EPA United States Environmental Protection Agency

Innovation through ORD's Partnerships and Collaborations

Pre-recorded material provided for the NASEM Committee on Anticipatory Research for EPA's Research and Development Enterprise to Inform Future Environmental Protection, in advance of November 17, 2021 Committee Meeting

The Importance of ORD's Collaborations

- ORD has asked the Committee to:
  - identify advancing scientific and technological areas important to ORD's work over the next several decades and how we may take advantage of them, and
  - provide recommendations on how ORD can strategically position itself to anticipate and respond to future research needs of the Agency.
- Collaborations are one way through which ORD advances science, innovates, and takes advantage of new scientific areas.
- Collaborations allow for more diverse, inclusive, interdisciplinary, and cross-sector expertise and resources to be brought to the table.

Examples of Innovative Collaborations

- This series of pre-recorded presentations will highlight a few examples of research areas in ORD where different types of collaborations have been leveraged to push forward into new scientific or technological fields, approaches, or innovations.
- These examples are meant to demonstrate for the Committee:
  - the different ways in which ORD collaborates, which could be mechanisms through which ORD takes advantage of the emerging scientific and technological areas identified by the Committee,
  - the characteristics that enable successful collaborations to drive innovation and advance into new scientific areas.

EPA United States Environmental Protection Agency

EPA's Non-Targeted Analysis Collaborative Efforts

Elin M. Ulrich (she/her)

Supervisory Chemist, Chief Advanced Analytical Chemistry Methods Branch

Center for Computational Toxicology and Exposure

Office of Research & Development

Provided for the NASEM committee on Anticipatory Research for EPA's Research and Development Enterprise to Inform Future Environmental Protection

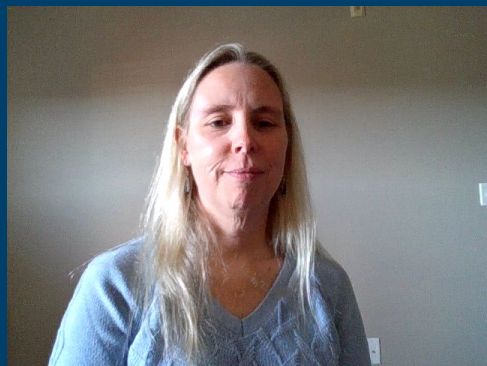
EPA's Non-Targeted Analysis Collaborative Efforts

Elin M. Ulrich (she/her)

Supervisory Chemist, Chief Advanced Analytical Chemistry Methods Branch

Center for Computational Toxicology and Exposure

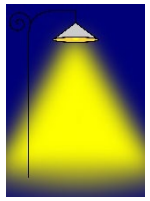
Office of Research & Development



Provided for the NASEM committee on Anticipatory Research for EPA's Research and Development Enterprise to Inform Future Environmental Protection

What is Non-Targeted Analysis?

- **Targeted Analysis**

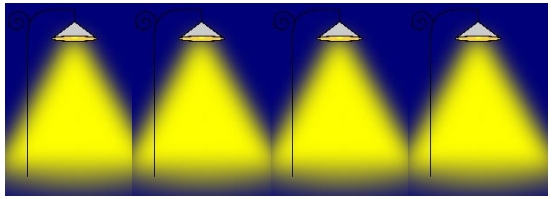
  

  "known knowns"

  Standards, calibration curves

- **Suspect Screening Analysis (SSA)**

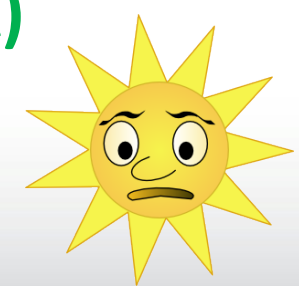
  "known unknowns"

