EPA United States Environmental Protection Agency

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

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], Louis Groff[2]

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

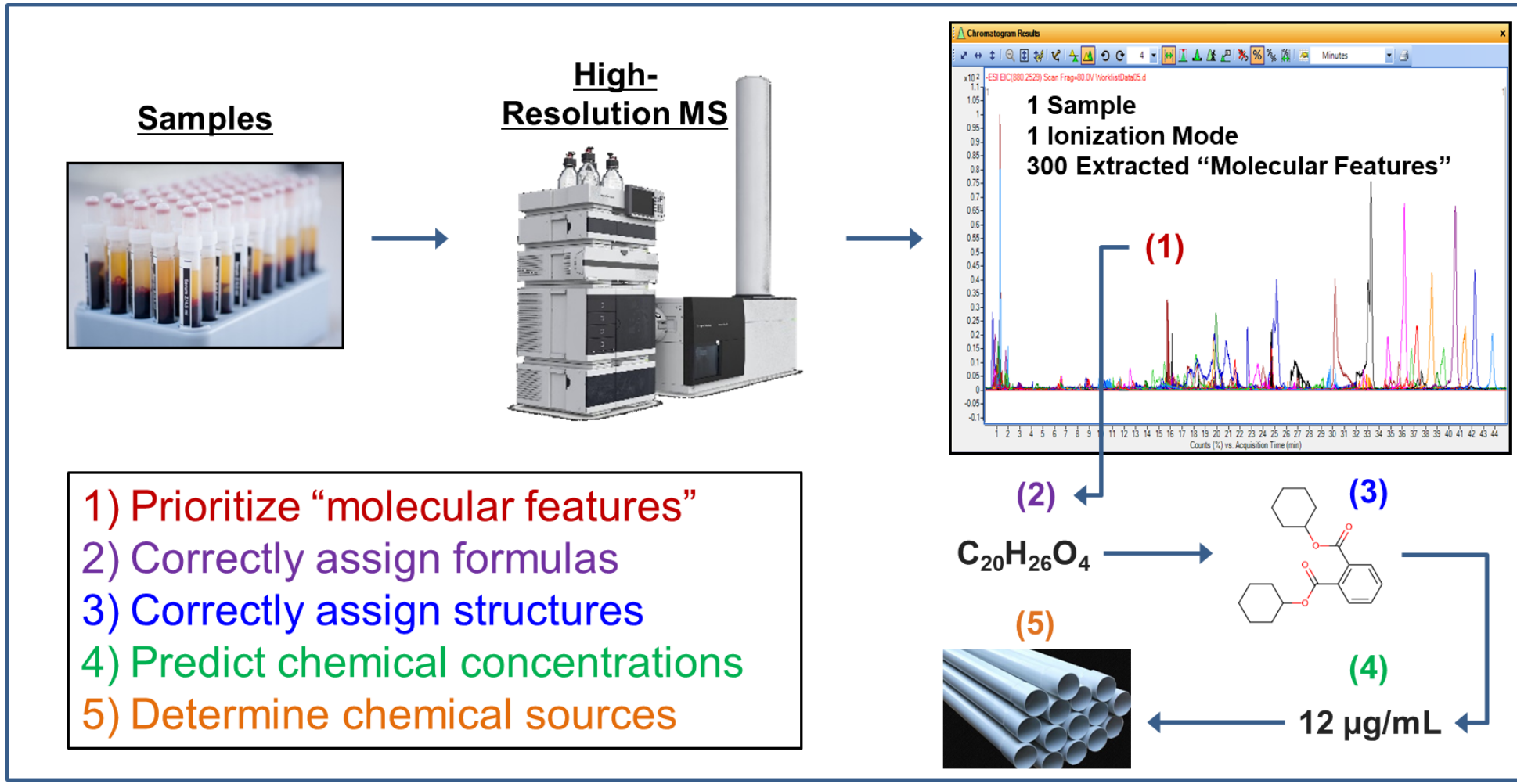
What's So Great About NTA?

Rapidly screen for "knowns"

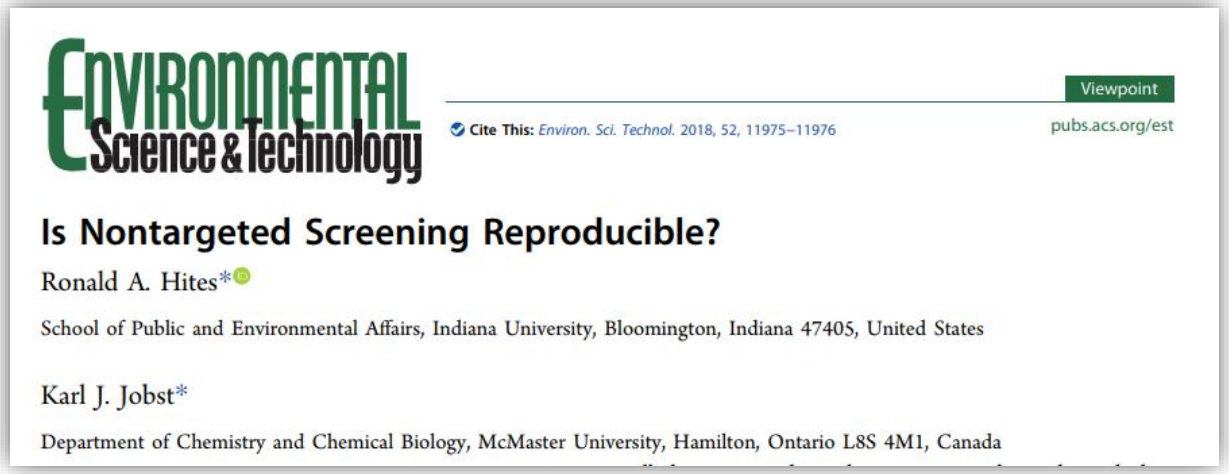
Discover "unknowns"

