mansouri [b, c, 1], Antony J. Williams [c], Andrew D. McEachran [b, c], Mark J. Strynar [a]

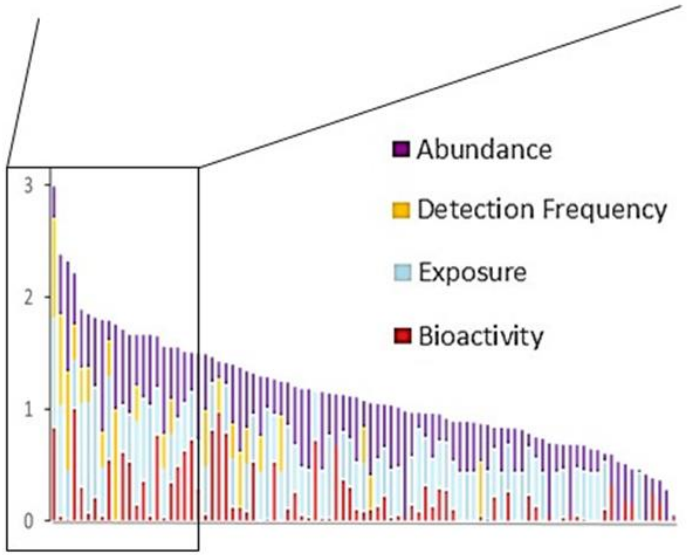
[a] United States Environmental Protection Agency, National Exposure Research Laboratory, Research Triangle Park, NC 27709, United States
[b] Oak Ridge Institute for Science and Education Research Participant, 109 T.W. Alexander Drive, Research Triangle Park, NC 27709, United States
[c] United States Environmental Protection Agency, National Center for Computational Toxicology, Research Triangle Park, NC 27709, United States

Top 20 Priority Compounds



#	Compound	ToxPi Score
1	1,2-Benzisothiazolin-3-one*	2.99
2	Diethyleneglycol	2.38
3	N-[3-(Dimethylamino)propyl] methacrylamide	2.32
4	Nonylparaben	2.22
5	Dipentyl phthalate	1.89
6	2-[2-(2-Butoxyethoxy) ethoxy]ethanol*	1.85
7	N,N-Dimethyldodecan-1-amine*	1.81
8	Sucralose	1.80
9	PFOS*	1.79
10	2-(2-Ethoxyethoxy) ethyl acetate*	1.76
11	TDCPP*	1.71
12	Zearalanol	1.67
13	PFOA*	1.66
14	Butylparaben	1.66
15	Noristerat	1.65
16	p-Synephrine	1.55
17	Alprostadil	1.55
18	Sclareol	1.55
19	PFDA*	1.51
20	Simvastatin	1.50

