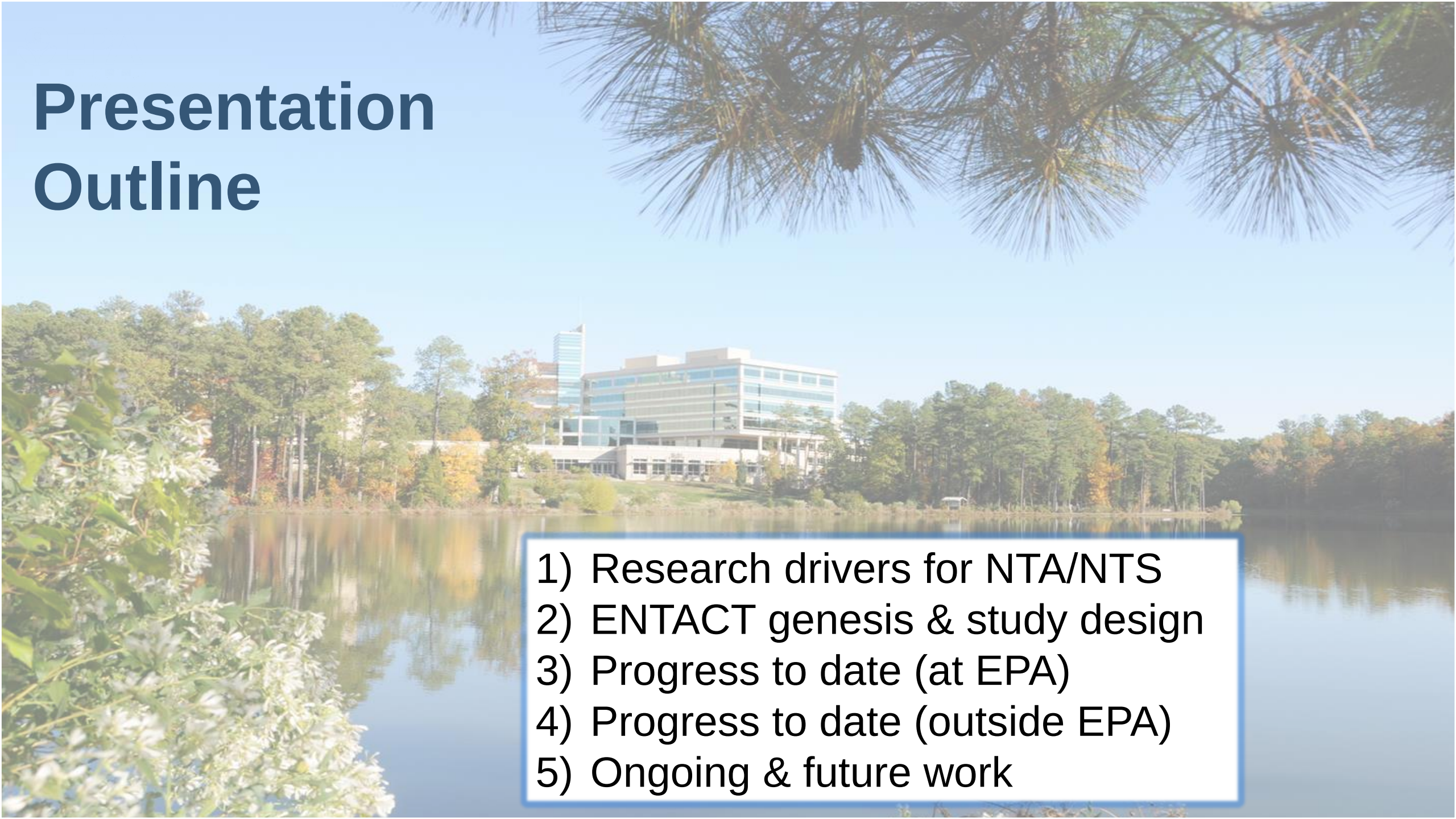
EPA United States Environmental Protection Agency

The ENTACT Story: Using US EPA Resources to Evaluate and Enhance Non-target Workflows

Jon Sobus[1], Elin Ulrich[1], Jarod Grossman[2], Alex Chao[2], Seth Newton[1], Antony Williams[1], Ann Richard[1],Chris Grulke[1], Andrew McEachran[2], Randolph Singh[2], Hussein Al-Ghoul[2]