Uncover historical exposures

Generate source fingerprints…



NTA State-of-the-Science



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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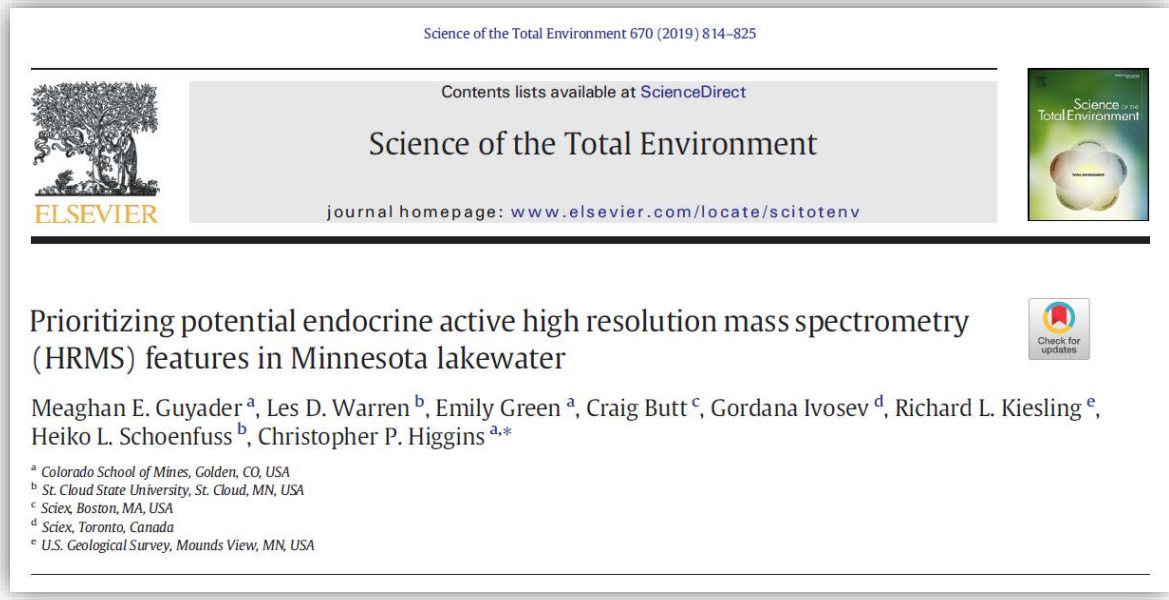
Karl J. Jobst*

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



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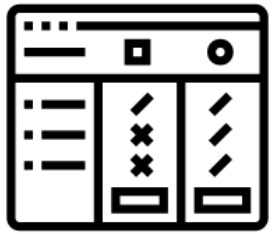
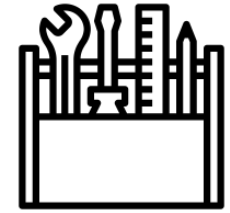
"*No single analytical technique is suitable for the analysis of all compounds, and successful nontargeted screening will require the development of multiplatform approaches, facilitated and validated through interlaboratory collaborations*."

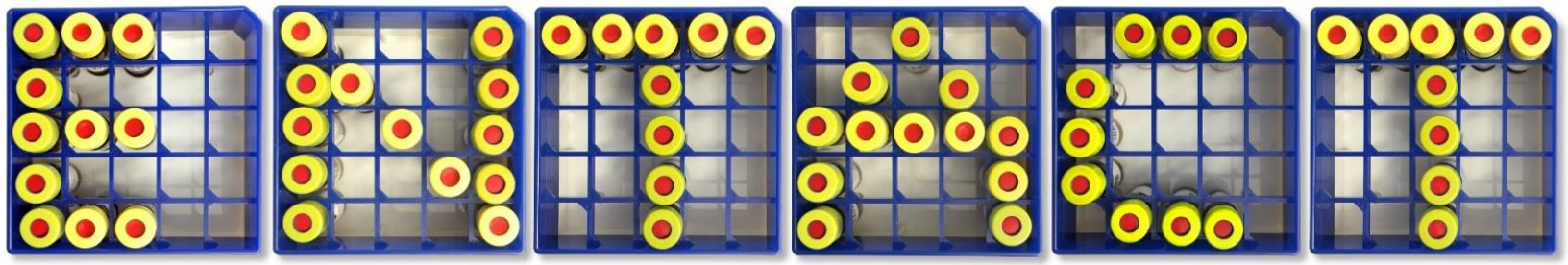
"*The novelty of nontarget analysis, particularly its current lack of implementation by regulatory agencies, has prevented the establishment of streamlined quality assurance and quality control (QA/QC) procedures.*"



Science Questions for Research Community

- How variable are tools and results from lab to lab?
- Are some methods/workflows 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

