EPA
United States
Environmental Protection
Agency

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

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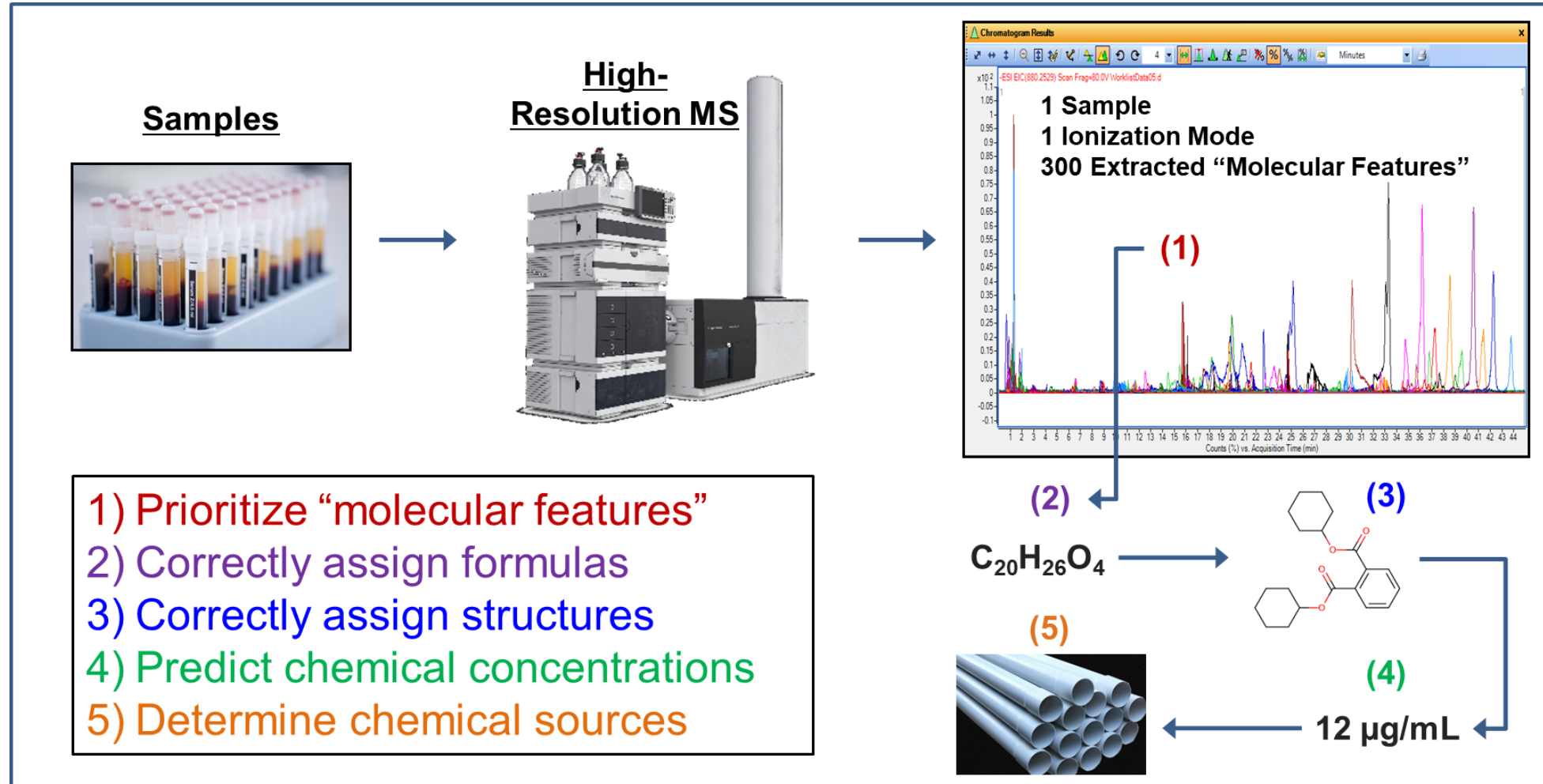[1] Center for Computational Toxicology and Exposure
[2] ORAU/ORISE Participant

Office of Research and Development

What's So Great About NTA?