[1] Center for Computational Toxicology and Exposure
[2] ORAU/ORISE Participant

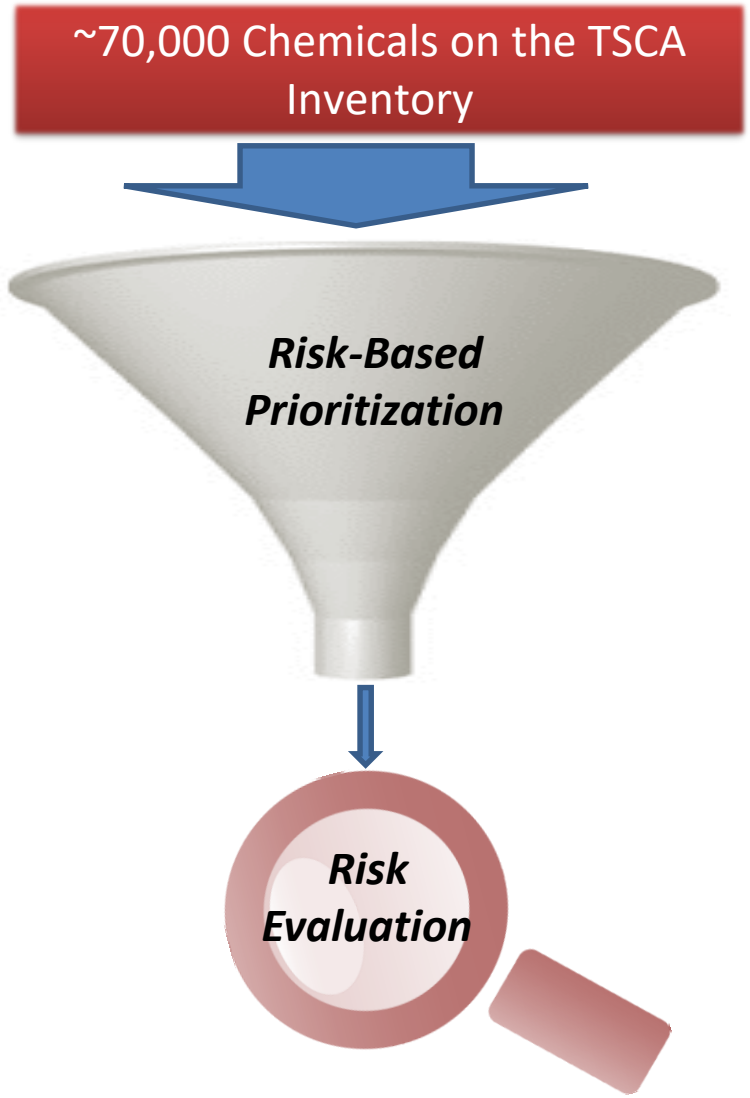
Presentation Outline



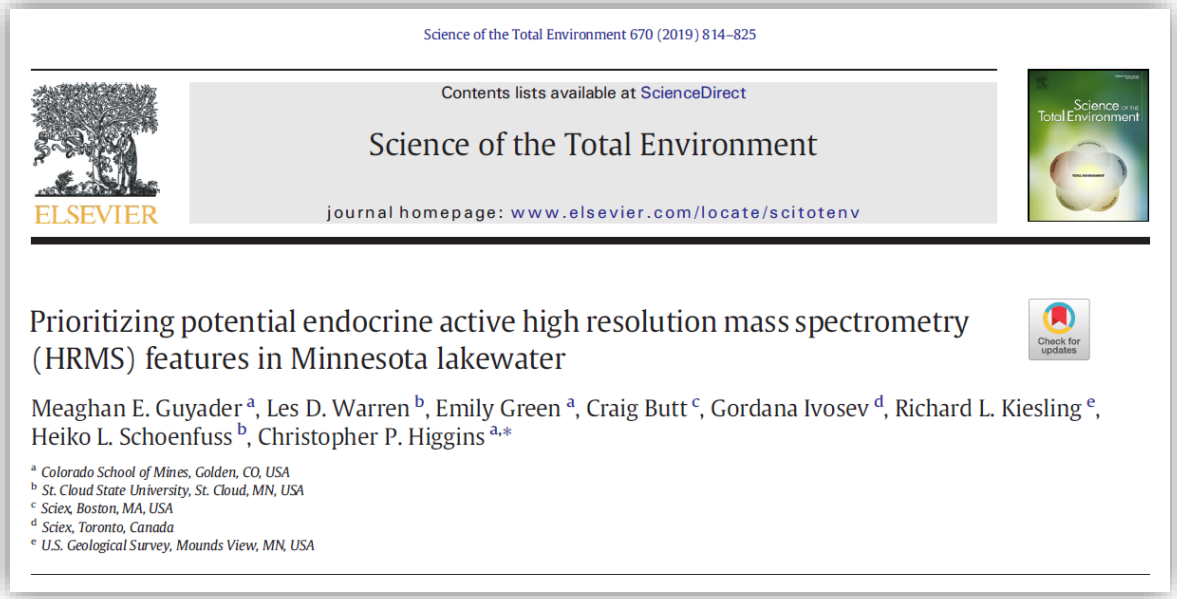
1) Research drivers for NTA/NTS
2) ENTACT genesis & study design
3) Progress to date (at EPA)
4) Progress to date (outside EPA)
5) Ongoing & future work

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>



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,*]

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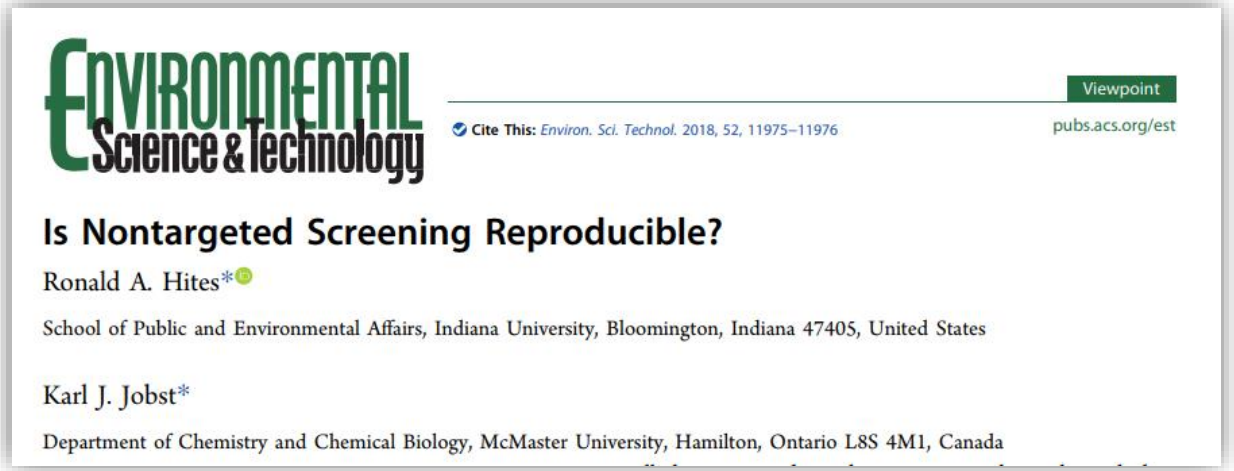
[d] Sciex, Toronto, Canada
[e] U.S. Geological Survey, Mounds View, MN, USA



Environmental Science & Technology

Viewpoint

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

pubs.acs.org/est

Is Nontargeted Screening Reproducible?

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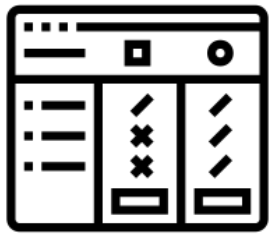
Karl J. Jobst*

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*."



Key Research Needs

Anal Bioanal Chem (2015) 407:6237–6255
DOI 10.1007/s00216-015-8681-7

REVIEW

Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis

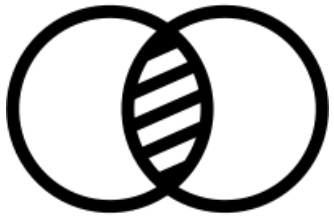
Emma L. Schymanski[1] · Heinz P. Singer[1] · Jaroslav Slobodnik[2] · Ildiko M. Ipolyi[2] · Peter Oswald[2] · Martin Krauss[3] · Tobias Schulze[3] · Peter Haglund[4] · Thomas Letzel[5] · Sylvia Grosse[5] · Nikolaos S. Thomaidis[6] · Anna Bletsou[6] · Christian Zwiener[7] · María Ibáñez[8] · Tania Portolés[8] · Ronald de Boer[9] · Malcolm J. Reid[10] · Matthias Onghena[11] · Uwe Kunkel[12] · Wolfgang Schulz[13] · Amélie Guillon[14] · Naïke Noyon[14] · Gaëla Leroy[15] · Philippe Bados[16] · Sara Bogialli[17] · Draženka Stipaničev[18] · Pawel Rostkowski[19] · Juliane Hollender[1,20]

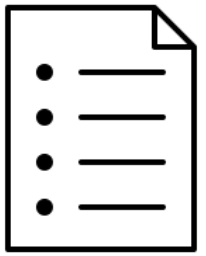
- 18 Institutes
- 12 Countries
- 1 river water extract

- <u>Workflows & Methods</u>:
- Analytical → well harmonized
- Data processing → not harmonized

<u>Clearly expressed needs for</u>:

1) More tightly defined interlaboratory comparisons
2) The use of spiked samples
3) The shared use of comprehensive suspect lists





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

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

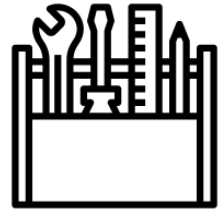
Published July 30, 2018

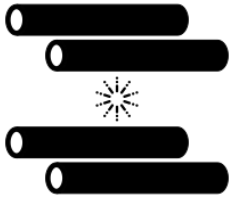
EPA scientists are leading a multi-phase project to evaluate the ability of non-targeted analysis laboratory methods to consistently and correctly identify unknown chemicals in samples. EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) was formed in late 2015 and includes nearly 30 academic, government,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