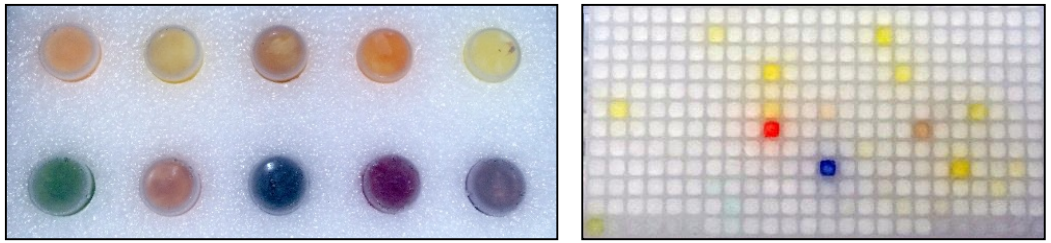
ENTACT Part 1

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

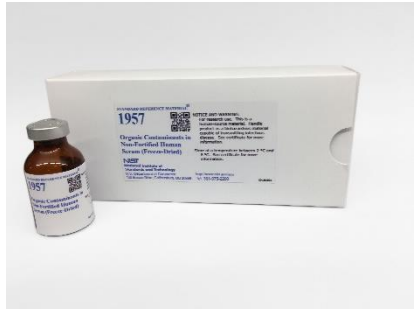
2nd: Unveiling of chemicals

3rd: Unblinded evaluation

ENTACT Part 2

Reference & Fortified House Dust

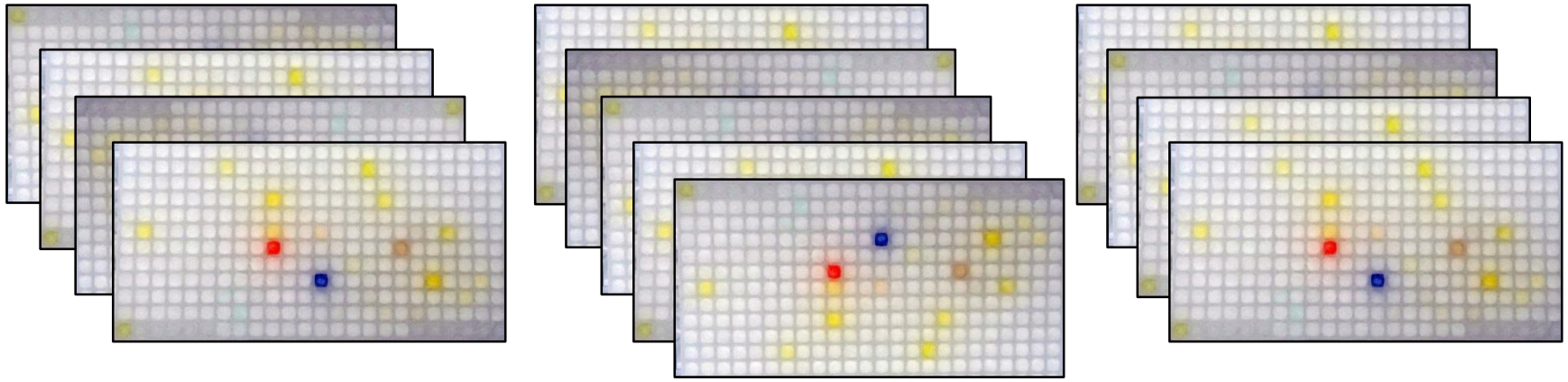
Reference & Fortified Human Serum



Reference & Fortified Silicone Wristbands

EPA United States Environmental Protection Agency

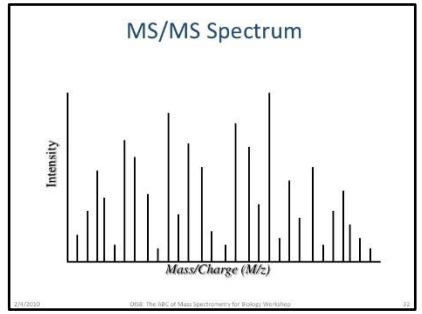
ENTACT Part 3



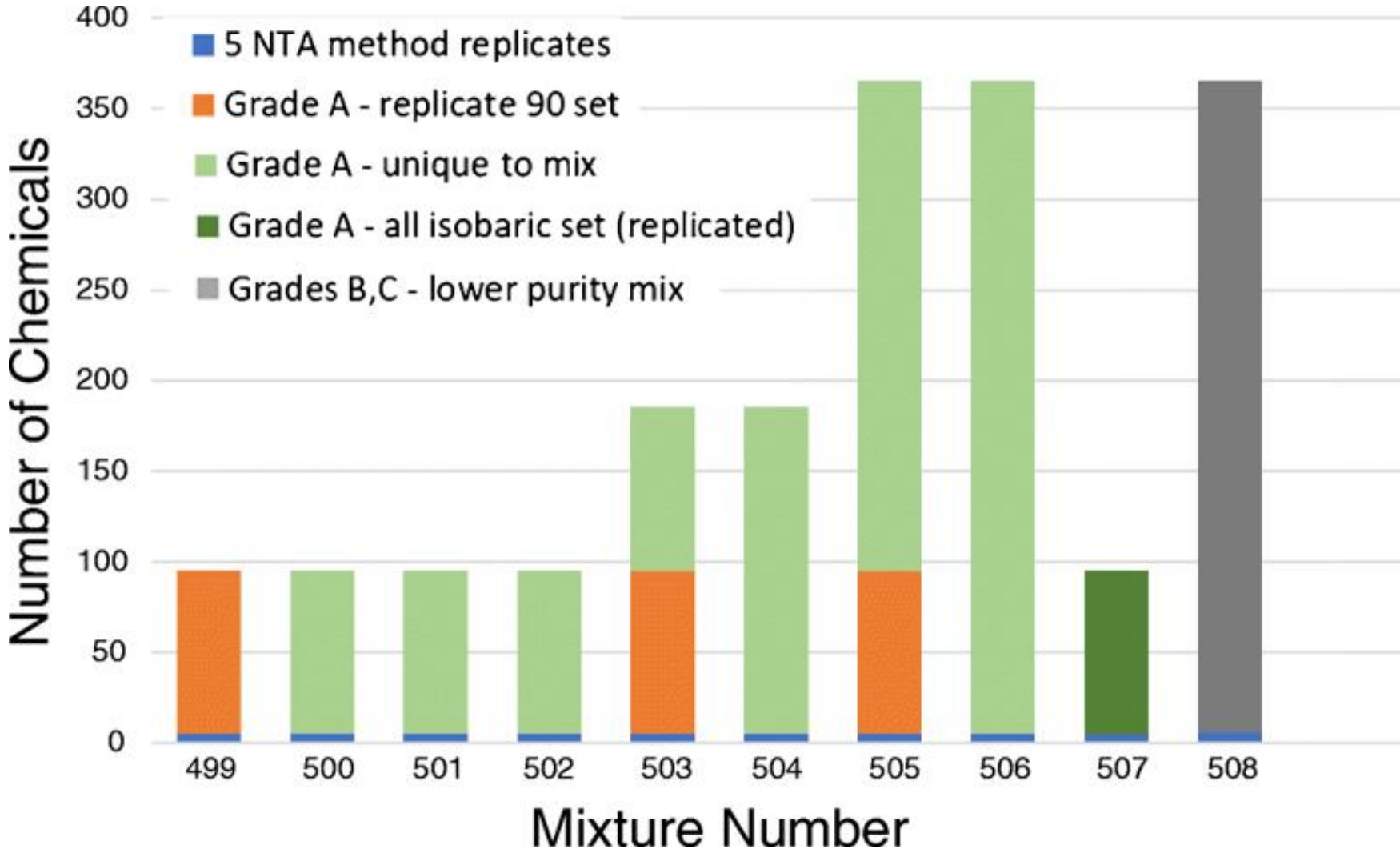
~4600 ToxCast substances

Instrument/software vendors & select labs

Reference libraries for the public



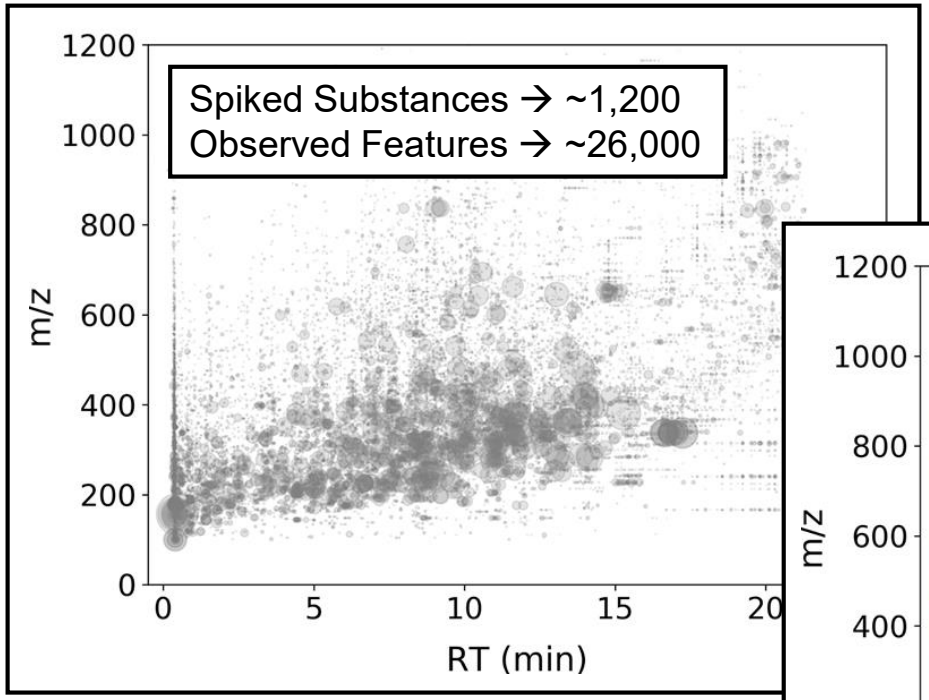
Design of ENTACT Mixtures

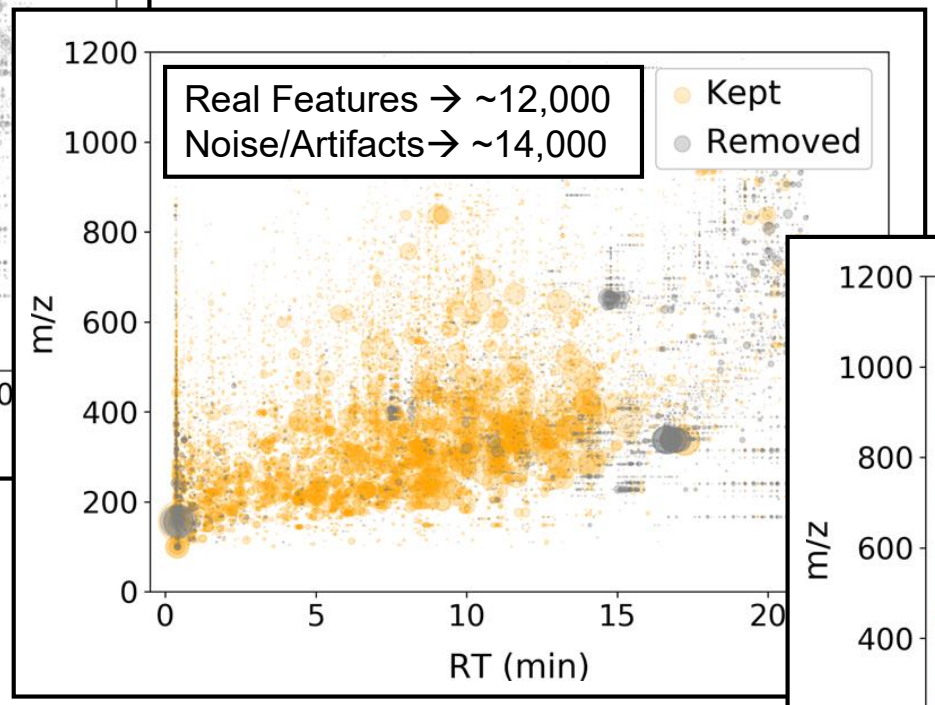


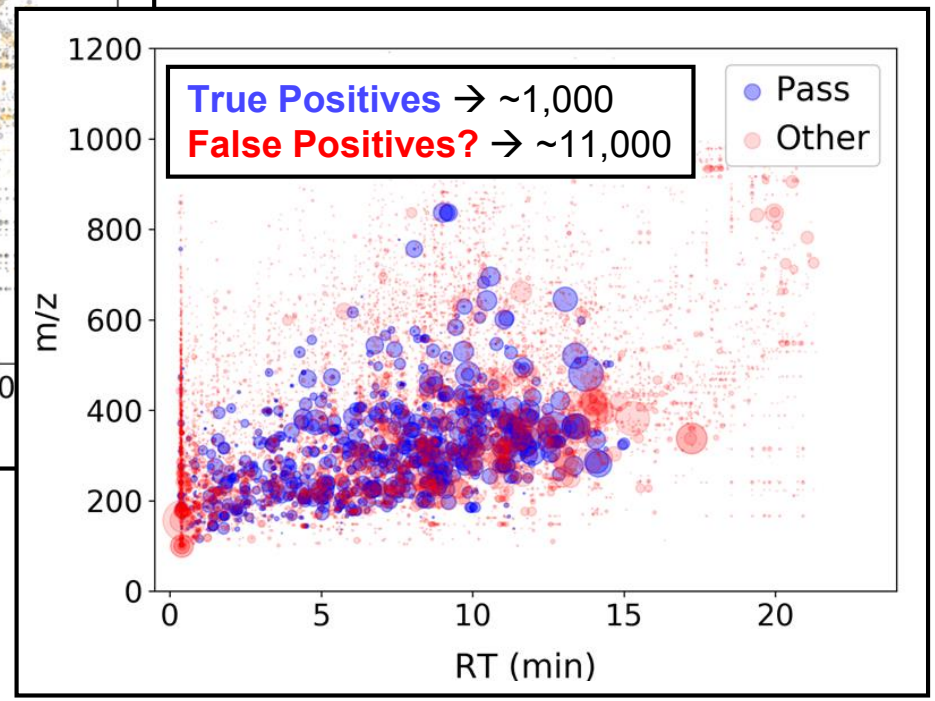
Ulrich et al. 2019. doi: 10.1007/s00216-018-1435-6

EPA Lab Results