Rapidly screen for "knowns"

Discover "unknowns"

Uncover historical exposures

Generate source fingerprints…

Samples

High-Resolution MS

1 Sample
1 Ionization Mode
300 Extracted "Molecular Features"

(1)

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)**

(5)

(4)

12 µg/mL

NTA State-of-the-Science

Viewpoint

pubs.acs.org/est

Is Nontargeted Screening Reproducible?

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Prioritizing potential endocrine active high resolution mass spectrometry (HRMS) features in Minnesota lakewater

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[a] Colorado School of Mines, Golden, CO, USA
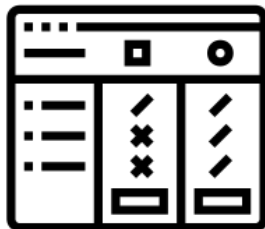[b] St. Cloud State University, St. Cloud, MN, USA
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"*No single analytical technique is suitable for the analysis of all compounds, and successful nontargeted screening will require the development of multiplatform approaches, facilitated and validated through interlaboratory collaborations.*"
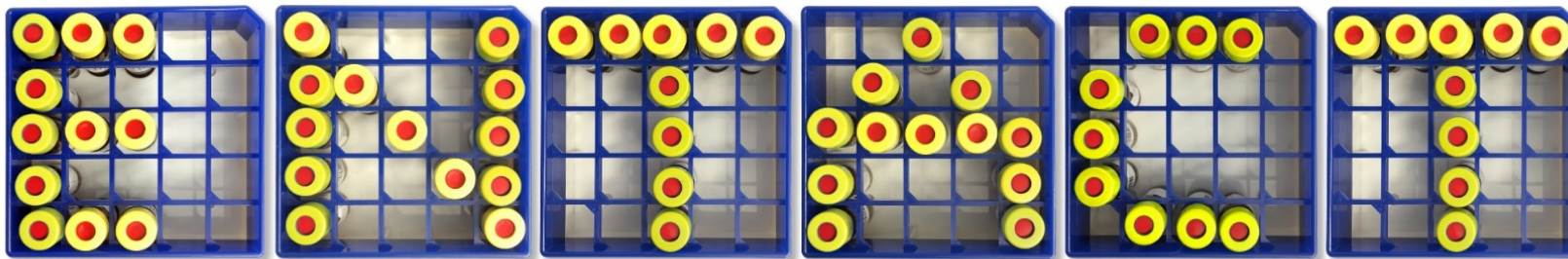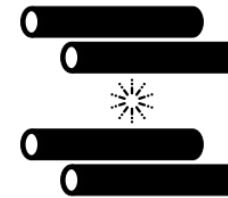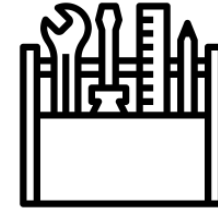
"*The novelty of nontarget analysis, particularly its current lack of implementation by regulatory agencies, has prevented the establishment of streamlined quality assurance and quality control (QA/QC) procedures.*"
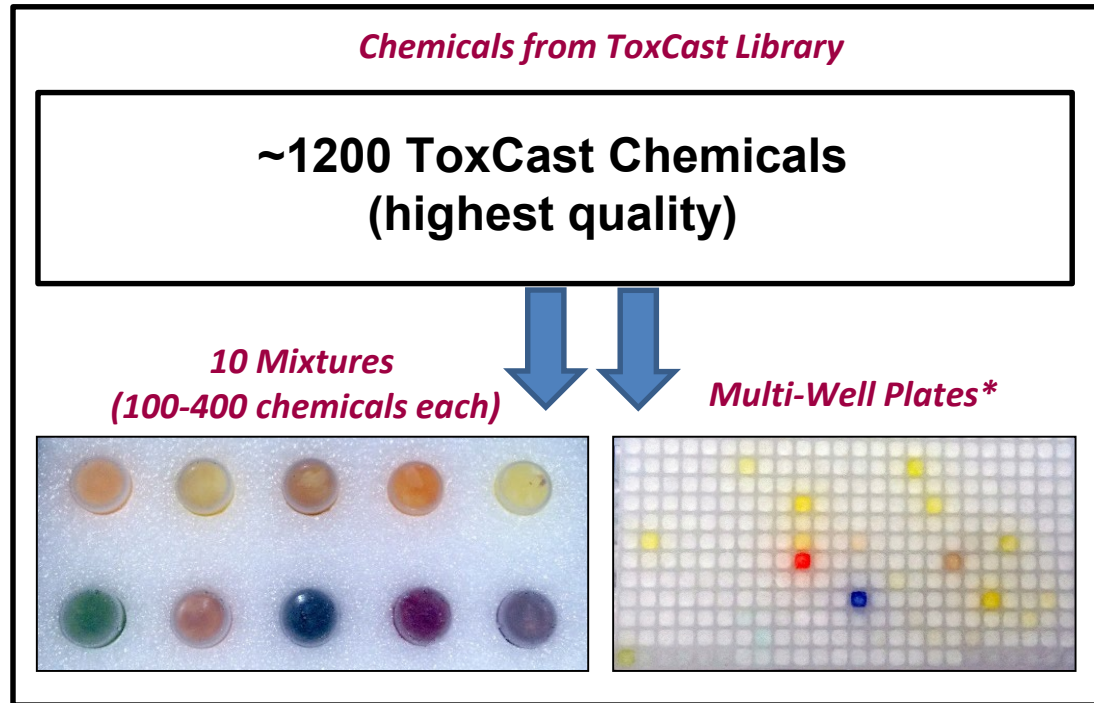
Science Questions for Research Community