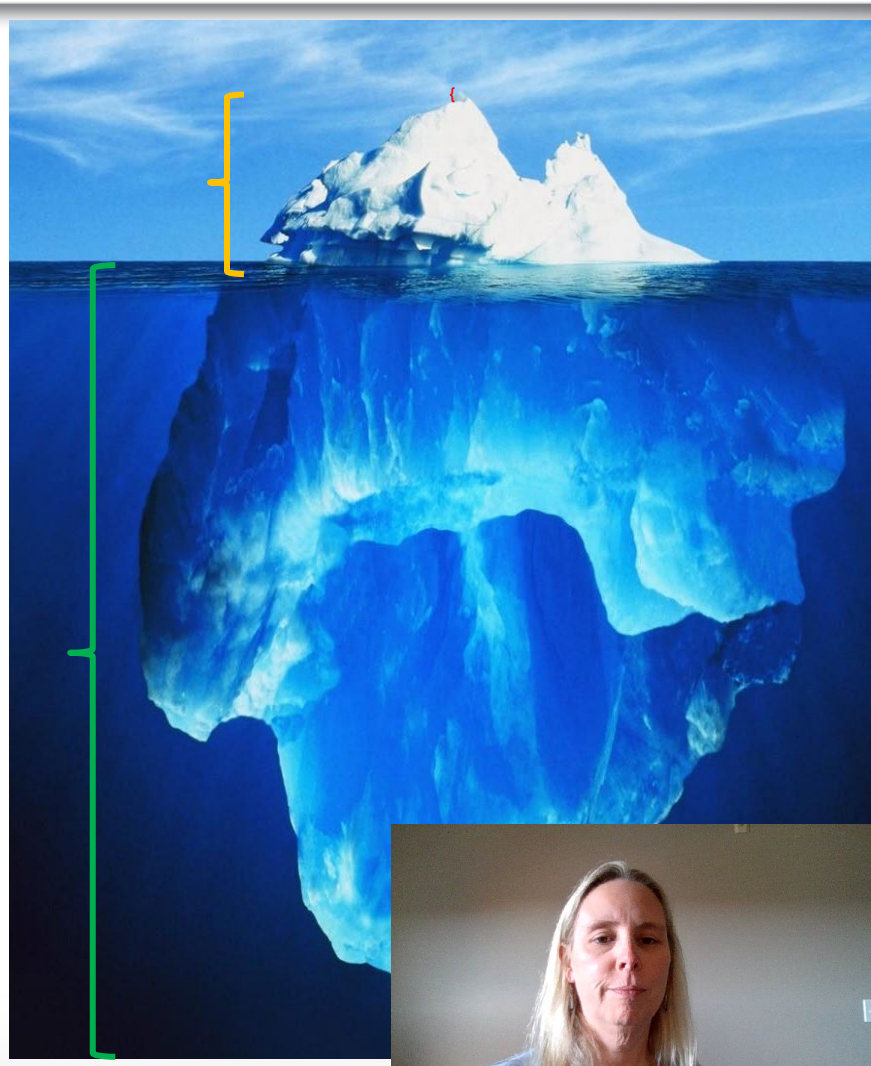
  Lists of compounds

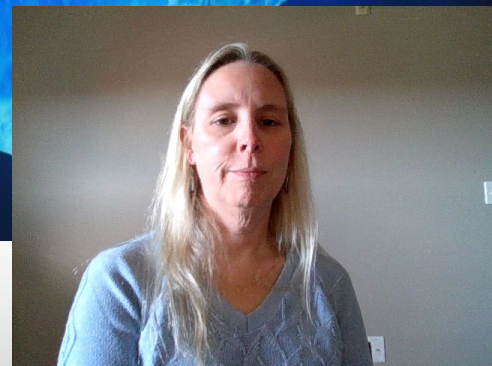
- **Non-Targeted Analysis (NTA)**

  "unknown unknowns"