LC-QTOF HRMS (ESI+ and ESI-)







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

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

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

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
DTSC Department of Toxic Substances Control
eawag aquatic research
United States Environmental Protection Agency
FIU

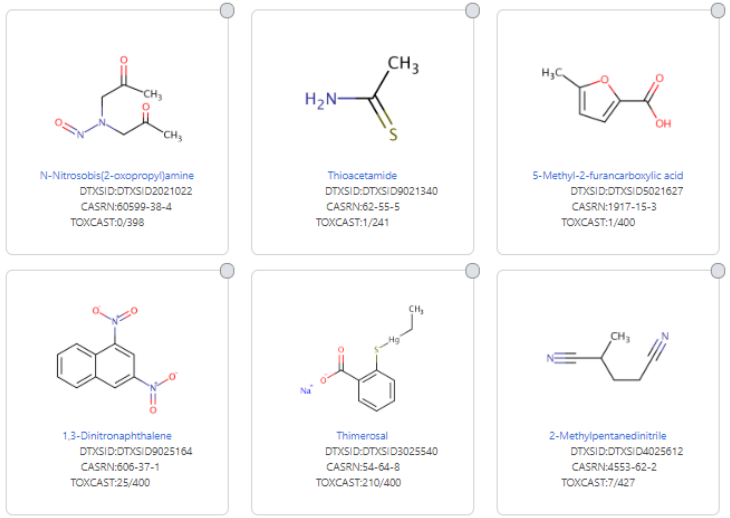
Processing ENTACT Data Submissions

- Individual methods treated separately (if appropriate)
- One candidate mass/formula/compound per feature
- Confidence level revised as needed (with consensus)
- Matching to spiked substances by mass, formula & structure
- "**Observed**" if structure or formula (no spiked isomers) match
- "**Identified**" if structure match
- "**Reproducible**" if correctly ID'd >50% of the time
  - For compounds spiked >1 time and identified ≥1 time