*Confirmed with standard



Top 100 Priority Compounds

Exposure Forensics for Recycled Products

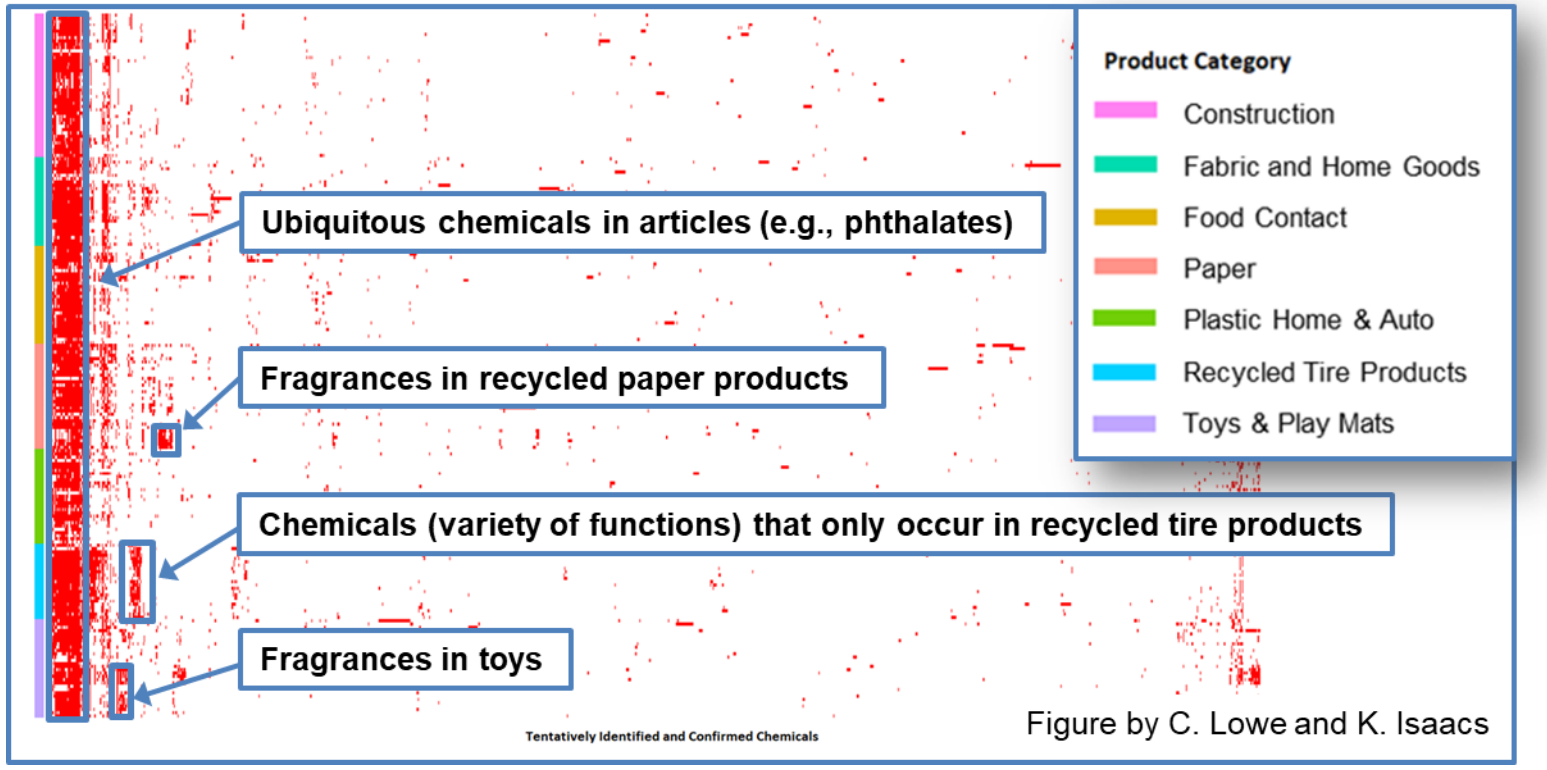
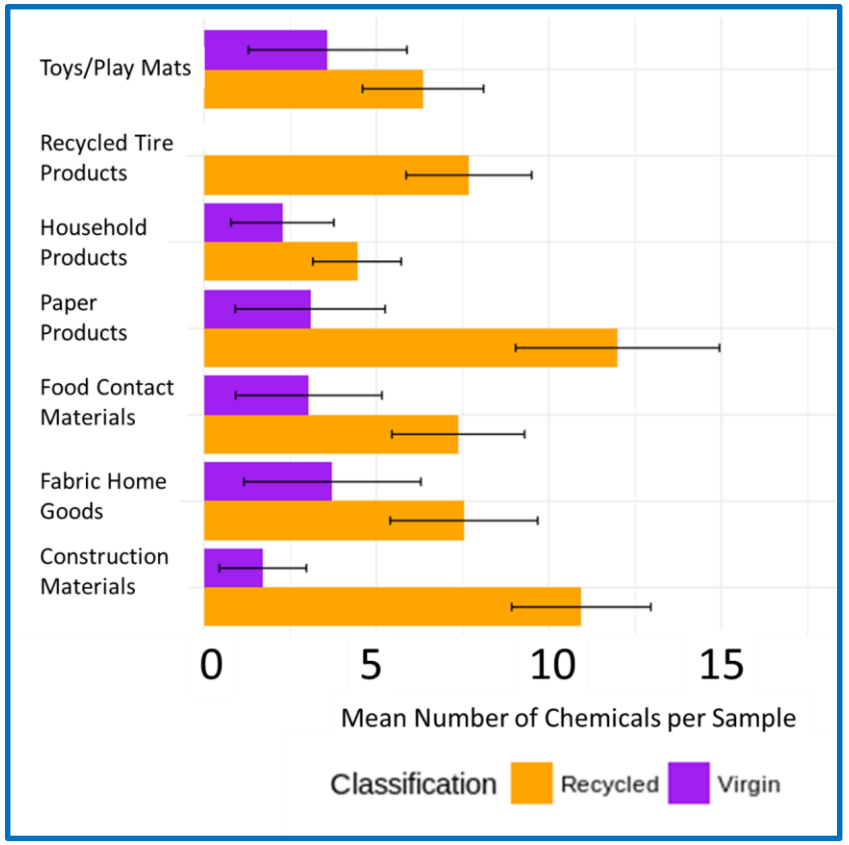