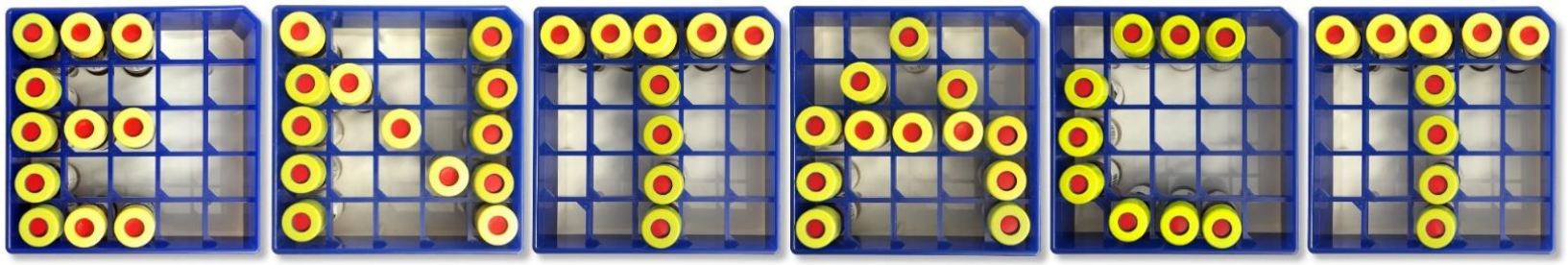
"One of our main goals is to figure out what scientists are doing with non-targeted analysis as a group at large, particularly which chemicals we correctly identify and why," says Elin Ulrich, an EPA scientist who co-leads ENTACT with EPA's Jon Sobus.

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?