- 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
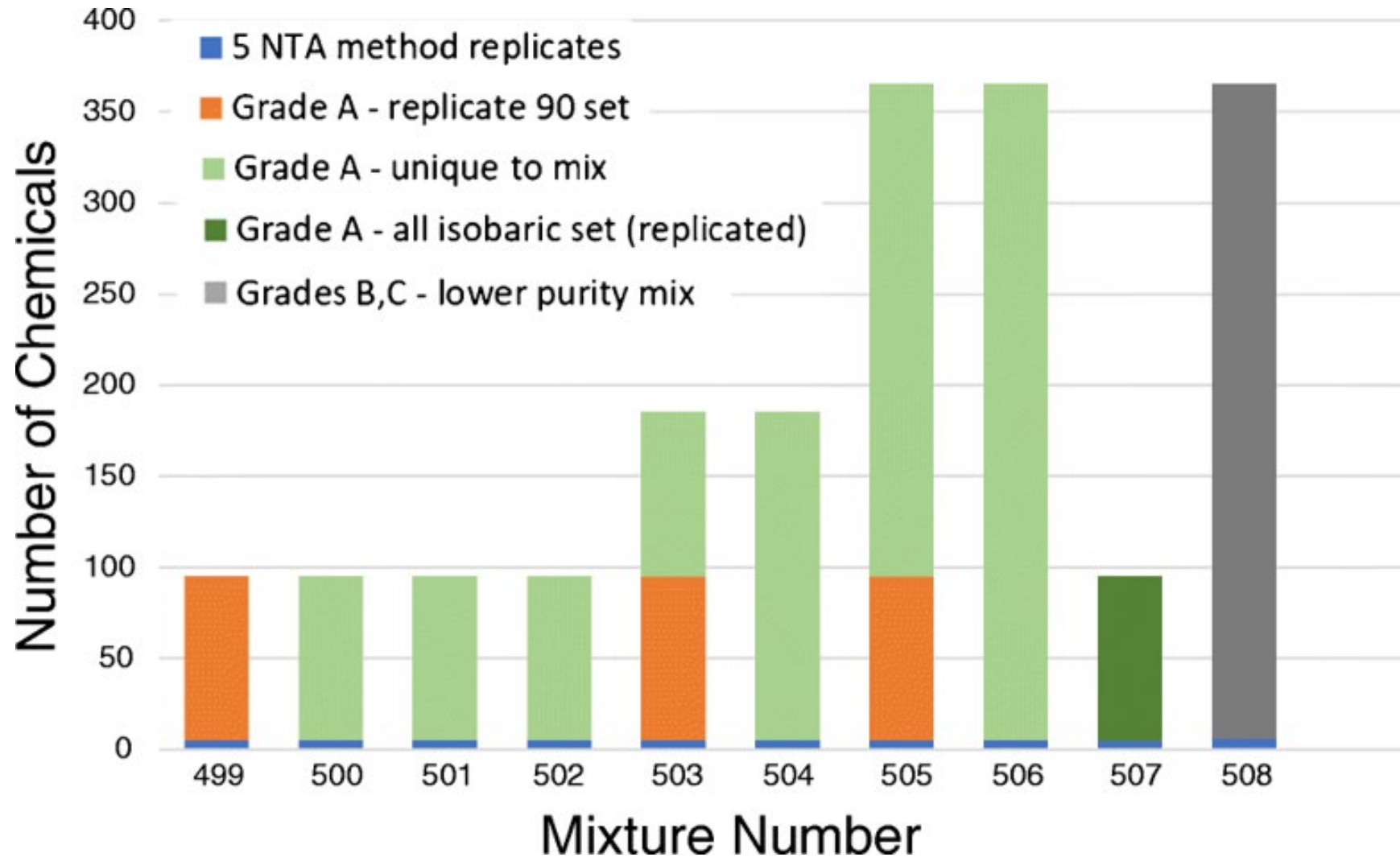
ENTACT Part 3