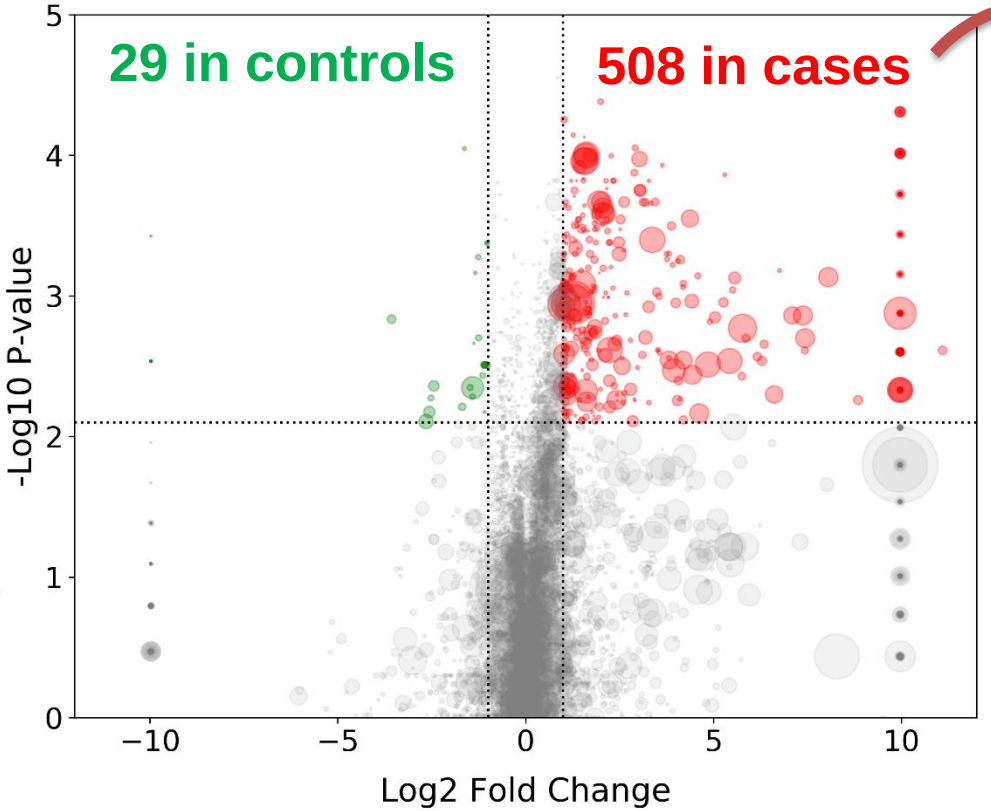
Figure by C. Lowe and K. Isaacs



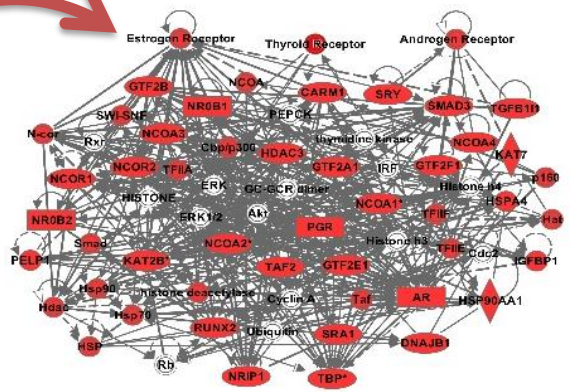
Biomarker Discovery for Placenta Samples

Different Environmental Exposures

The Placental Exposome (via LC-HRMS)



Altered Cell Signaling

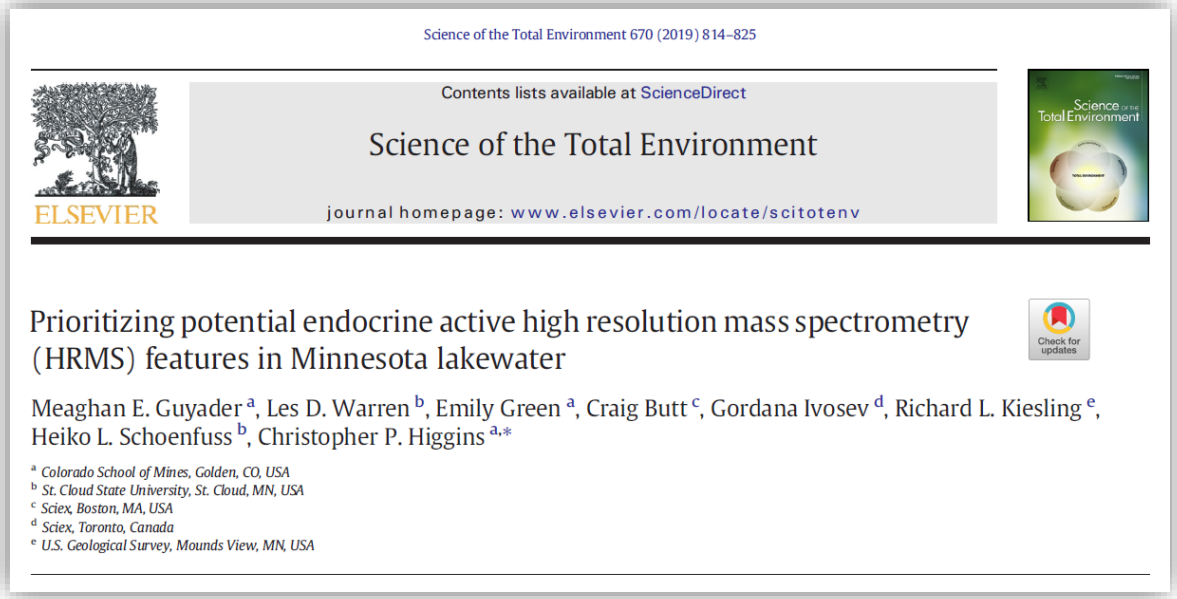


Impaired Angiogenesis

Preeclampsia

Collaboration with J. Rager (UNC Chapel Hill) and J. Grossman (Agilent)

NTA State-of-the-Science



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

Contents lists available at ScienceDirect

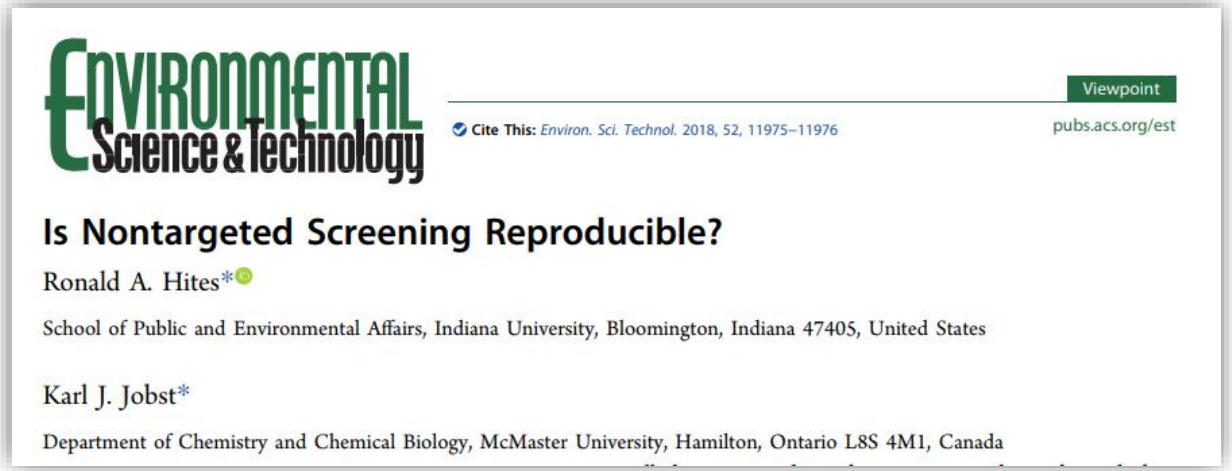
Science of the Total Environment

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*

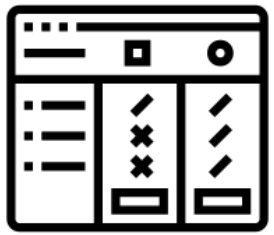
School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana 47405, United States

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/ORD Takes a Leadership Role

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.