Lab Comparison: “Observed” Compounds

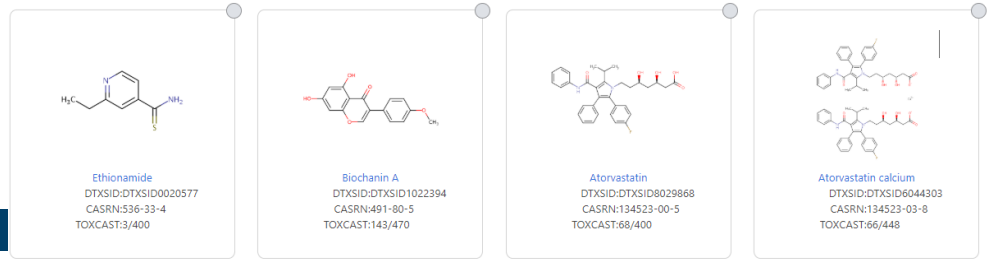
7 Labs, 12 Methods

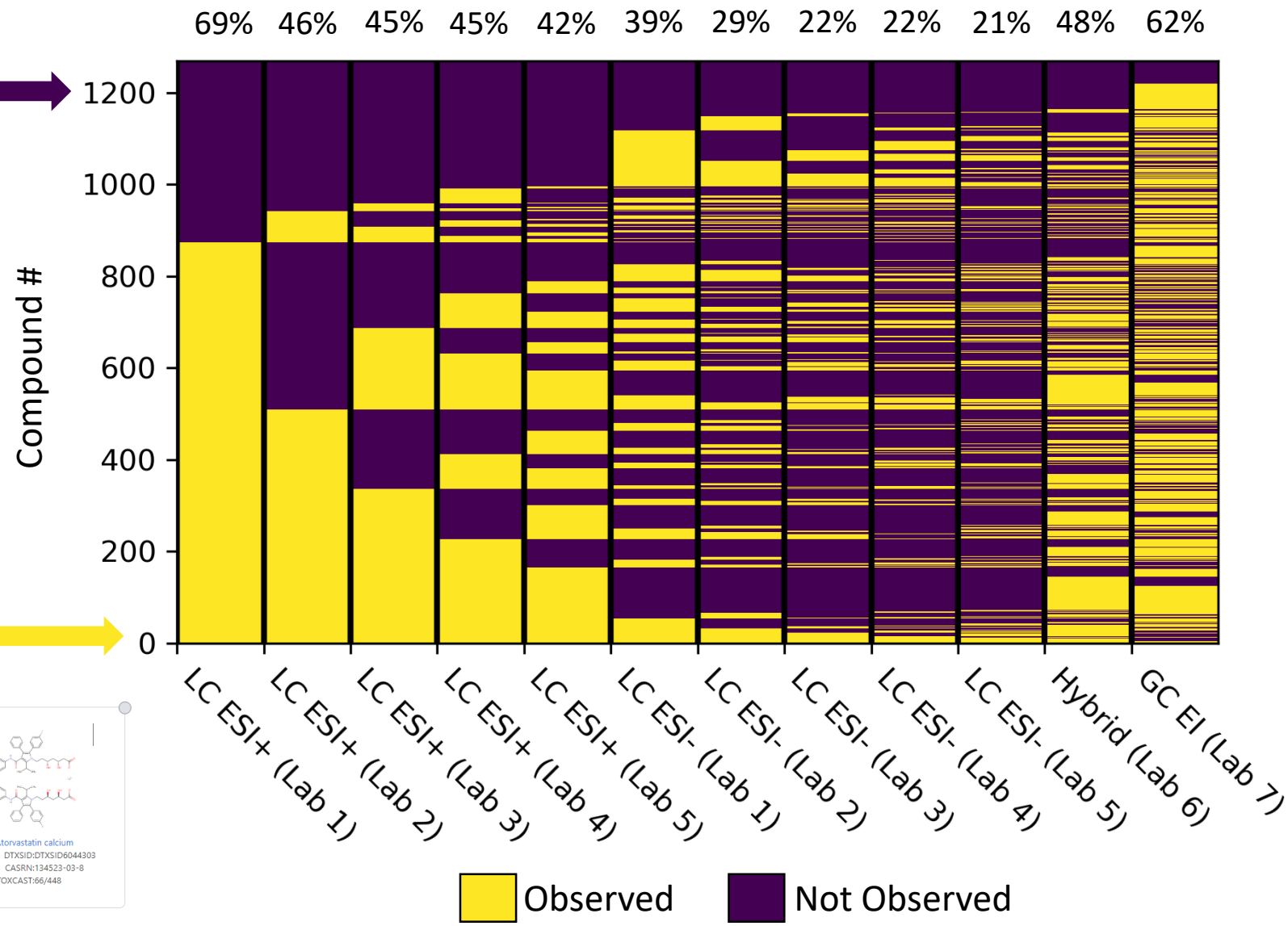
~5% Not Observed by Any Method



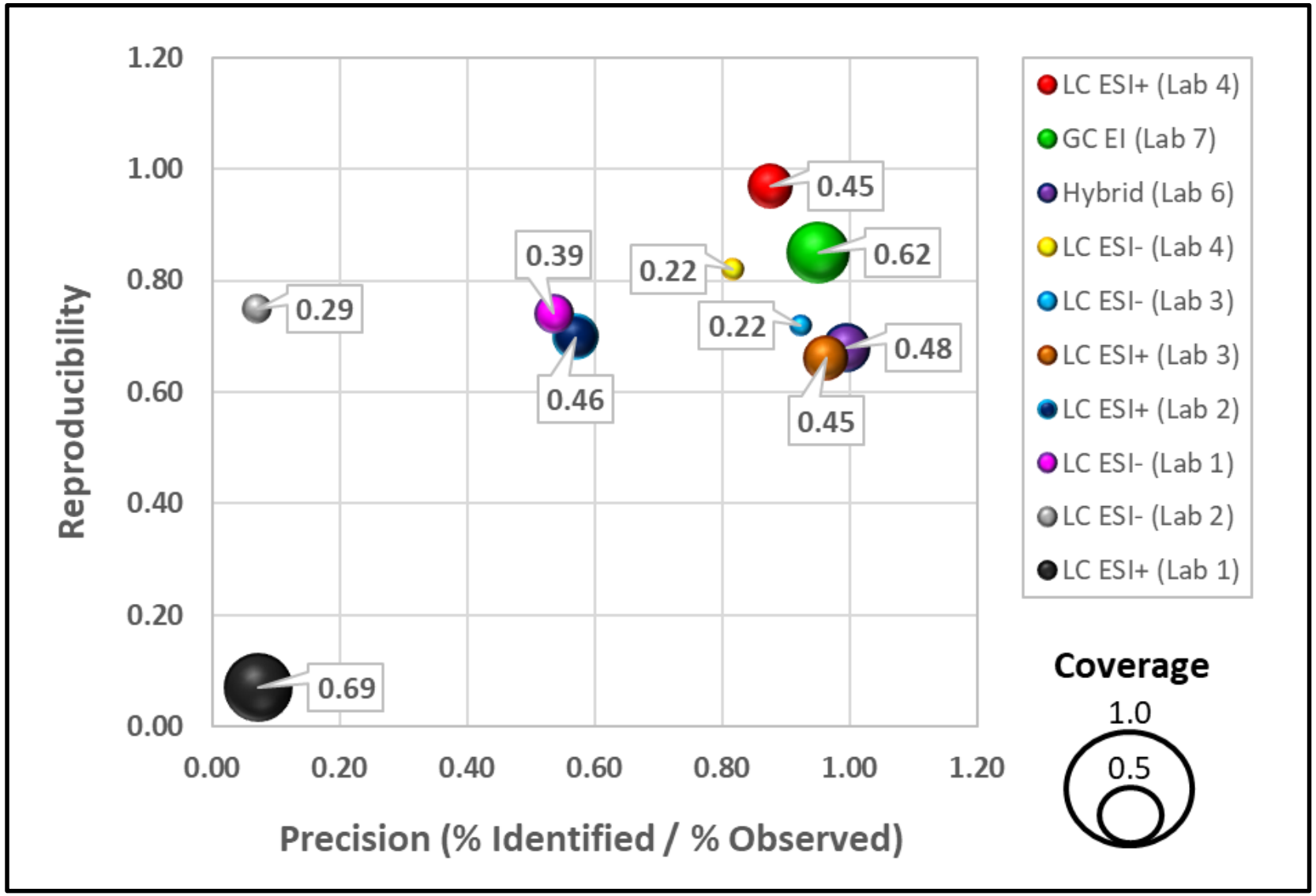
...