~4600 ToxCast substances

Instrument/software vendors & select labs
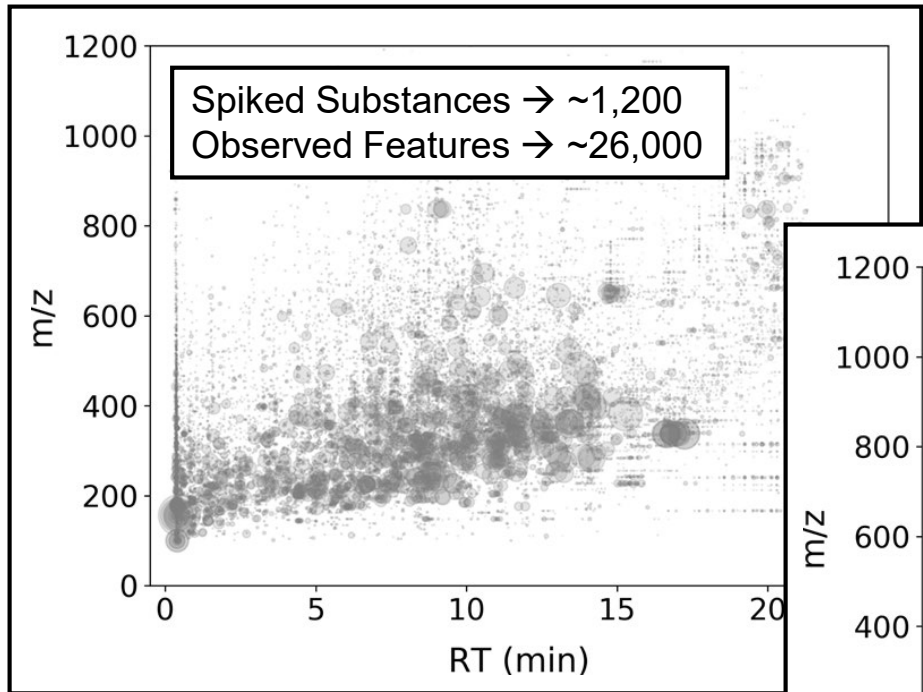
Reference libraries for the public

Design of ENTACT Mixtures

EPA Lab Results

United States Environmental Protection Agency

LC-QTOF HRMS (ESI+ and ESI-)

Spiked Substances → ~1,200
Observed Features → ~26,000

Real Features → ~12,000
Noise/Artifacts → ~14,000

- Kept
- Removed

True Positives → ~1,000
False Positives? → ~11,000

- Pass
- Other

Substance Spiked?

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

Substance Identified?