EPA science in ACTION
www.epa.gov/research
INNOVATIVE RESEARCH FOR A SUSTAINABLE FUTURE

EPA'S NON-TARGETED ANALYSIS COLLABORATIVE TRIAL (ENTACT)

Environmental Protection Agency (EPA) 2018

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

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.

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

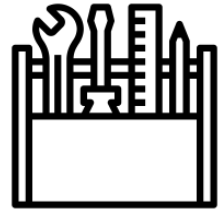
Published July 30, 2018

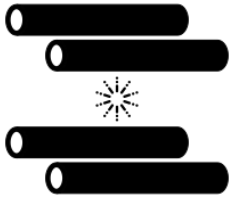
EPA scientists are leading a multi-phase project to evaluate the ability of non-targeted analysis laboratory methods to consistently and correctly identify unknown chemicals in samples. EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) was formed in late 2015 and includes nearly 30 academic, government, 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.

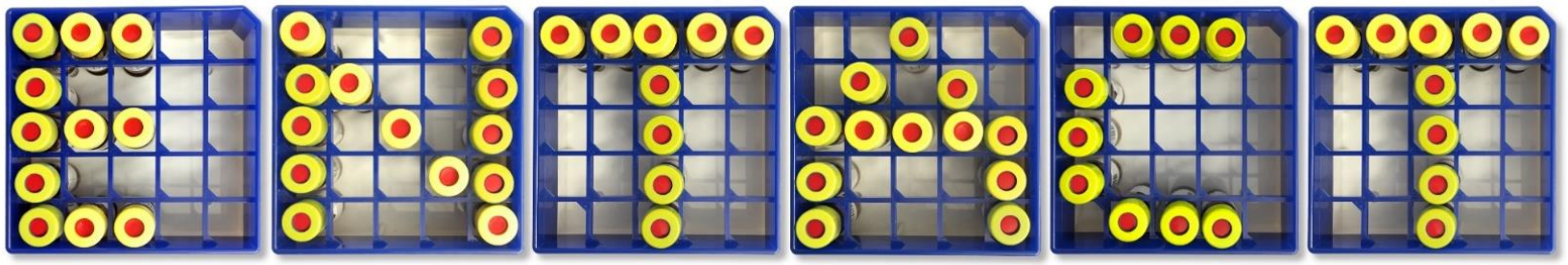
"One of our main goals is to figure out what scientists are doing with non-targeted analysis as a group at large, particularly which chemicals we correctly identify and why," says Elin Ulrich, an EPA scientist who co-leads ENTACT with EPA's Jon Sobus.

Science Questions for 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?