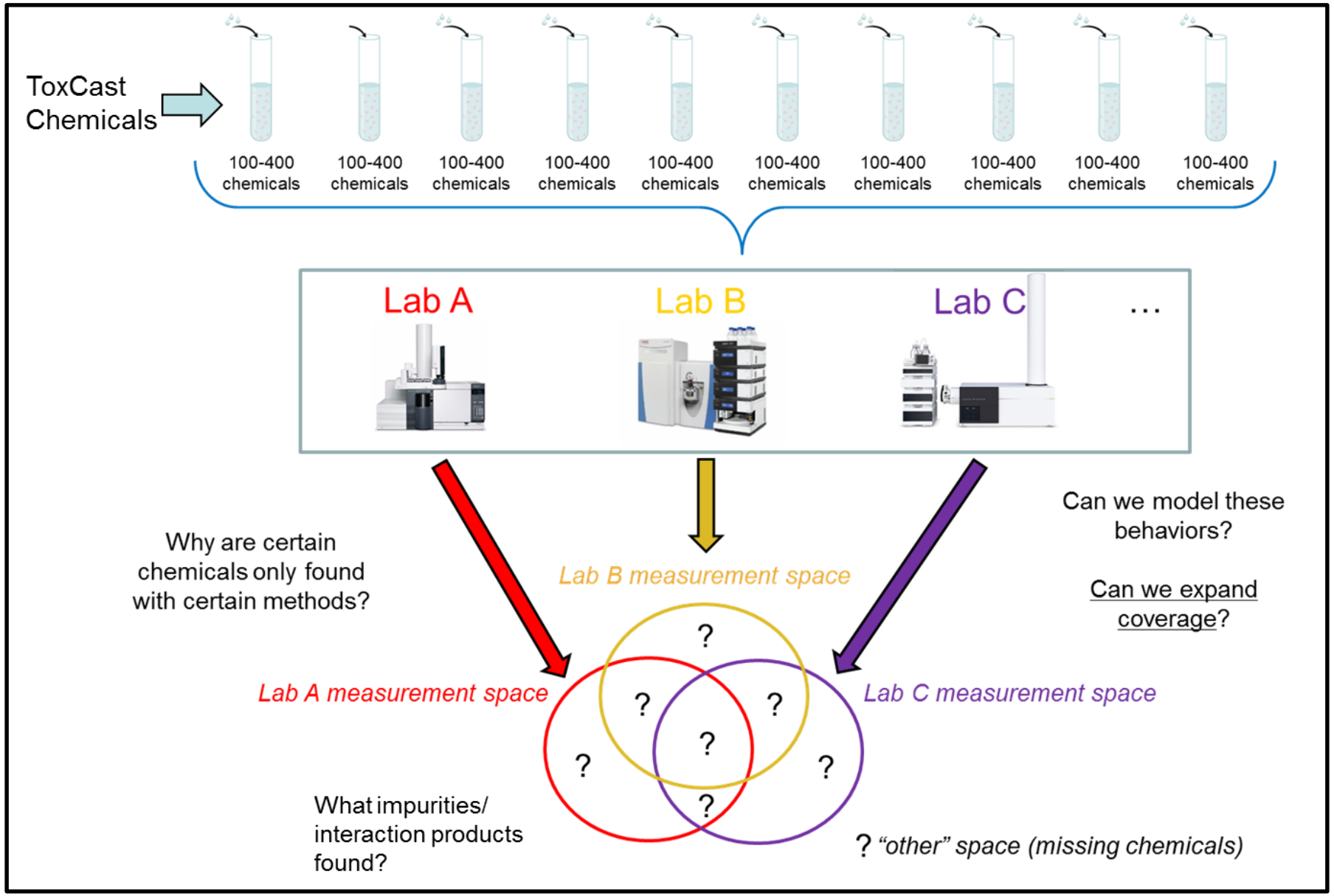






EPA's Non-Targeted Analysis Collaborative Trial

Original ENTACT Concept



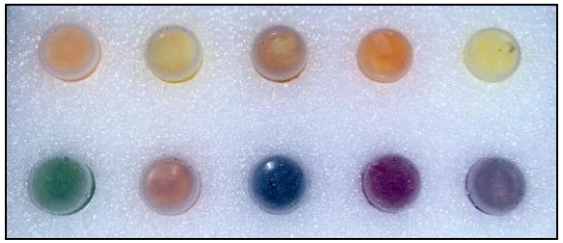
ENTACT Part 1

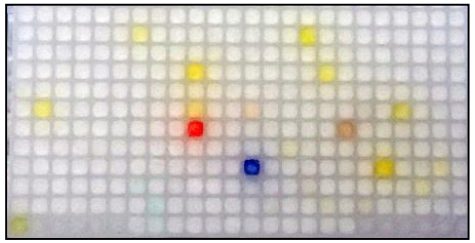
Chemicals from ToxCast Library

~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

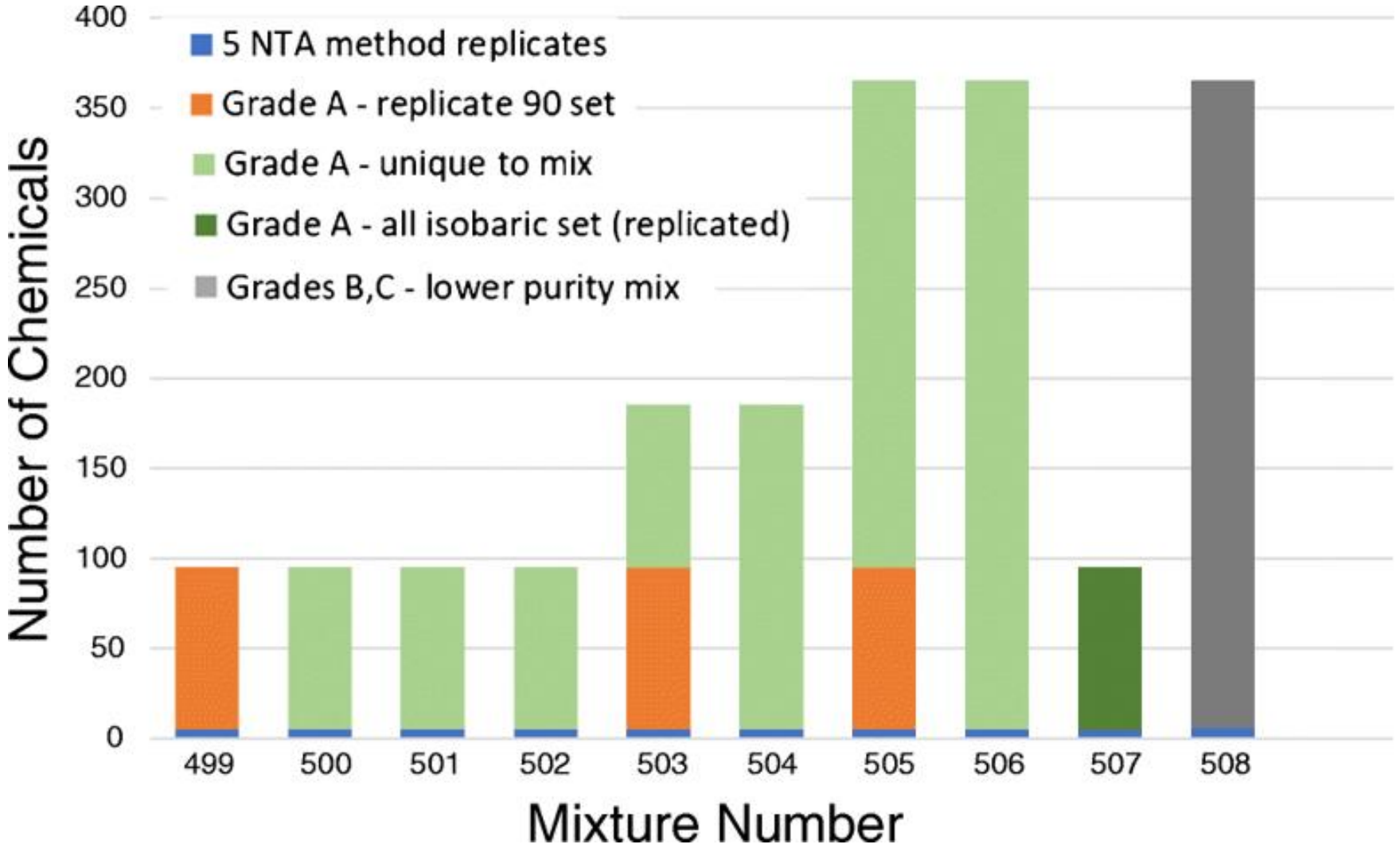
ENTACT Part 2

Reference & Fortified House Dust

Reference & Fortified Human Serum

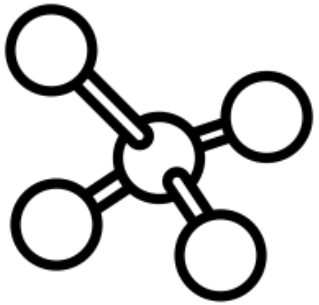
Reference & Fortified Silicone Wristbands

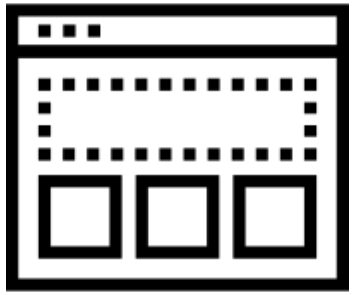
Design of ENTACT Mixtures



Resources Provided to Participants

- SOPs for sample handling, analysis, and data submission
- Procedures used for sample preparation
- Up to 16 samples with eventual (unblinded) chemical mappings
- MS-Ready DSSTox list (671,852 unique) with .mol files
- MS-Ready ToxCast list (4,248 unique) with .mol files
- Method and Data reporting templates
- FTP site, accounts, and instructions





EPA Methods for ENTACT Mixtures

Agilent 6530B Q-TOF

Agilent ZORBAX Eclipse Plus C8 column (2.1 x 50 mm, 1.8 µm)

A: 5% methanol, 95% water (0.4 mM ammonium formate)

B: 95% methanol, 5% water (0.4 mM ammonium formate)

10 ENTACT Mixtures

Waters Acquity UPLC® BEH C_{18} column (2.1 x 50 mm, 1.7 µm)

A: water (0.1% formic acid)

B: acetonitrile (0.1% formic acid)

3 ENTACT Mixtures

EPA Analysis Workflow

Sample Preparation
3 Dilutions, 3 Replicate Injections, 6 Blanks

Sample Analysis
LC-QTOF/HRMS: ESI+ and ESI-, MS[1]

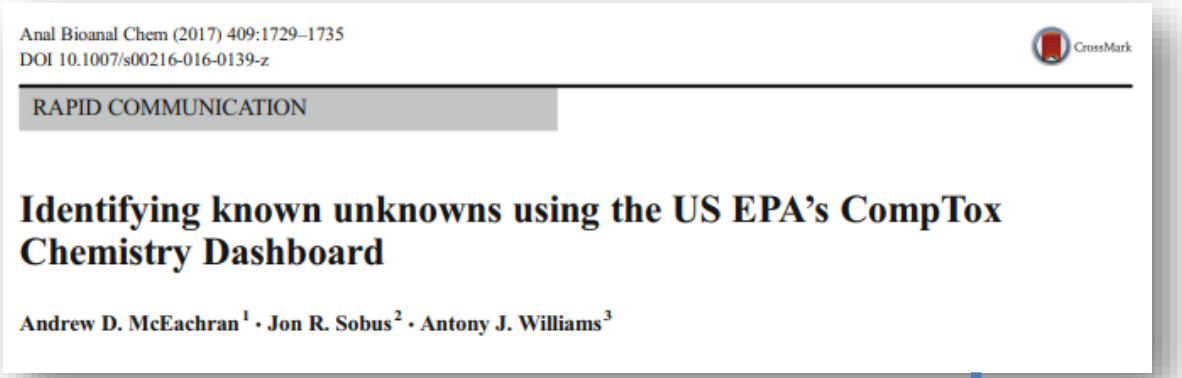
Peak Picking & Alignment
Agilent MassHunter Profinder Software

Formula Assignment
Agilent Mass Profiler Professional Software,
DSSTox Unique MS-Ready Formula List

Feature Filtering & Flagging
Custom Script:
Blank Subtraction, Fold-Change Thresholds,
Formula Match Score Cut-Off

Candidate Structure Selection
EPA CompTox Chemicals Dashboard:
Batch Search, Data Source Ranking

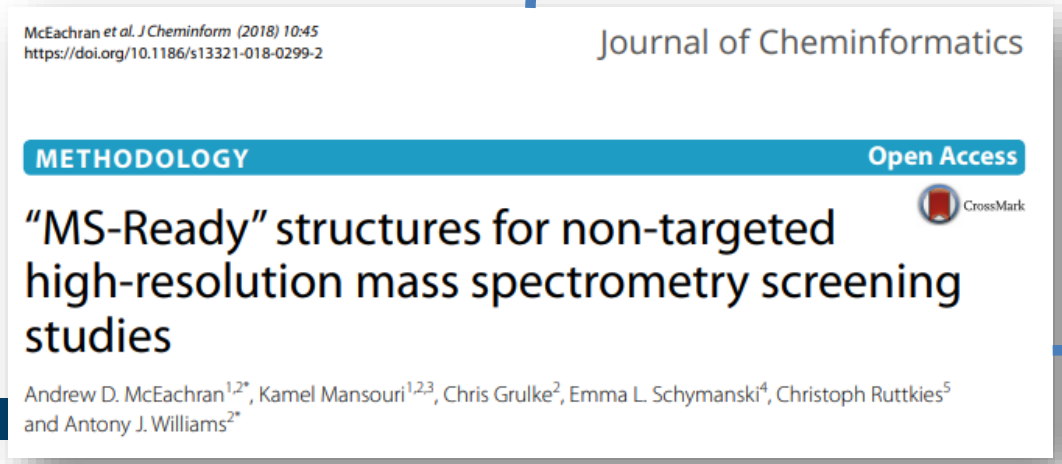
Chemical Structure Corroboration
LC-QTOF/HRMS:
DDA MS^2 Using Preferred Ions List,
Agilent Reference MS^2 libraries