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
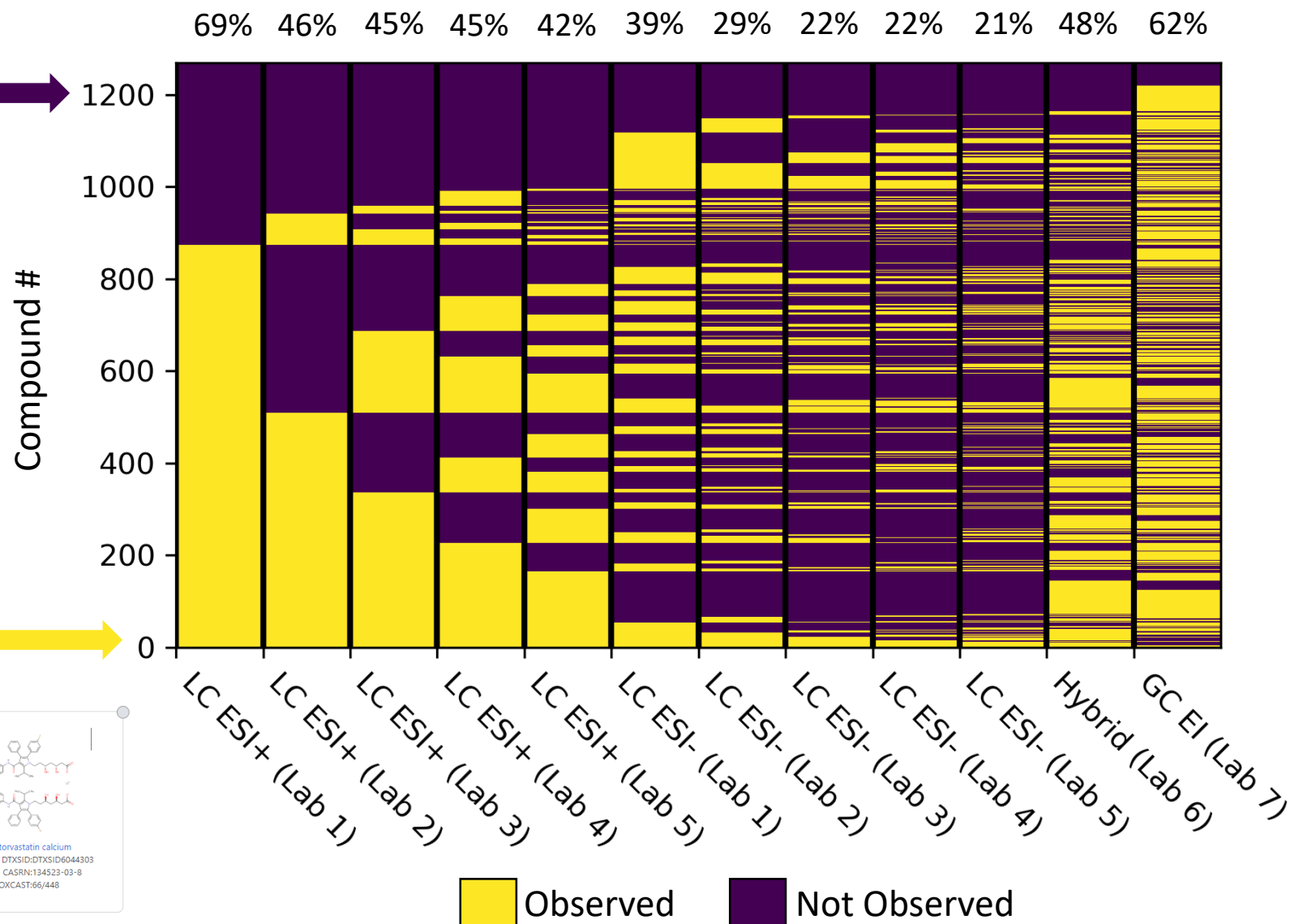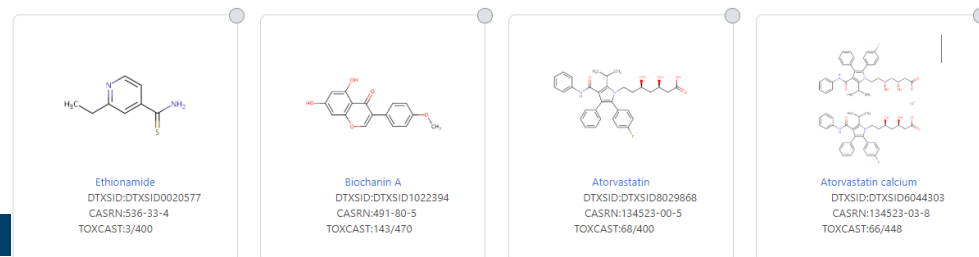 - For compounds spiked >1 time and identified ≥1 time