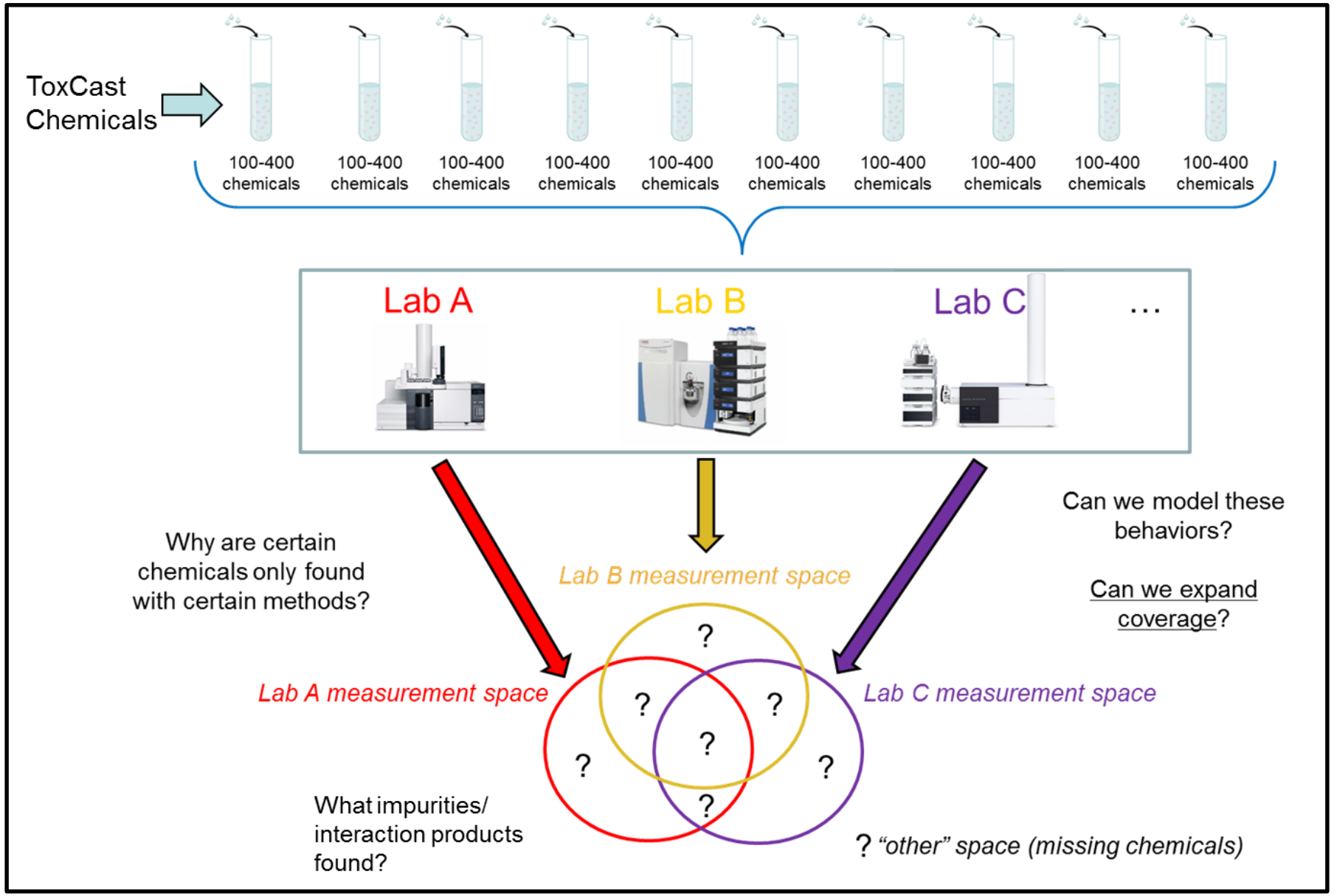






EPA's Non-Targeted Analysis Collaborative Trial

Original ENTACT Concept



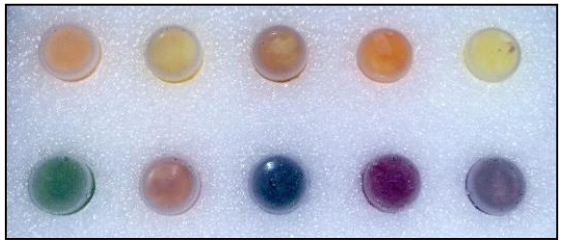
ENTACT Part 1

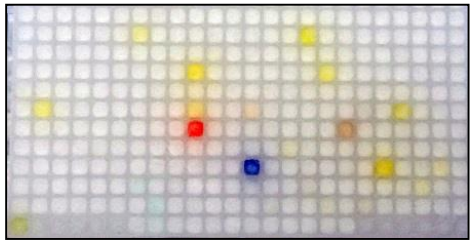
Chemicals from ToxCast Library

~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

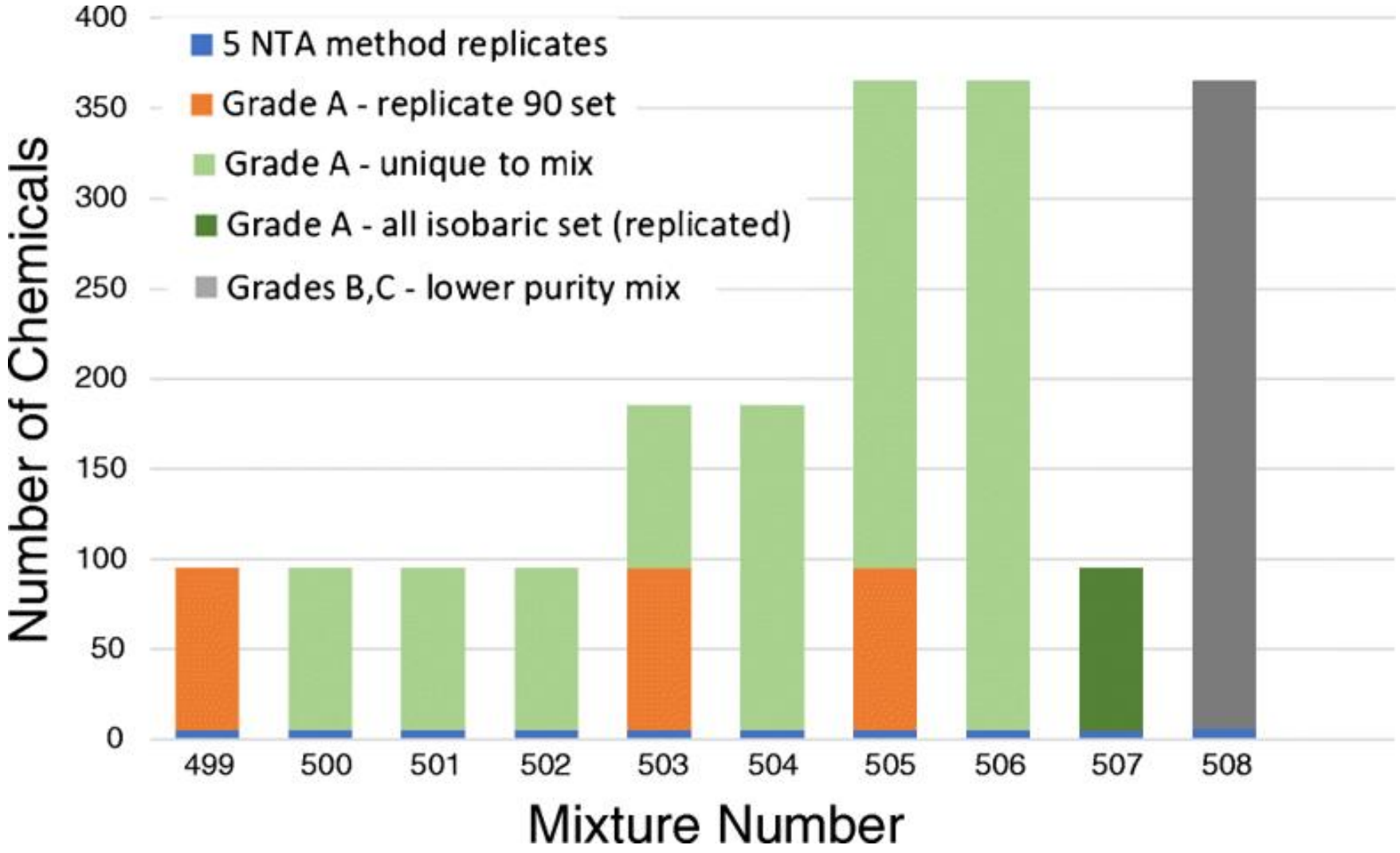
ENTACT Part 2

Reference & Fortified House Dust

Reference & Fortified Human Serum

Reference & Fortified Silicone Wristbands

Design of ENTACT Mixtures



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