Anal Bioanal Chem (2017) 409:1729–1735
DOI 10.1007/s00216-016-0139-z

RAPID COMMUNICATION

Identifying known unknowns using the US EPA's CompTox Chemistry Dashboard

Andrew D. McEachran[1] · Jon R. Sobus[2] · Antony J. Williams[3]


McEachran *et al. J Cheminform (2018) 10:45*
https://doi.org/10.1186/s13321-018-0299-2

Journal of Cheminformatics

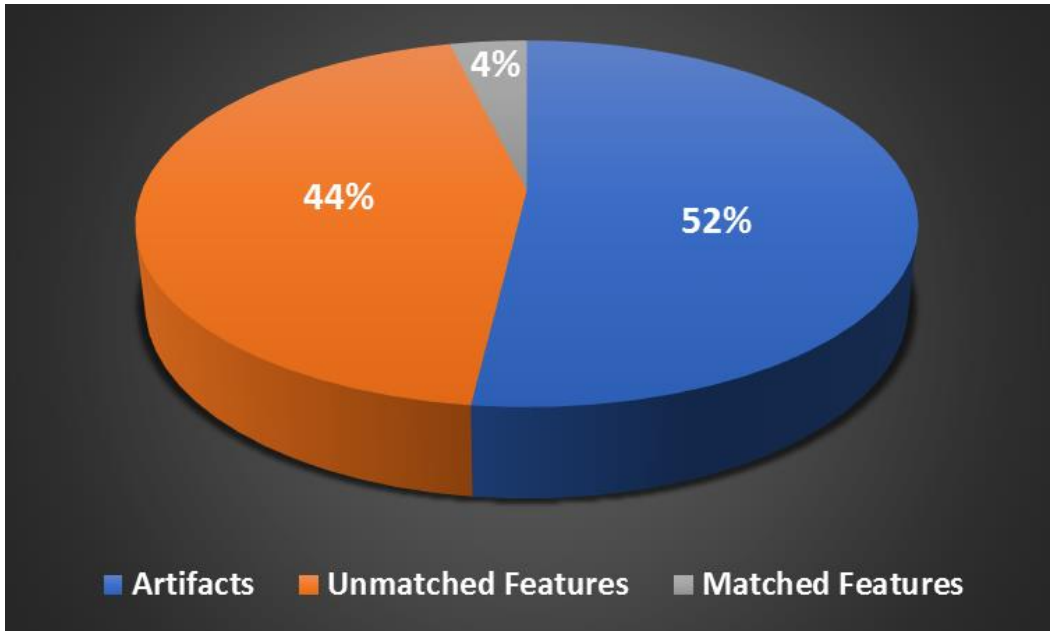
METHODOLOGY Open Access

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

Andrew D. McEachran[1,2*], Kamel Mansouri[1,2,3], Chris Grulke[2], Emma L. Schymanski[4], Christoph Ruttkies[5] and Antony J. Williams[2*]

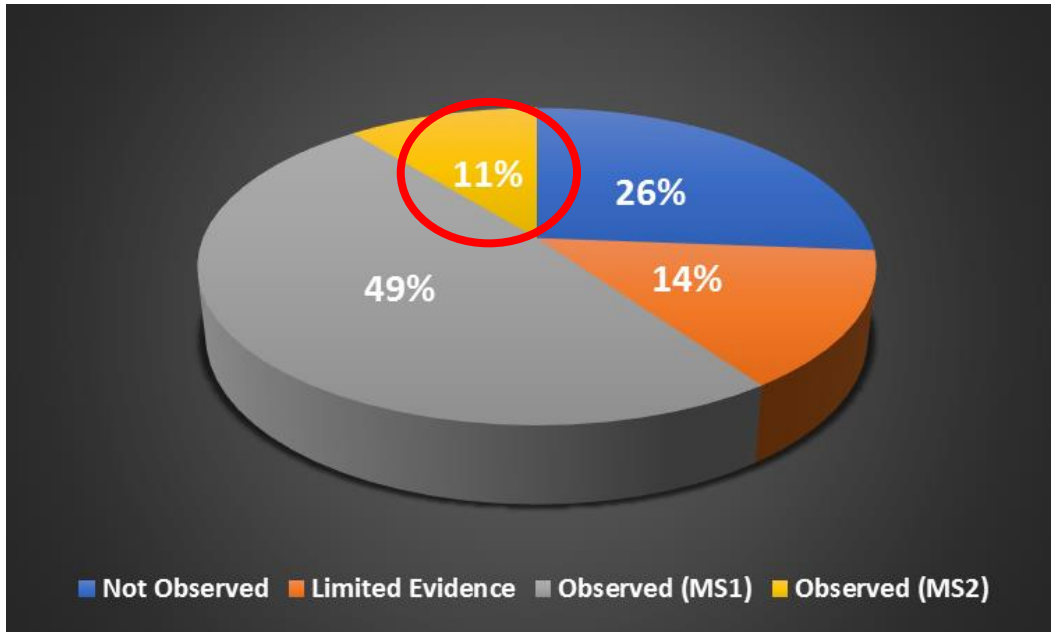
EPA Initial Results

By Feature (total = 26K)



< 5% of Observed Features Matched to a Spiked Substance

By Substance (total = 1,269)



~ 75% of Spiked Substances were Observed

*Only 48% of ENTACT substances were in reference MS^2 library

Generation of *in silico* Spectra

CFM-ID v2.0

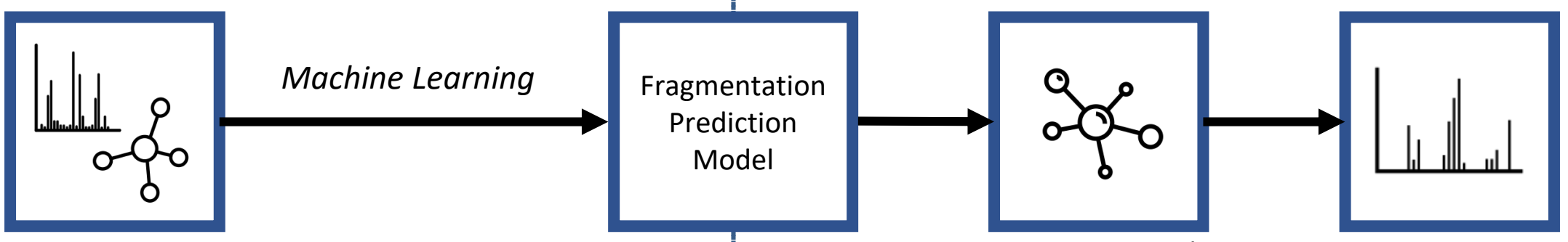
Competitive fragmentation modeling of ESI-MS/MS spectra for putative metabolite identification