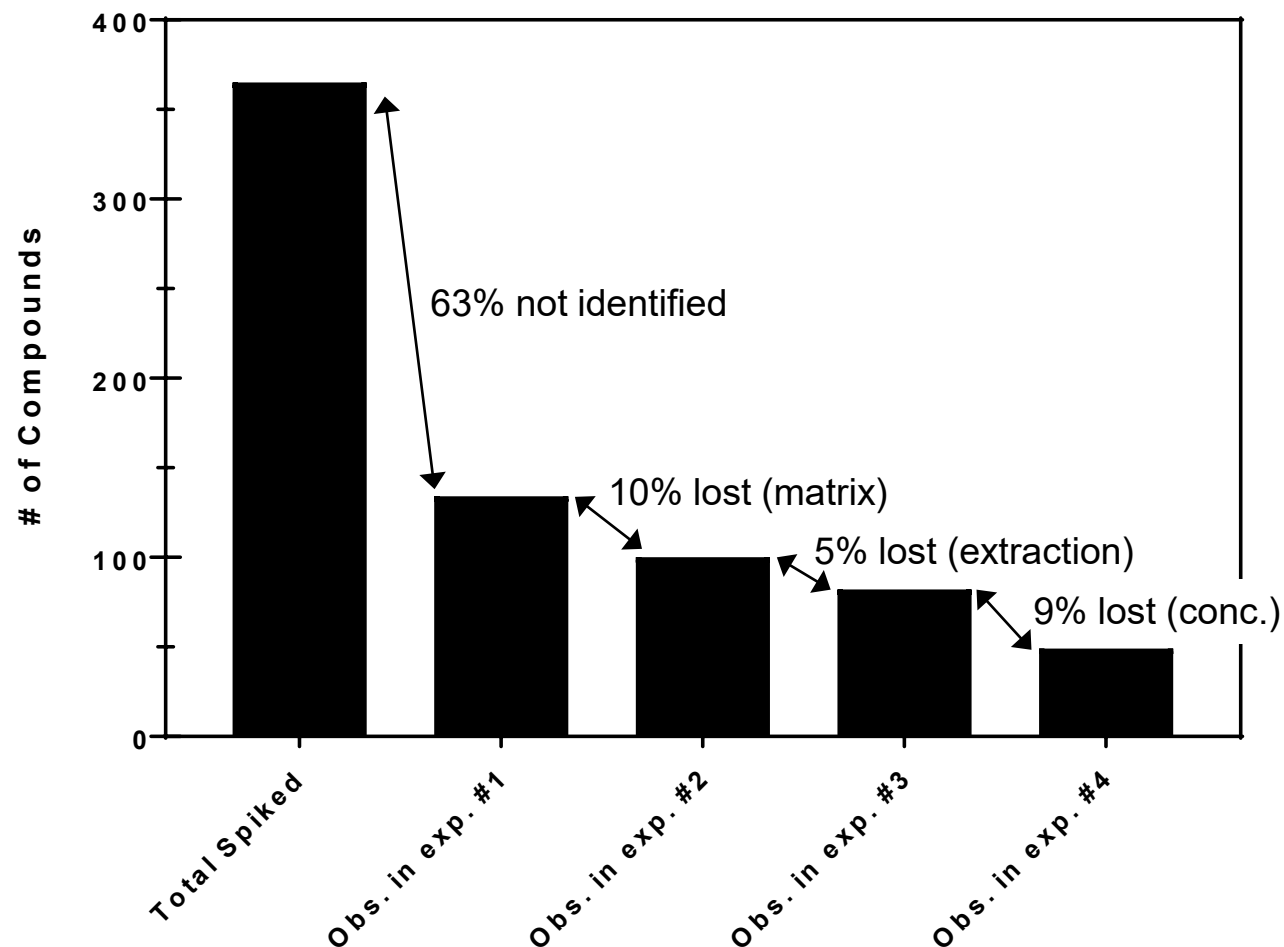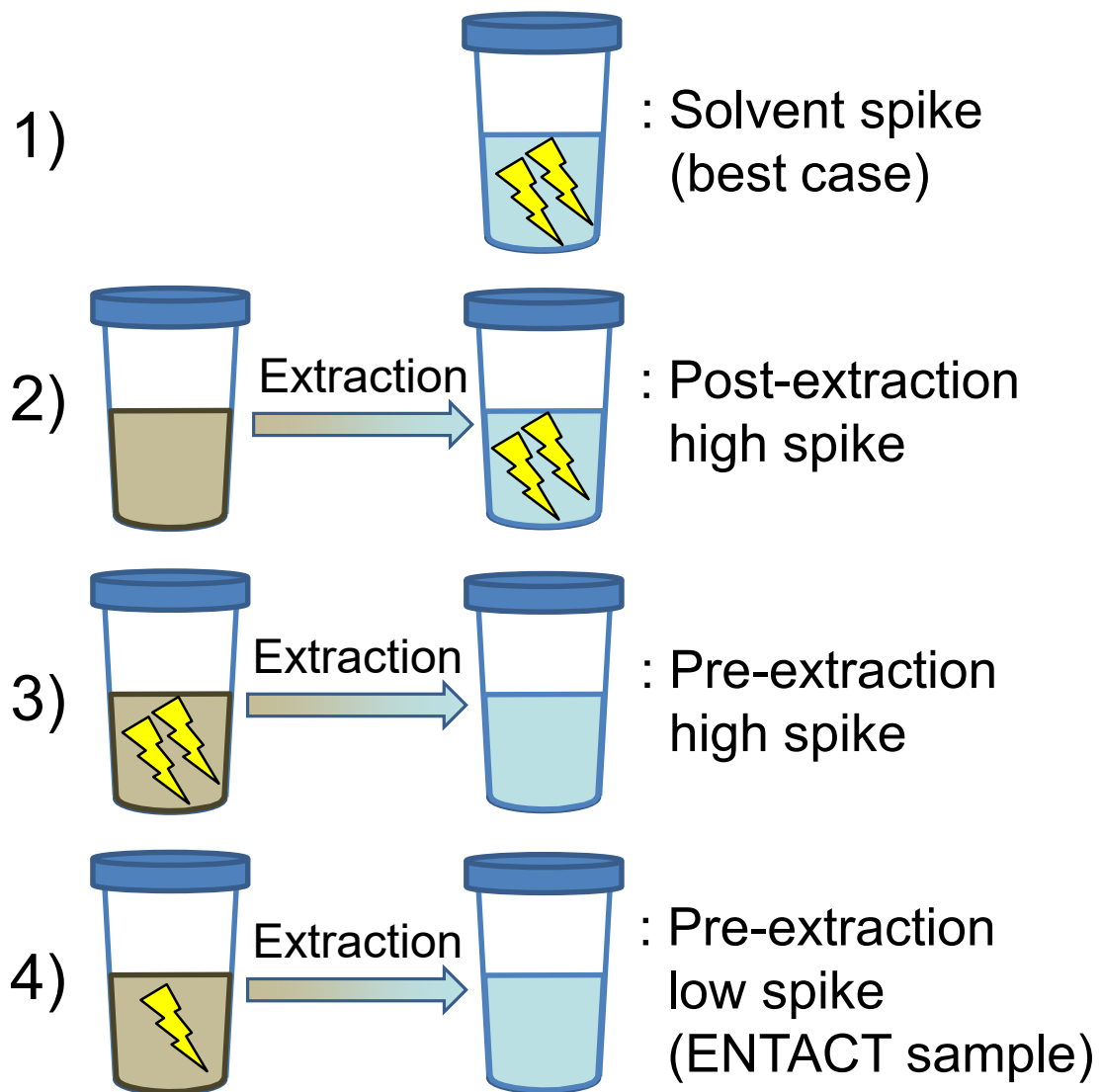Lab Comparison: "Observed" Compounds
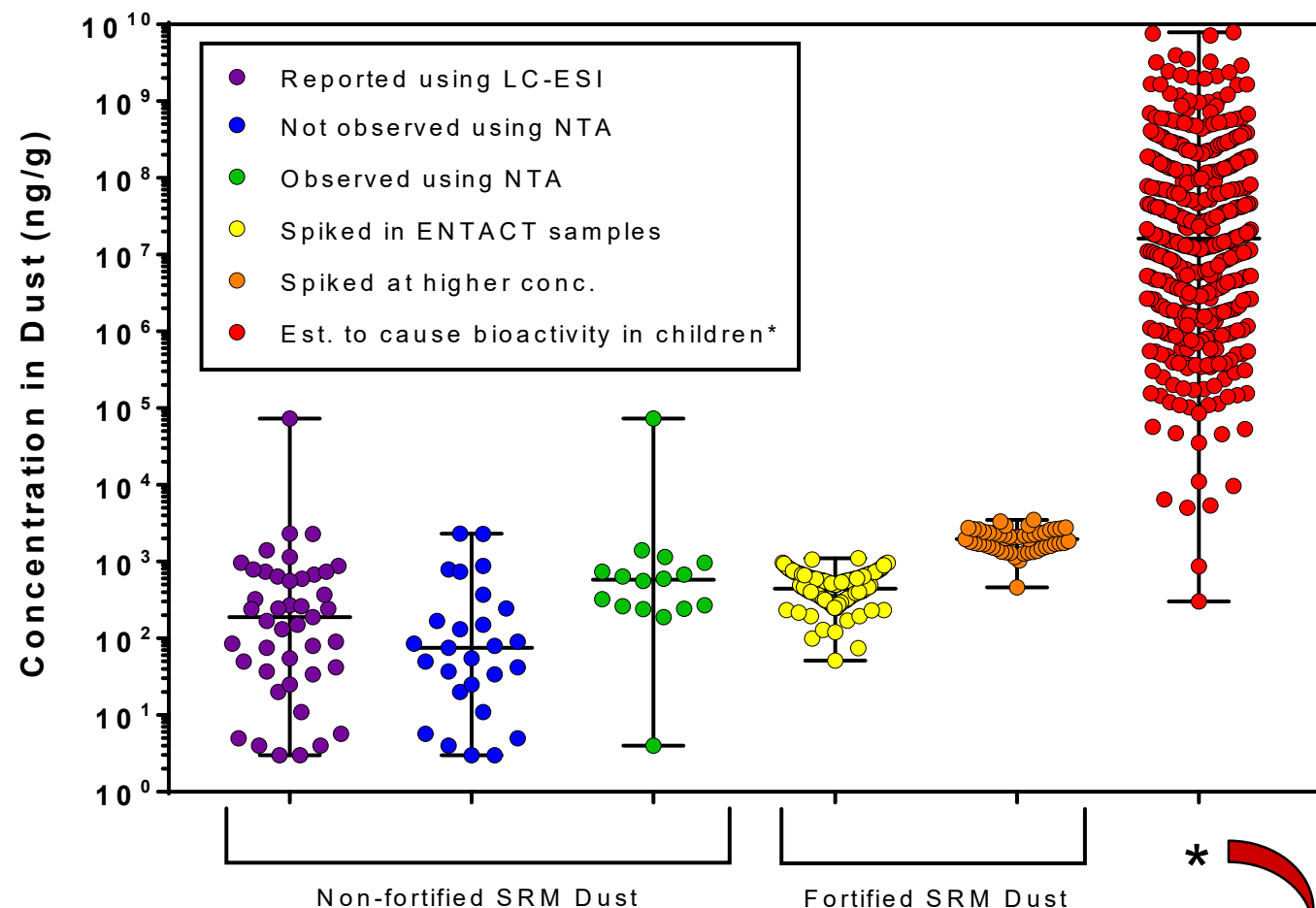
Example Performance Report

Experiments with SRM Dust

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

2585 Organic Contaminants in House Dust

Concentration in Dust (ng/g)

- Reported using LC-ESI
- Not observed using NTA
- Observed using NTA
- Spiked in ENTACT samples
- Spiked at higher conc.
- Est. to cause bioactivity in children*

Non-fortified SRM Dust

Fortified SRM Dust

*

* "..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."
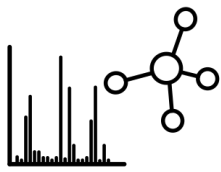
Evaluation of *in silico* Spectra

Summary of ENTACT Findings

- NTA methods are suitable for <u>many</u> ToxCast chemicals
  - ~5% of ENTACT compounds not observed across all methods

- Performance determined across 3 categories:
  - **<u>Coverage</u>** = Ability to Observe → (Range = 21% to 69%)
  - **<u>Precision</u>** = Ability to Identify those Observed → (Range = 7% to 99%)
  - **<u>Reproducibility</u>** = 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

Credit: the Research Triangle Foundation

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Questions?

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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.