<1% Observed by All 12 Methods





Lab Comparison: Total Performance



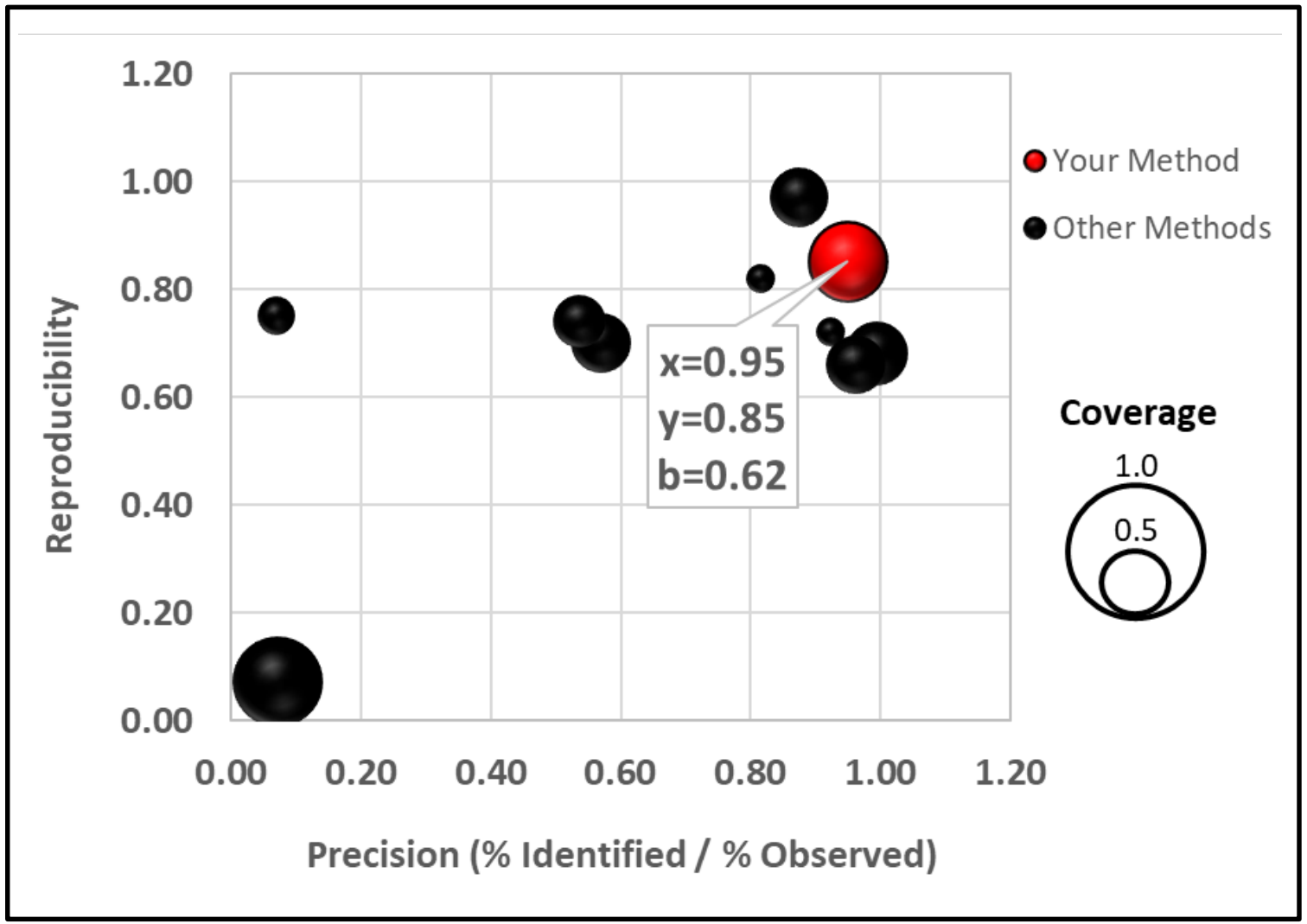
Metrics (all %):

X-Axis →
How often correct?

Y-Axis →
How consistent?

Bubble Size →
How much coverage?