Authors | Authors and affiliations

Felicity Allen, Russ Greiner, David Wishart

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

Andrew D. McEachran, Ilya Balabin, Tommy Cathey, Thomas R. Transue, Hussein Al-Ghoul, Chris Grulke, Jon R. Sobus & Antony J. Williams



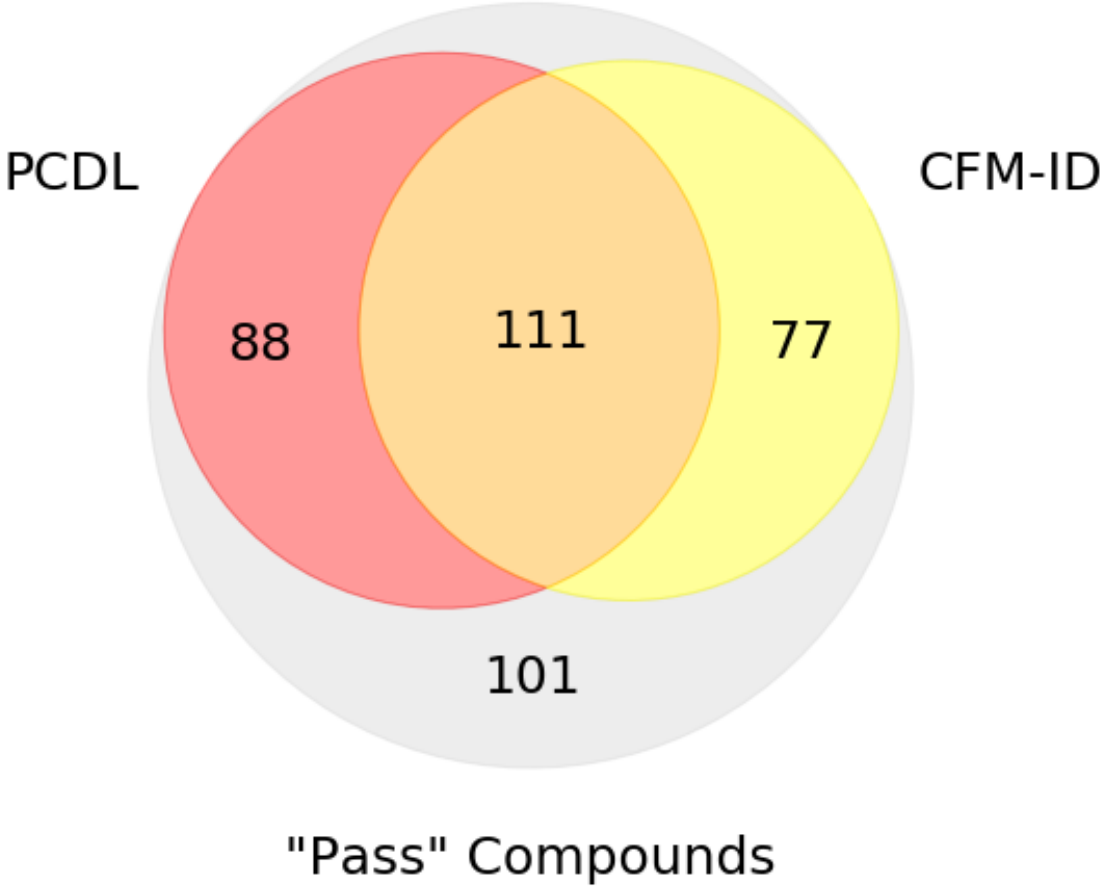
Training Set: Metlin MS2 spectra and structures

DSSTox MS-Ready Structures (~765,000)

DSSTox MS2 spectra (10, 20, 40v)

McEachran, Andrew D., et al. *Scientific data* 6.1 (2019): 1-9
Allen, Felicity, et al. *Metabolomics* 11.1 (2015): 98-110.

Reference vs. *in silico* Library Coverage



MS2 Library	% of "Pass" Compounds Identified
Agilent PCDL	53%
CFM-ID Top Hit	50%
PCDL and/or CFM-ID Top Hit	73%

PCDL → Agilent reference MS^2 library

"Pass" compounds (n=377) → ENTACT chemicals observed with MS^2 data

Who Else 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

Comparing Reported Features (n=16 labs)

	ToxCast Mixtures										Fortified Matrices		
	1	2	3	4	5	6	7	8	9	10	Dust	Serum	Band
Act.	95	95	95	95	185	185	365	365	95	365	365	95	185
Lab 1	128	148	166	187	292	269	**318**	470	177	**410**	NR	NR	NR
2	142	154	**102**	129	250	242	**401**	**399**	**105**	**452**	NR	NR	NR
3	48	40	48	59	110	101	97	130	37	**109**	NR	NR	NR
4	301	130	375	341	408	404	719	687	198	**327**	NR	NR	NR
5	*65*	*66*	**74**	**72**	*105*	*118*	*193*	*215*	*54*	*162*	NR	NR	NR
6	587	552	596	554	798	846	1327	1274	509	1176	NR	NR	NR
7	**93**	**114**	**116**	**106**	**182**	**201**	**360**	**374**	**73**	**330**	*236*	**92**	*124*
8	337	372	303	365	321	363	466	505	510	463	*259*	222	313
9	135	130	125	154	**188**	**195**	**284**	**295**	**100**	*153*	*270*	*54*	*101*
10	*70*	*57*	*64*	*66*	*105*	*115*	*176*	*125*	*35*	*159*	NR	NR	NR
11a	595	486	571	630	746	669	899	910	588	792	1009	614	NR
11b	*66*	170	*51*	*41*	272	*116*	*214*	*101*	163	**404**	861	145	557
12	*51*	*37*	*35*	*39*	*74*	*59*	*124*	*109*	*42*	*105*	*124*	*52*	*76*
13	137	*65*	*45*	**74**	*68*	234	**413**	**408**	120	**317**	**389**	178	*88*
14	215	249	212	249	**207**	275	*245*	*254*	140	*253*	NR	NR	NR
15	1298	1258	1304	1209	1651	1641	2520	2538	1202	2193	NR	NR	NR
16	153	217	221	199	254	321	523	651	496	**396**	NR	NR	NR