  MS first principles

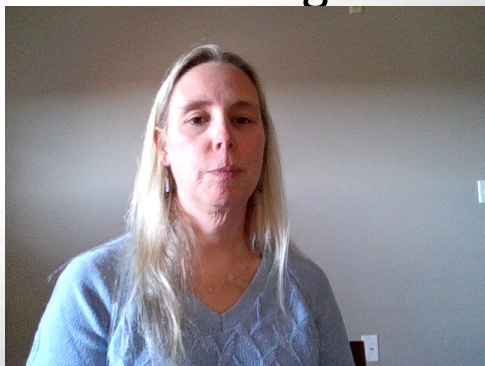




EPA's Non-Targeted Analysis Collaborative Trial (ENTACT)

Background:

- August 2015 workshop at EPA to discuss the state of the science for suspect screening analysis (SSA) and non-targeted analysis (NTA) for exposure applications
- Approximately 200 people attended, ~140 in person, ~60 by webinar
- Sessions on research and regulatory drivers, NTA in environmental and biological matrices, emerging techniques, databases/informatics tools with 26 platform and poster presentations on NTA research
- Half-day discussion on how to use EPA's resources from ToxCast– genesis of ENTACT
- In his introductory remarks, Tom Burke challenged the group to "Make non-targeted the new targeted"



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

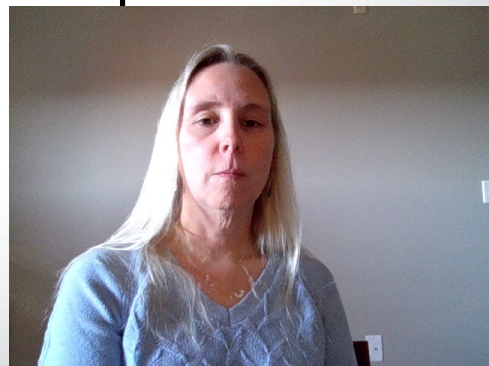
ENTACT Objectives

- Characterize current method performance characteristics (e.g., % true/false positives)
- Establish performance benchmarks for SSA and NTA
- Establish benchmark methods for SSA and NTA
- Increase compounds/spectra available in reference libraries (with participants and publicly available)
- Develop reporting standards for studies using SSA and NTA methods
- And so much more…

	October 2017	Nov 2021
Compounds	6,810	19,613
Spectra	2,379,547	8,635,344
Trees	10,658	27,213