Example Performance Report



Performance Scores:
(% of max score)

Precision: **95%**

min [▭] max

Reproducibility: **87%**

min [▭] max

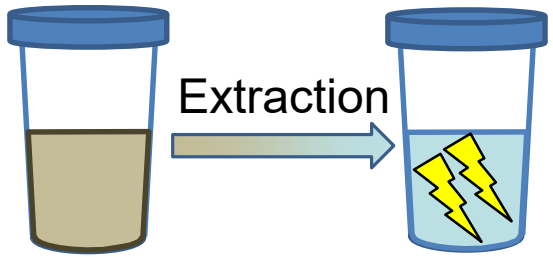
Coverage: **86%**

min [▭] max

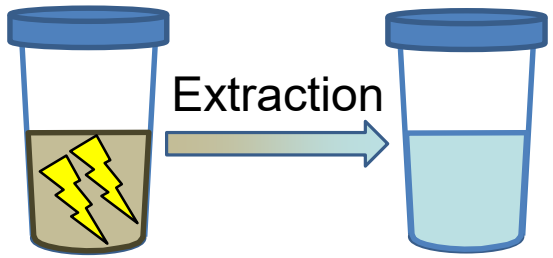
Experiments with SRM Dust

1) : Solvent spike (best case)

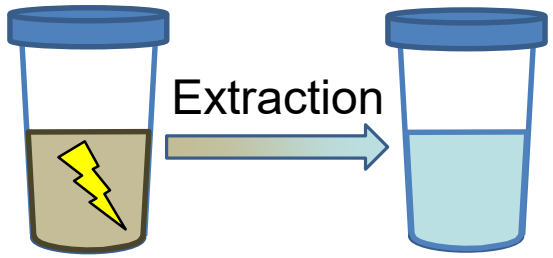
2) : Post-extraction high spike

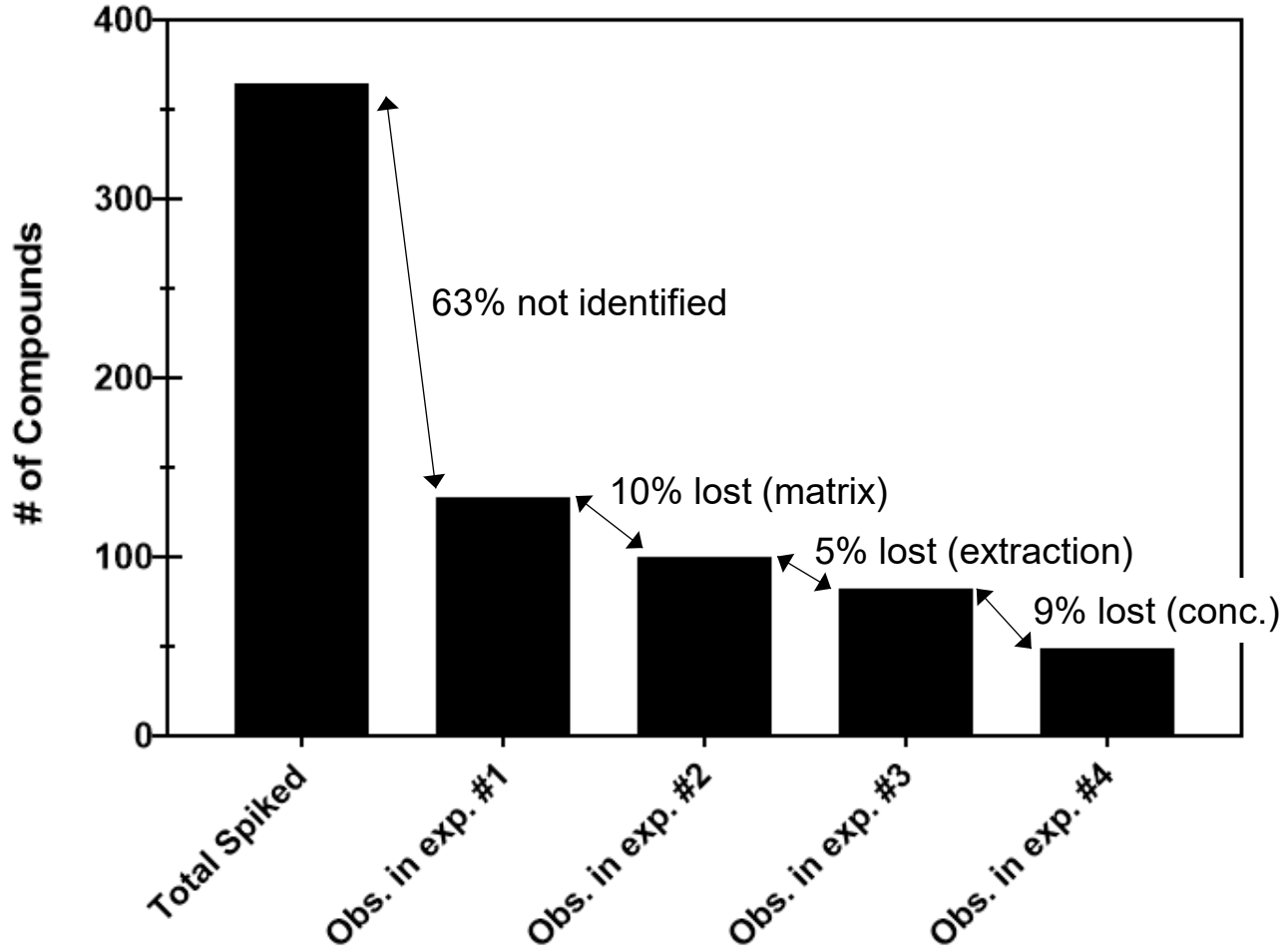


3) : Pre-extraction high spike



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



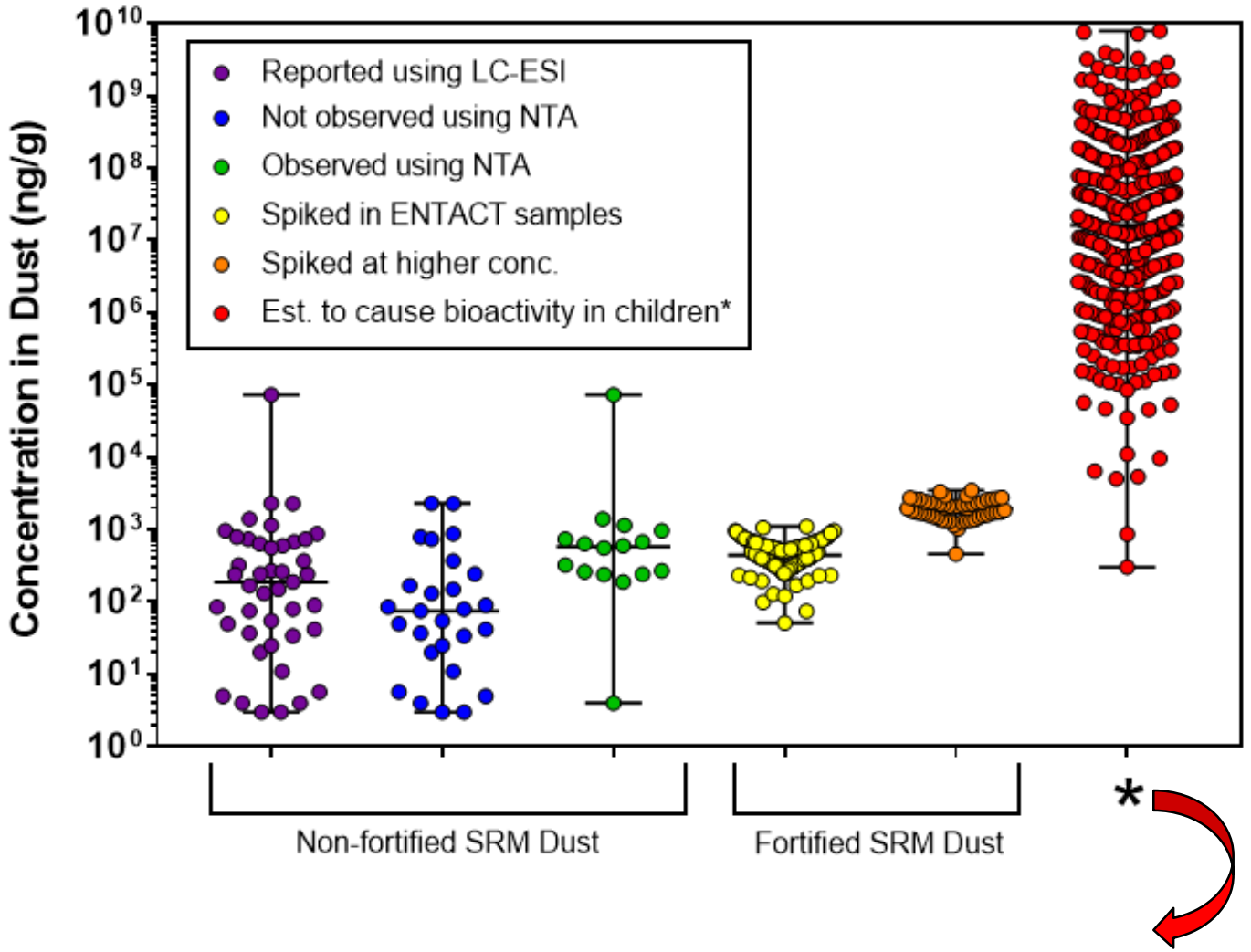


Newton et al. 2020. doi: 10.1007/s00216-020-02658-w

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 concentration that would be needed (for 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. 2020. doi: 10.1007/s00216-020-02658-w

Evaluation of *in silico* Spectra

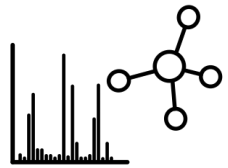
Metabolomics (2015) 11:98–110
DOI 10.1007/s11306-014-0676-4

ORIGINAL ARTICLE

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

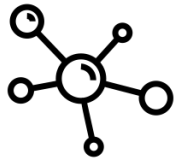
Felicity Allen · Russ Greiner · David Wishart

Training Set

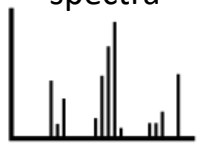


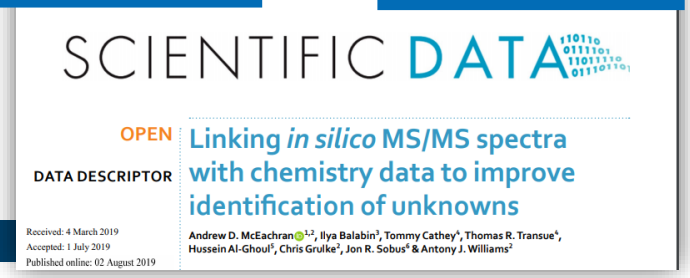
Fragmentation Prediction Model

DSSTox structures

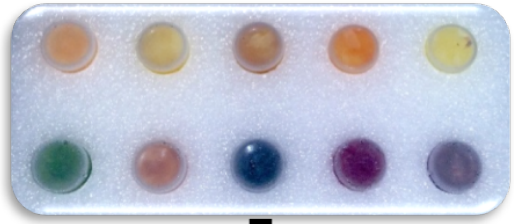


DSSTox MS2 spectra





10 Synthetic Mixtures:
1,269 Unique ToxCast Substances