= under reported

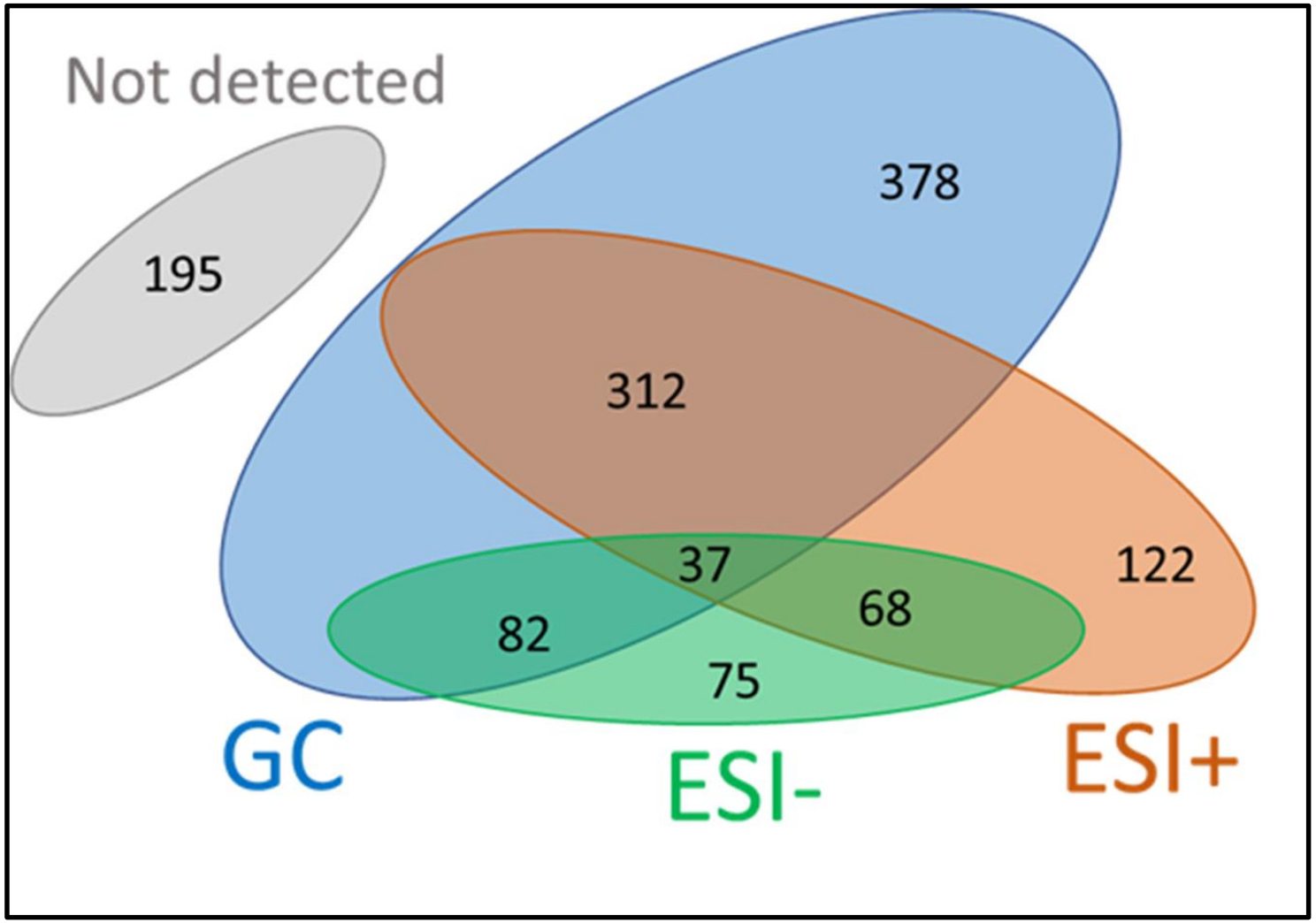
= near actual

= over reported

NR = not reported

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

Comparing Identified Compounds (n=3 labs)



1,269 Spiked Substances

GC = gas chromatography

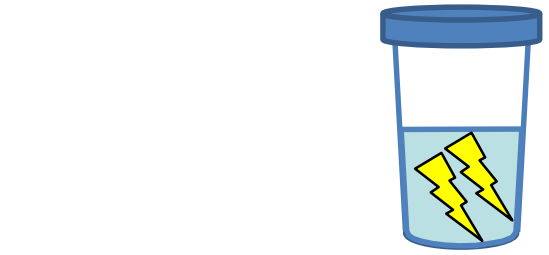
ESI- = neg. electrospray ionization (liquid chromatography)

ESI+ = pos. electrospray ionization (liquid chromatography)

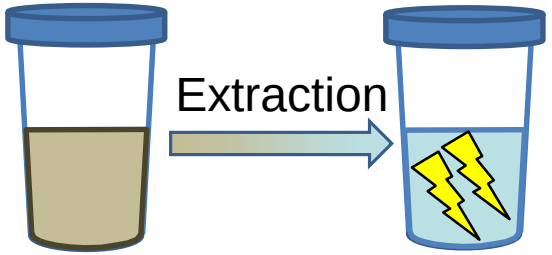
Ulrich et al. 2019. https://doi.org/10.1007/s00216-018-1435-6

Experiments with SRM Dust

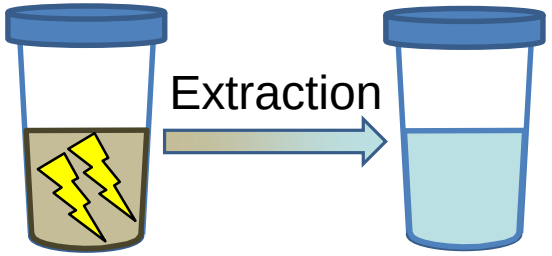
1) : Solvent spike (best case)