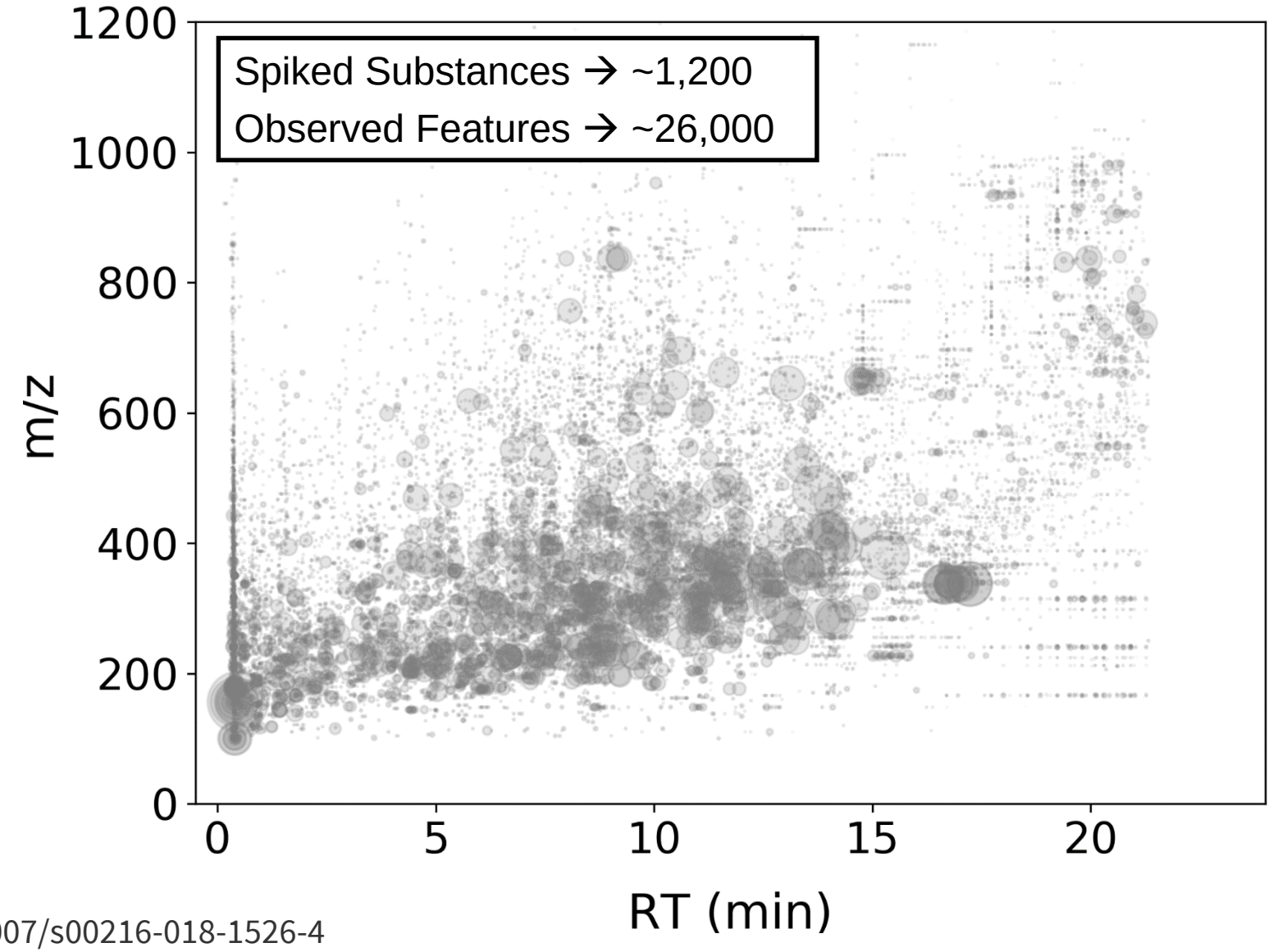
Vendors:

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

General Participants:

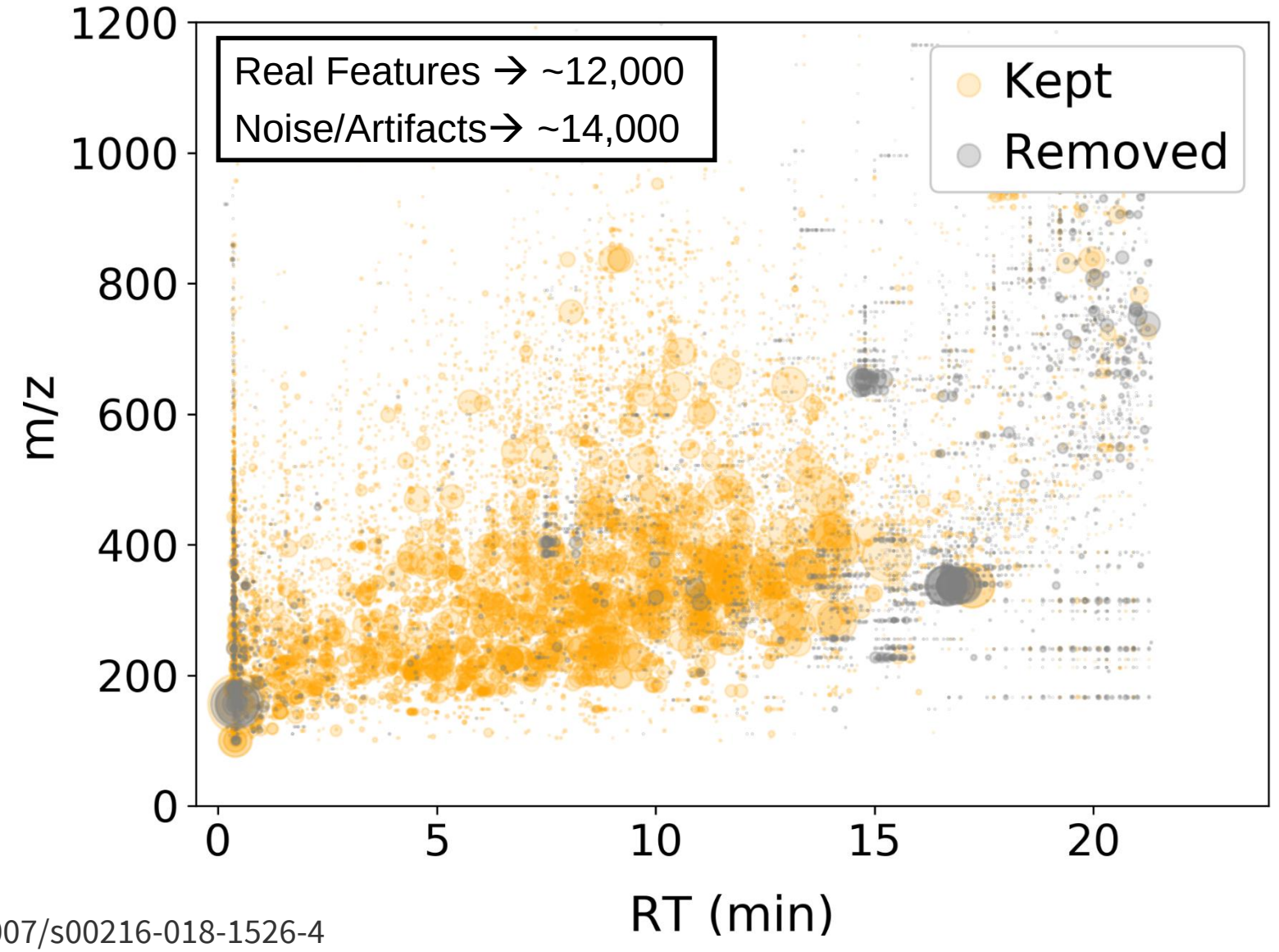
Université du Luxembourg
University of Florida
Wisconsin State Laboratory of Hygiene, University of Wisconsin–Madison
NIST
NC State University
RECETOX
Cornell University
UW Tacoma
Mount Sinai
University of Birmingham
CalEPA California Environmental Protection Agency
eawag aquatic research
United States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