LC-QTOF HRMS:
Data Dependent Acquisition

MS2 Reference Library

No Library Matches

Probable Structures

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

Tentative Structures

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

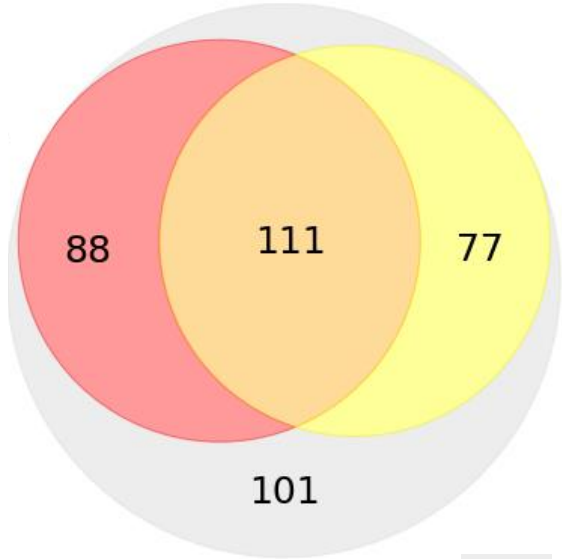
RESEARCH PAPER

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

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

377 ENTACT Compounds with MS2 Spectra

Top Reference Library Match



Top *in silico* Library Match

Not Top Match

Summary of ENTACT Findings

- NTA methods are suitable for many ToxCast chemicals
  - ~5% of ENTACT compounds not observed across all methods
- Performance determined across 3 categories:
  - **Coverage** = Ability to Observe → (Range = 21% to 69%)
  - **Precision** = Ability to Identify those Observed → (Range = 7% to 99%)
  - **Reproducibility** = Ability to Consistently Identify → (Range = 7% to 97%)
- Multiple methods required for broad characterization
  - No “one size fits all” method
  - <1% of ENTACT compounds observed using all methods
- Concentration, media, and extraction techniques will affect performance
- Mixtures/Data are highly valuable for NTA method development/evaluation

Contributing Researchers

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

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