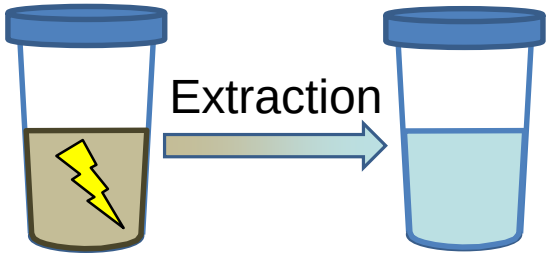
2) : Post-extraction high spike

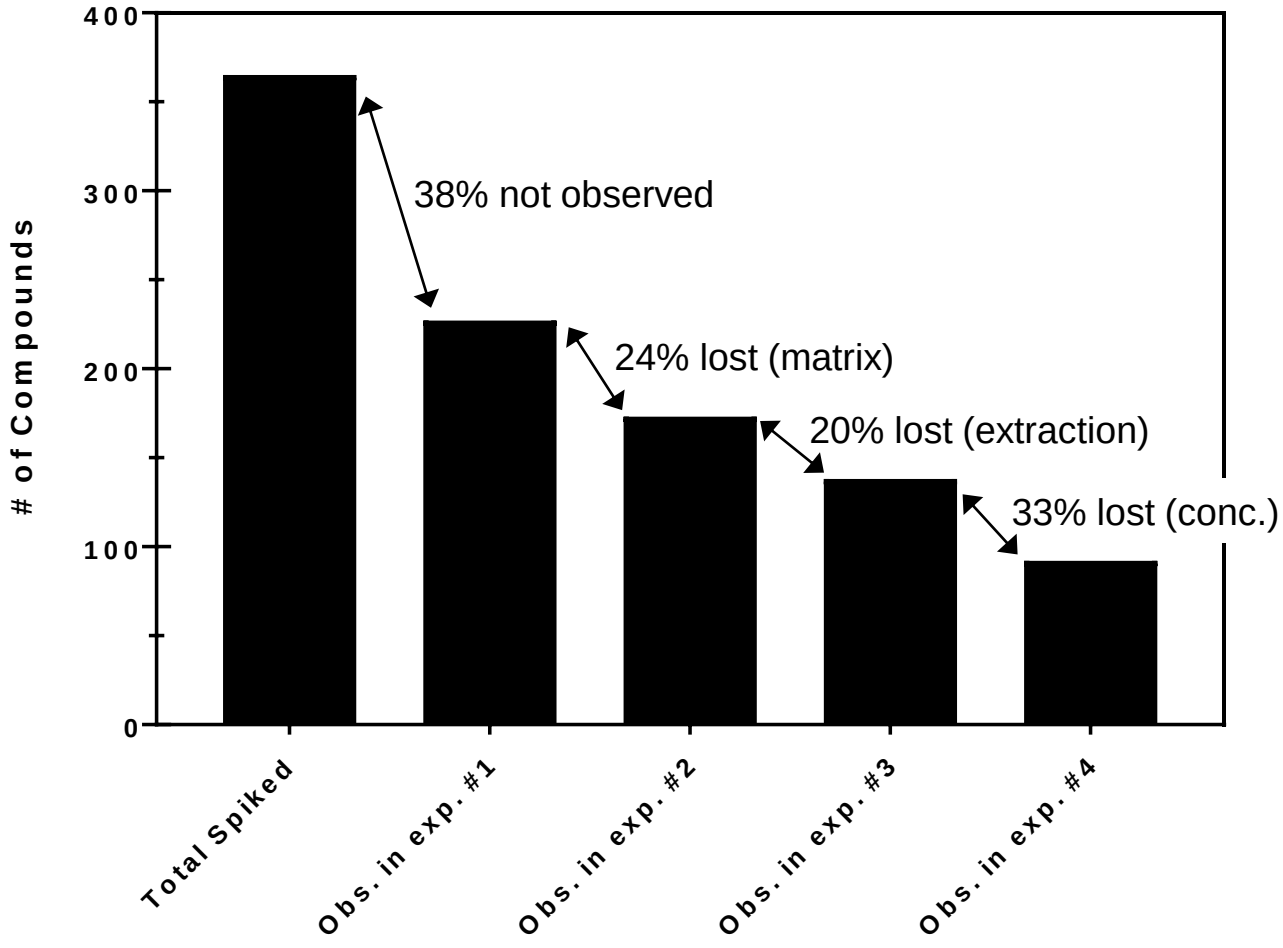


3) : Pre-extraction high spike



4) : Pre-extraction low spike (ENTACT sample)



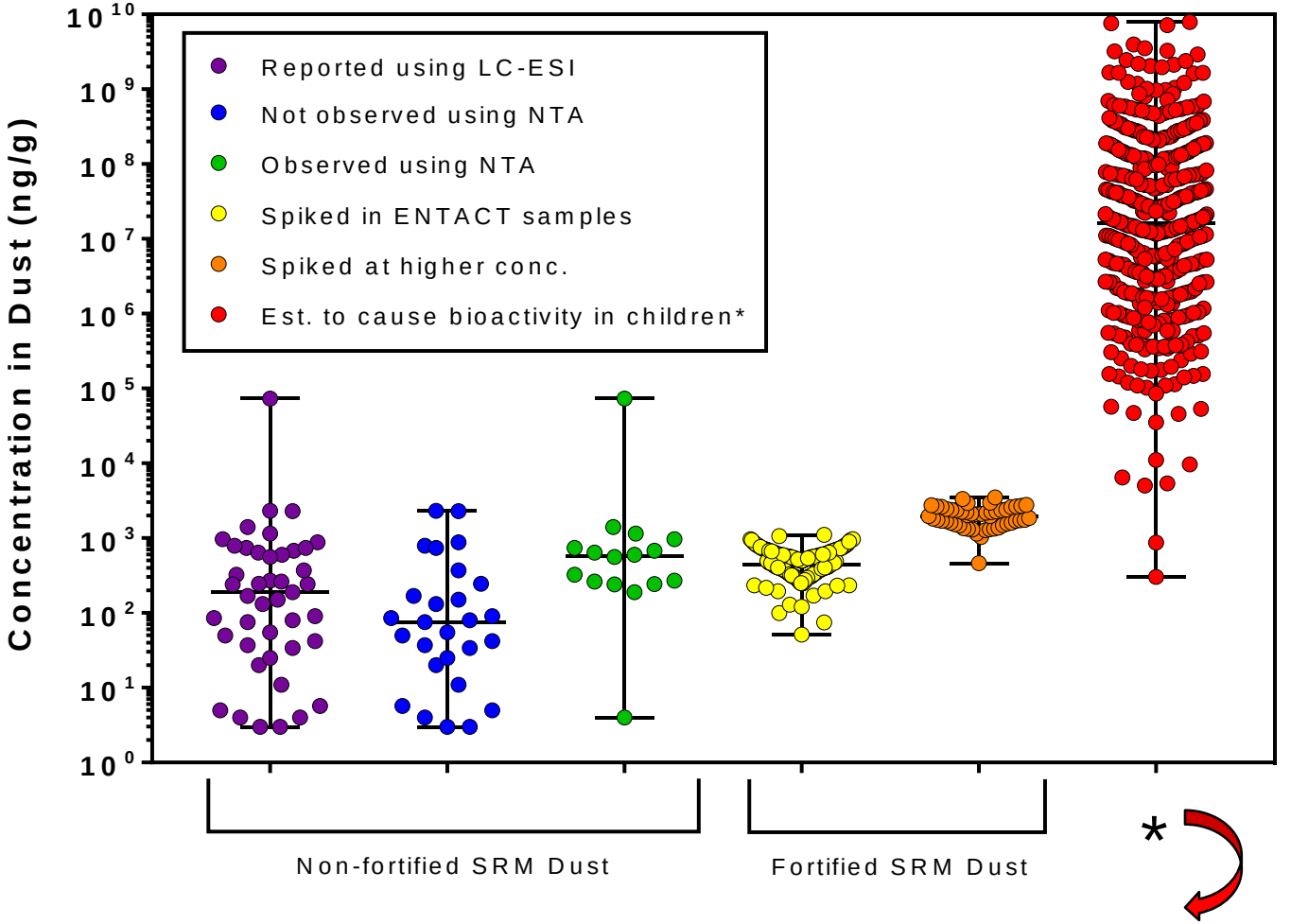


Newton et al. [in preparation]

Experiments with SRM Dust

Results for Unfortified SRM Dust

Chemical Class	All Reported Compounds	Reported Using LC-ESI	Observed Using NTA
PAHs	69	0	0
PCBs	44	0	0
PFAS	31	31	12
BFRs	30	3	0
OCPs	15	0	0
OPEs	12	9	4
Phthalates	7	0	2
Total	208	**43**	**18**



* "..the dose that would be needed in the most-sensitive 5% of the population to produce a steady-state plasma concentration equal to [the 10th] percentile of the ToxCast AC50 distribution across assays for the given chemical."

Newton et al. [in preparation]

Ring et al.: https://doi.org/10.1016/j.envint.2017.06.004

Publications to date

Analytical and Bioanalytical Chemistry (2019) 411:853–866
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RESEARCH PAPER

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

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RESEARCH PAPER

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

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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

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

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Summary of ENTACT Findings

- NTA methods are suitable for detecting many ToxCast chemicals
- False positives can greatly outweigh true positives
  - False Pos / True Pos ~ 10×
  - Work needed on feature credentialing
- True Positives: ≤75%
  - Will miss some chemicals that are present in samples
    - Why? Which ones? Always?
- Multiple methods required for broad characterization
  - No “one size fits all” method
  - Subtle method changes affect measurable chemical space
- Concentration, media, and extraction techniques will affect performance
- Goal reached when we can make these statements:
  - “When a compound is observed, we’re confident it’s really there!”
  - “When a compound isn’t observed, we’re confident it’s not there!”

Ongoing and Future Work

- Full cross-lab performance evaluation
  - Primary focus → true positives, false negatives, confidence levels
  - Secondary focus → unexpected true positives
- Database development
  - Enable user queries, additional analyses, model development
- Global summary report
  - Provide guidance and acceptance criteria for NTA studies
- <u>The benefits of ENTACT will be proportional to the level of effort</u>!

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