EPA Results for 10 Synthetic Mixtures



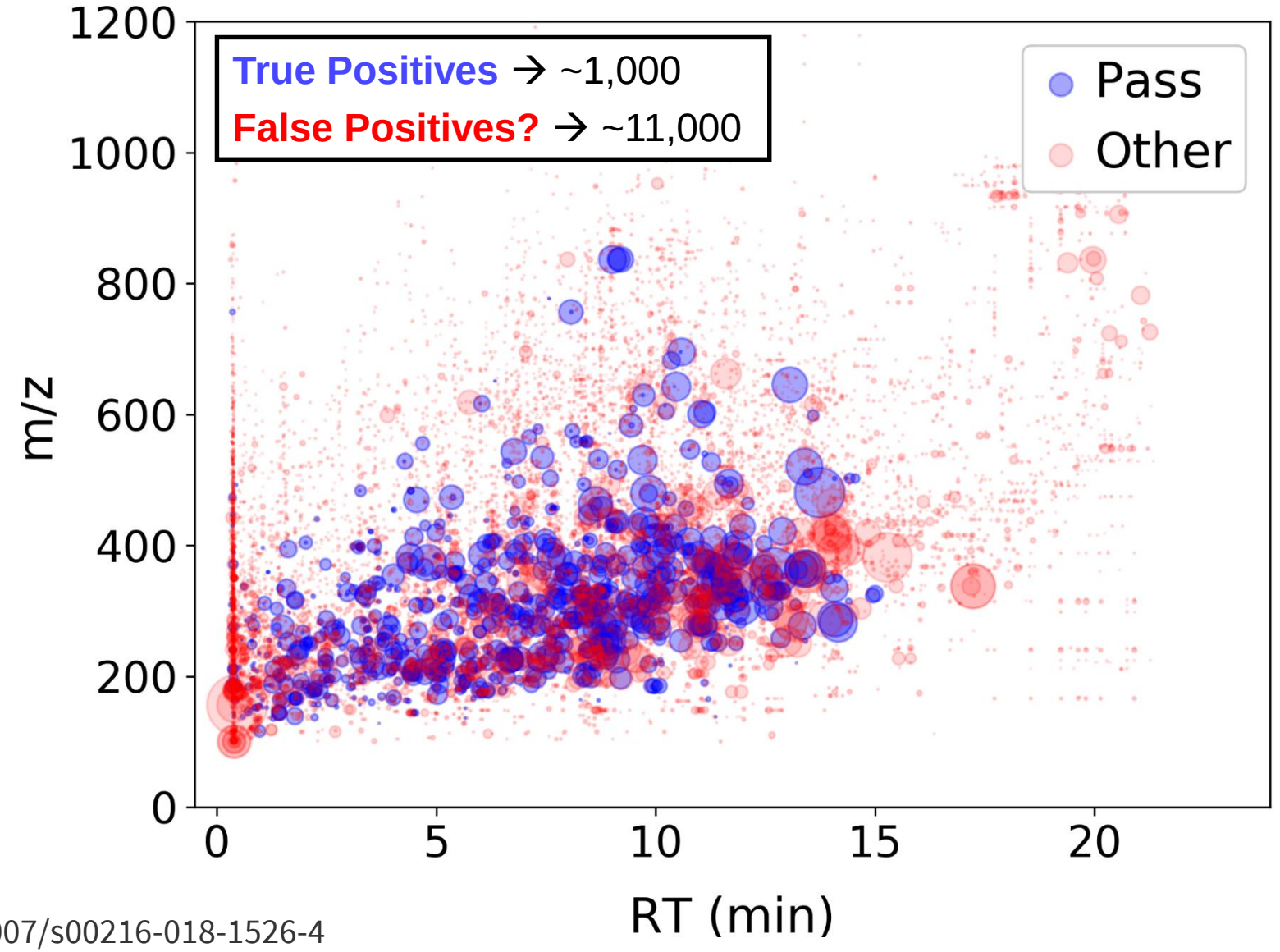
Sobus et al. 2019.
https://doi.org/10.1007/s00216-018-1526-4