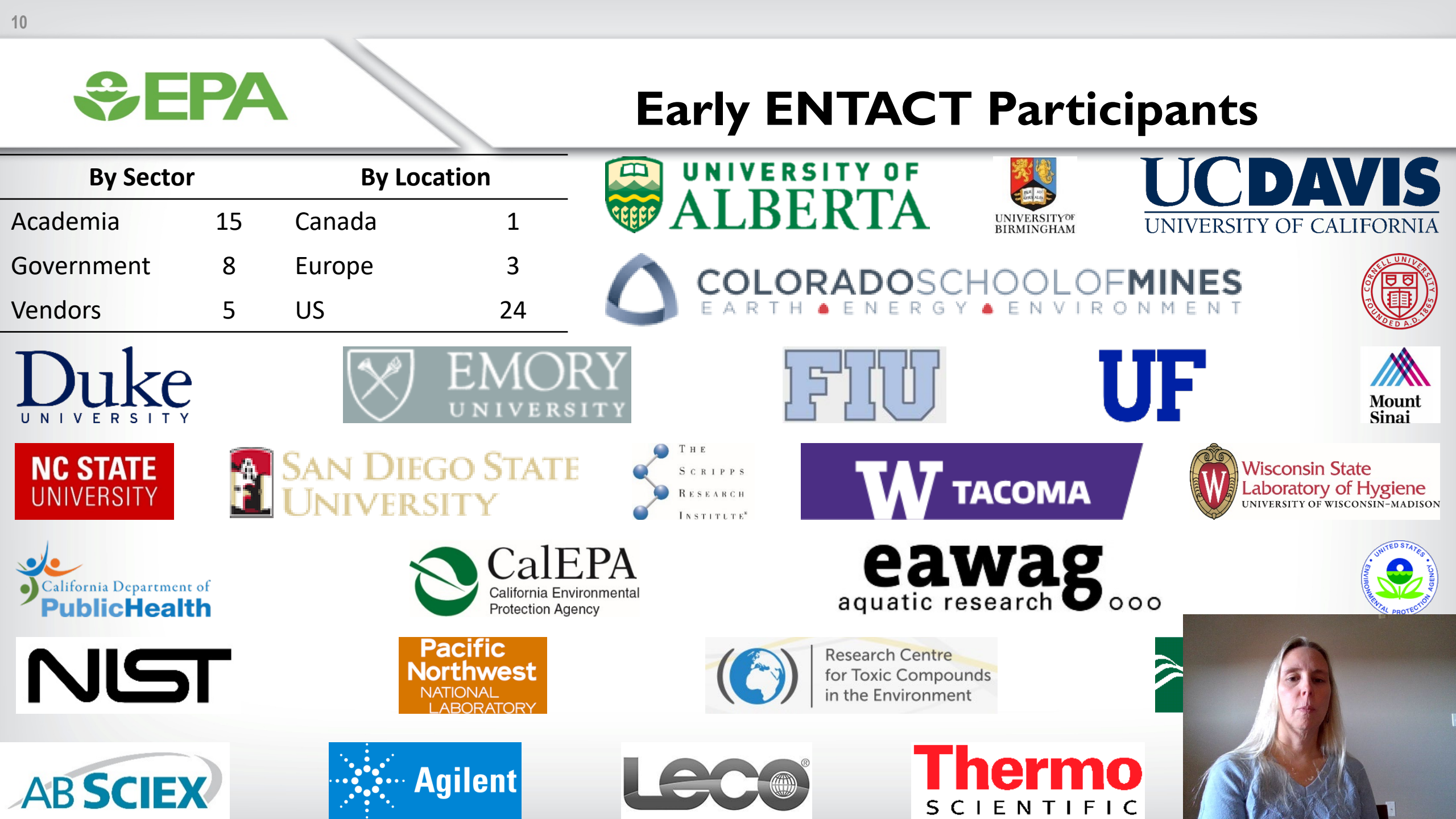
NTA Critical Needs Identified

- Tightly-defined ring trials to evaluate NTA method performance
  - 10 prepared mixtures, 6 extracts, and 4 blanks
- Availability of custom-made spiked samples for ring trials
  - ToxCast chemical library previously used for high-throughput toxicity testing
- Exchange of comprehensive suspect lists to enable interoperability
  - DSSTox- List of chemicals of interest to the agency, basis of CompTox Chemicals Dashboard
- Retrospective analysis of data
  - Participants are eventually unblinded to intentionally spiked substances in samples
- Need to benchmark non-targeted analysis for performance and coverage
  - How often is ID correct? How consistent are IDs?
    How many chemicals can be IDd?



Early ENTACT Participants

By Sector		By Location	
Academia	15	Canada	1
Government	8	Europe	3
Vendors	5	US	24



Resulting publications (so far)

Research Paper | Published: 06 December 2018

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

Elin M. Ulrich, Jon R. Sobus, Christopher M. Grulke, Ann M. Rich, Kamel Mansouri & Antony J. Williams

Analytical and Bioanalytical Chemistry **411**, 853–866 (2019)

Research Paper | Published: 05 January 2019

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

Jon R. Sobus, Jarod N. Grossman, Alex Chao, Randolph Singh, Antony J. Williams, Christopher M. Grulke, Ann M. Richard, Seth R. Newton, Andrew D. McEachran & Elin M. Ulrich

Analytical and Bioanalytical Chemistry **411**, 835–851 (2019) | Cite this article

Research Paper | Published: 03 June 2020

Expanded coverage of non-targeted LC-HRMS using atmospheric pressure chemical ionization: a case study with ENTACT mixtures

Randolph R. Singh, Alex Chao, Katherine A. Phillips, Xin Rui Xia, Damian Shea, Jon R. Sobus, Emma L. Schymanski & Elin M. Ulrich

Analytical and Bioanalytical Chemistry **412**, 4931–4939 (2020) | Cite this article

J Chem Inf Model. 2020 Dec 28;60(12):6251-6257. doi: 10.1021/acs.jcim.0c00899. Epub 2020 Dec 7.

***Chespa*: Streamlining Expansive Chemical Space Evaluation of Molecular Sets**

Jamie R Nuñez [1,2], Monee Mcgrady [1], Yasemin Yesiltepe [1,2], Thomas O Metz [1]

Research Paper | Published: 14 October 2021

Predicting compound amenability with liquid chromatography-mass spectrometry to improve non-targeted analysis

Charles N. Lowe, Kristin K. Isaacs, Andrew McEachran, Christopher M. Grulke, Jon R. Sobus, Elin M. Ulrich, Ann Richard, Alex Chao, John Wambaugh & Antony J. Williams

Analytical and Bioanalytical Chemistry

Research Paper | Open Access | Published: 22 January 2020

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

Alex Chao, Hussein Al-Ghoul, Andrew D. McEachran, Ilya Balabin, Tom Transue, Grossman, Randolph R. Singh, Elin M. Ulrich, Antony J. Williams & Jon R. Sobus

Analytical and Bioanalytical Chemistry **412**, 1303–1315 (2020) | Cite this article

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*

Cite this: *J. Chem. Inf. Model.* 2019, 59, 9, 4052–4060

Publication Date: June 25, 2019

https://doi.org/10.1021/acs.jcim.9b00444

Copyright © 2019 American Chemical Society

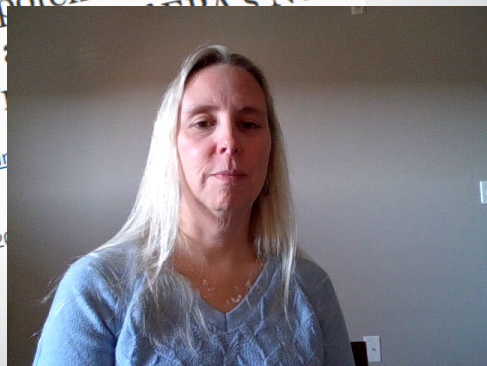
Article Views 491 | Altmetric 6 | LEARN ABOUT THESE MET

Paper in Forefront | Published: 25 April 2020

Examining NTA performance and potential using fortified and reference house dust ... Targeted Analysis Collaborative T...

Seth R. Newton, Jon R. Sobus, Elin M. Ulrich, Randolph R. Sin... Laughlin-Toth & Mark Strynar

Analytical and Bioanalytical Chemistry **412**, 4221–4233 (202...



Benchmarking and Publications for Non-Targeted Analysis (BP4NTA)

- Formed in August 2018 at ENTACT workshop
- Initially led by Elin Ulrich (EPA) and Ben Place (NIST)
- Currently led by Christine Fisher (FDA) and Ruth Marfil-Vega (Shimadzu)

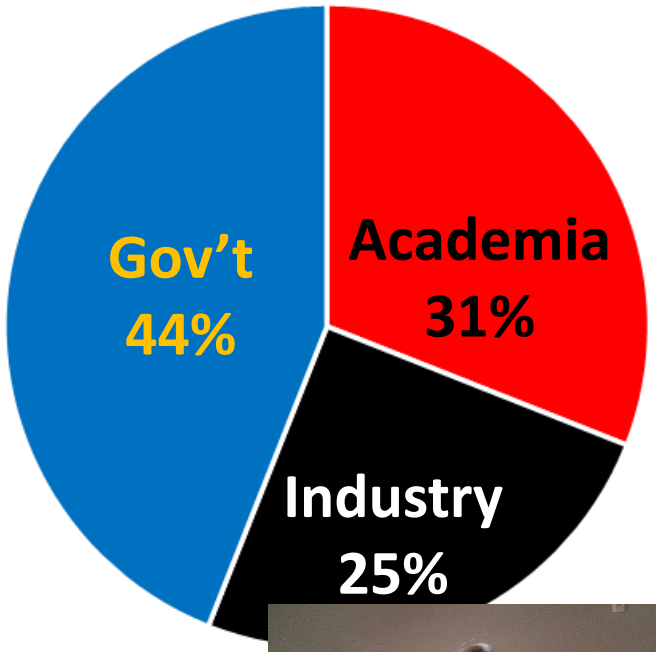
Interested? Contact us!