EPA Results for 10 Synthetic Mixtures



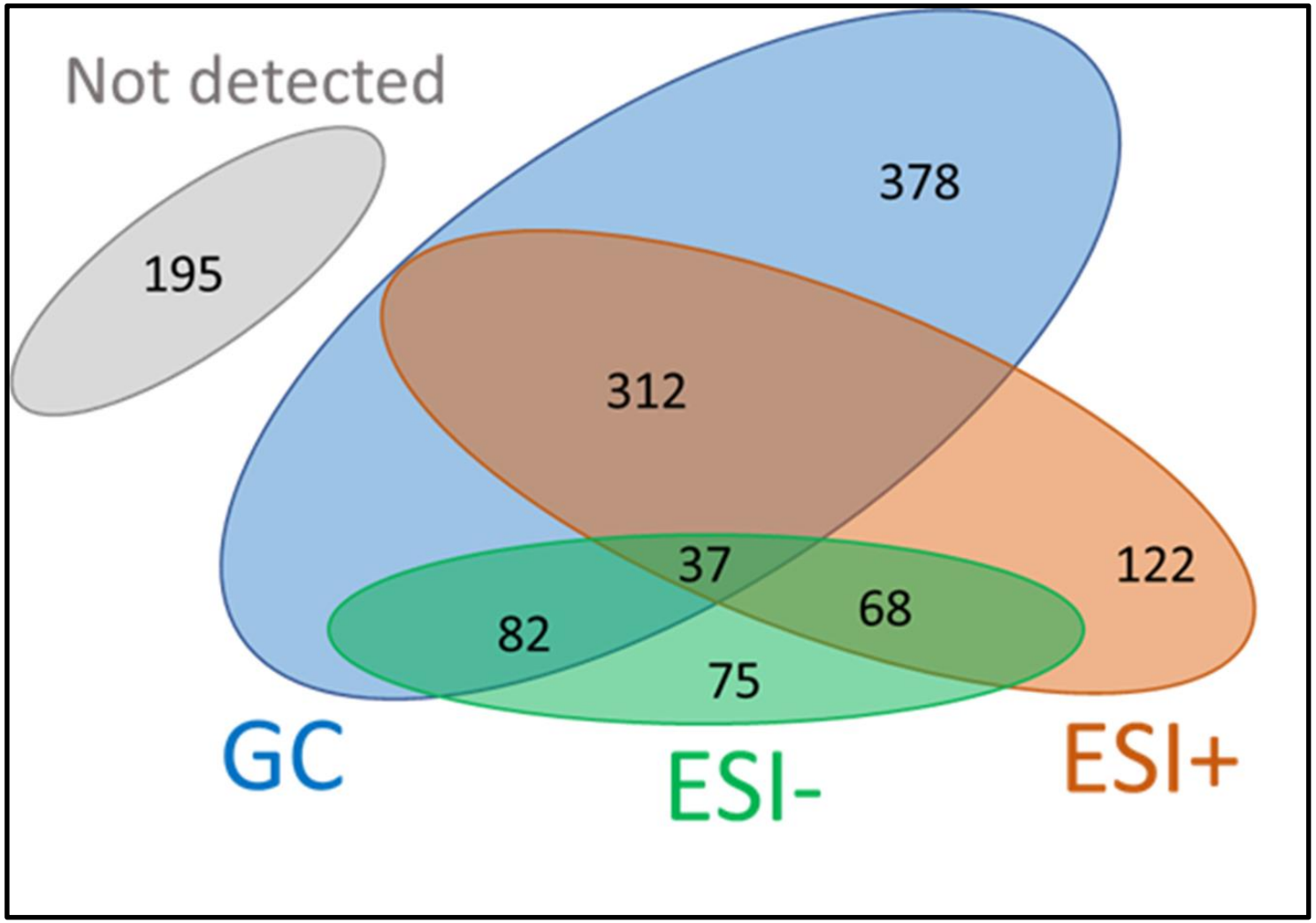
Sobus et al. 2019.
https://doi.org/10.1007/s00216-018-1526-4

EPA Results for 10 Synthetic Mixtures



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

Method Comparison (n=3 methods)



1,269 Spiked Substances

GC = gas chromatography

ESI- = neg. electrospray ionization (liquid chromatography)

ESI+ = pos. electrospray ionization (liquid chromatography)

Ulrich et al. 2019. https://doi.org/10.1007/s00216-018-1435-6

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

Summary and Conclusions

- 21st century exposure science demands higher-throughput monitoring techniques
- HRMS enables rapid chemical characterization in all tested media
- NTA methods represent a viable “first-pass” monitoring solution
  - Methods must be selected and implemented with care
  - Not a panacea, but a means of collecting provisional exposure data
- NTA well-suited for current “research” endeavors
  - Much more evaluation needed to establish “reference” methods
- Successful implementation requires close coordination between
  - Analytical chemists
  - Environmental/exposure modelers
  - Cheminformaticians
  - Programmers/Developers
  - Subject matter experts
  - and others…

Contributing Researchers

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

EPA ORD
Hussein Al-Ghoul*
Alex Chao*
Jarod Grossman*
Kristin Isaacs
Sarah Laughlin*
Charles Lowe
James McCord
Jeff Minucci
Seth Newton
Katherine Phillips
Tom Purucker
Randolph Singh*
Mark Strynar
Elin Ulrich

* = ORISE/ORAU

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

Agilent
Jarod Grossman
Andrew McEachran

GDIT
Ilya Balabin
Tom Transue
Tommy Cathey

Questions?

sobus.jon@epa.gov

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