Christine.ODonnell@fda.hhs.gov

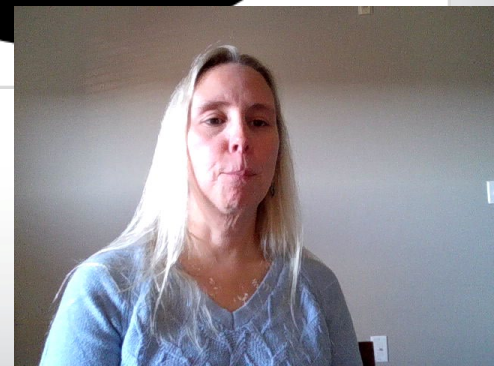
rmmarfilvega@shimadzu.com

Membership

~110 international members



- Membership based on interest in NTA
  - Experience with NTA varies from beginners to experts
  - Wide range of applications: metabolomics, exposure, food, biological, medical devices, environmental



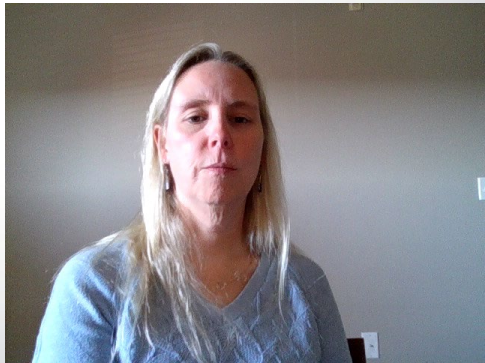
BP4NTA Objectives

Overarching goals and needs:

- Harmonize/standardize approaches and reporting practices, as possible
- Improve determination, calculation, and communication of performance metrics
- Share best practices (including QA/QC) within the NTA community
- Improve the transparency and reproducibility of peer-reviewed NTA studies

Long-term goals:

- Address gaps in data, methods, and computational tools within the community
- Move the NTA field toward measurable standards for proficiency testing
- Build and maintain coalitions and communications with other groups



Recently Delivered Products

- Introductory manuscript with Analytical Chemistry
- NTA terms, concepts, and performance calculations, with consensus definitions

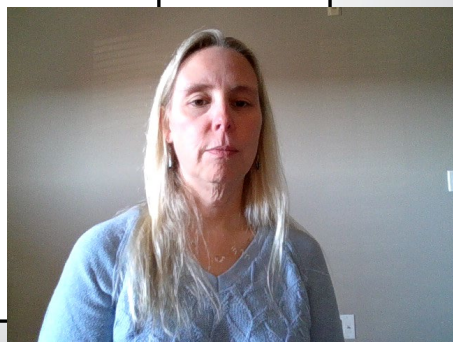
  https://nontargetedanalysis.org/
- Resources for new NTA researchers traversing the learning curve

  https://nontargetedanalysis.org/additional-resources/
- Study reporting tool to aid the design of NTA studies, review of research proposals/manuscripts

  https://doi.org/10.1021/acs.analchem.1c02621

BP4NTA Study Reporting Tool

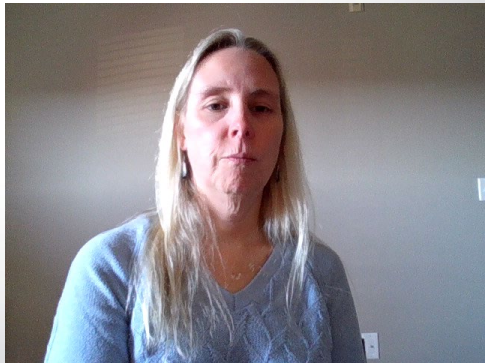
Section	Category	Sub-Category	Score	Rationale
Methods	Study Design	Objectives & Scope	Scores selected from drop-down menus for each sub-category NA 0 1 2 3	Space for reviewer to explain assigned score in each sub-category
		Sample Info & Prep		
		QC Spike & Samples		
	Data Acquisition	Analytical Sequence		
		Chromatography		
		Mass Spectrometry		
	Data Processing & Analysis	Data Processing		
		Statistical & Chemometric Analysis		
		Annotation & Identification		
Results	Data Outputs	Statistical & Chemometric Outputs		
		Identification & Confidence Levels		
	QA/QC Metrics	Data Acquisition QA/QC		
		Data Processing & Analysis QA/QC		



Upcoming Projects and Long-Term Goals

- Increasing awareness of SRT to journal editors, reviewers, and the scientific community
- NTA special issues- ET&C coming soon
- Build social media presence: Twitter @BP4NTA and Facebook group /bp4nta
- Meet with stakeholders (regulators, decision makers, toxicologists, epidemiologists)
  - What does NTA provide and how we can make results more useful?
- Publications on:
  - Performance metrics
  - Bounding chemical space
- Chemical reference standards for NTA with an eye towards performance
- Move toward proficiency testing levels for SSA and NTA (ASTM/ISO):
  - Define proficiency expert, competent, etc. (10 years out)

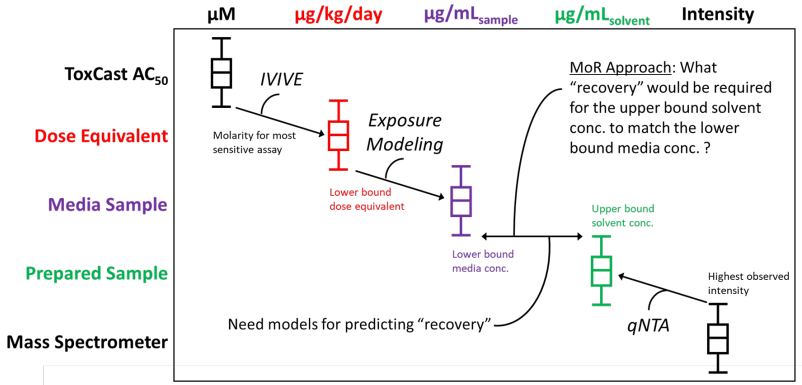
BP4NTA
BENCHMARKING AND PUBLICATIONS FOR NON-TARGETED ANALYSIS

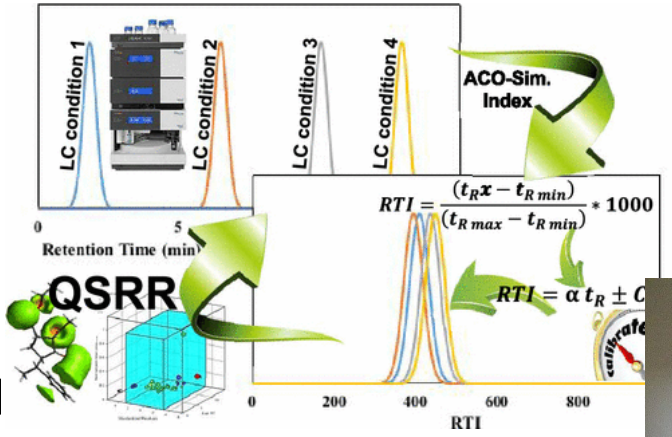


Conclusions

Benefits

- The entire field of NTA is moving forward scientifically
  - Benchmarking methods and performance
  - Improved communications and reporting
  - Resources for newcomers and experienced practitioners
    - MS libraries, data processing tools, qNTA, method amenability predictions, etc.
- Network of NTA practitioners is bigger and stronger
  - It's easier to solve hard problems with more people





Challenges

- Prioritizing what to work on
- We've picked the low hanging fruit, what's ahead is really hard
- Timelines, unforeseen issues (instruments down, COVID)
- Overlapping what stakeholders want/need with what NTA can provide