Non-Targeted Analysis at the U.S. Environmental Protection Agency

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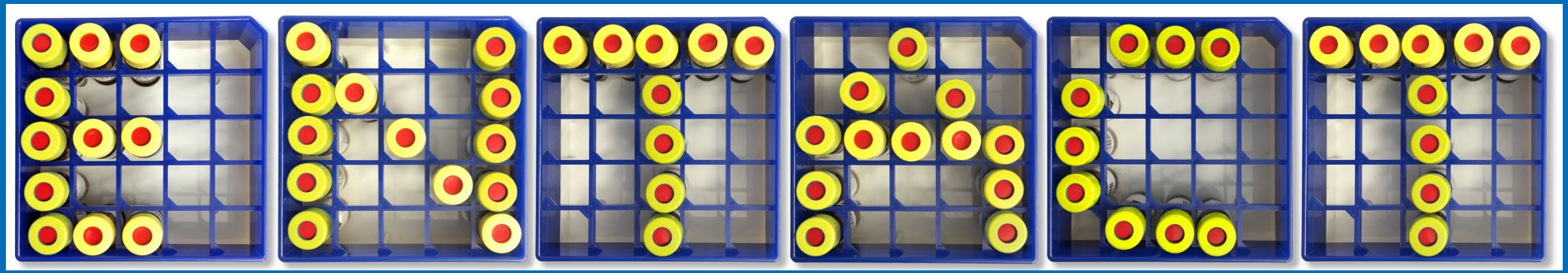

The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.
Dust samples were determined to be environmental in nature and not human subjects research.

EPA NTA Research Contributors

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- **Contracts:** EvoTec, General Dynamics Information Technology

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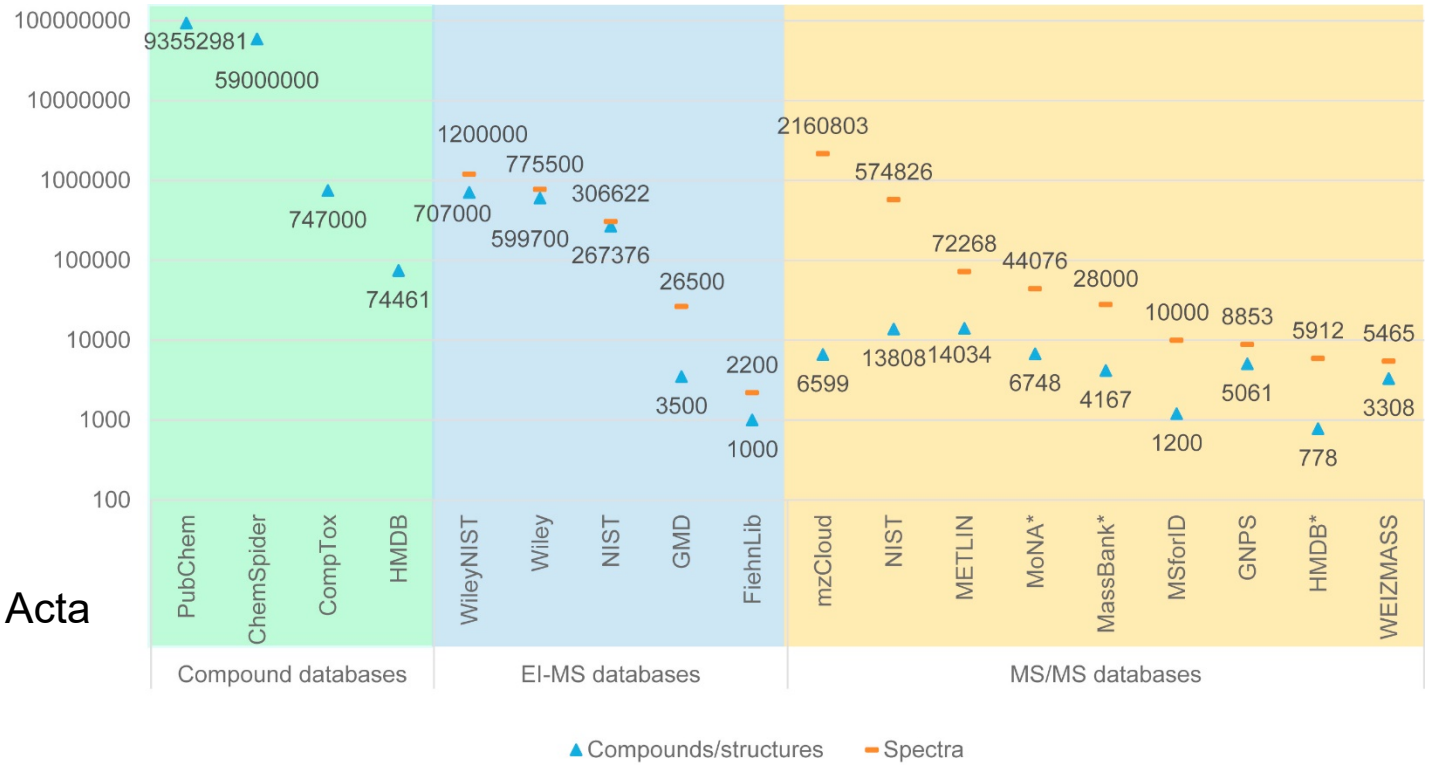
Outline

- **EPA's Non-Targeted Analysis Collaborative Trial (ENTACT)**
  - About ENTACT
  - Initial results
    - Big picture
    - Reverse phase vs. HILIC chromatgraphy
  - APCI vs. ESI
- **NTA Applications and QA at EPA**
- **Benchmarks and Publications for Non-Targeted Analysis**
    - EPA specific ENTACT results
  - CFM-ID for MS/MS fragmentation prediction

EPA's Non-Targeted Analysis Collaborative Trial

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



Peisl BYL, Schymanski EL, Wilmes P (2018) Anal. Chim. Acta 1037:13-27. https://doi.org/10.1016/j.aca.2017.12.034

NTA Critical Needs Identified

- Tightly-defined ring trials to evaluate NTA method performance
- Availability of custom-made spiked samples for ring trials
- Exchange of comprehensive suspect lists to enable interoperability
- Retrospective analysis of data

Resources Provided

- SOPs for sample handling, analysis, data return
- Procedures used for exposure matrix samples
- 16 samples provided; ToxCast/ENTACT well plates and maps
- MS-Ready DSSTox and ToxCast chemical lists; Dashboard; .mol files
- Method and Data templates; FTP site, accounts, instructions
- Mixture/Spike contents after submission of blinded analysis data

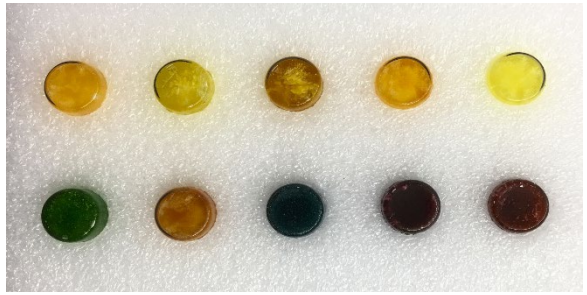
ENTACT Participants

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

ENTACT Sample Overview

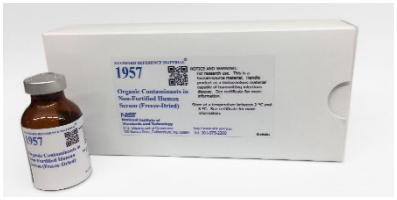
Part 1. Ten ToxCast mixtures

95, 185 or 365 substances/mixture

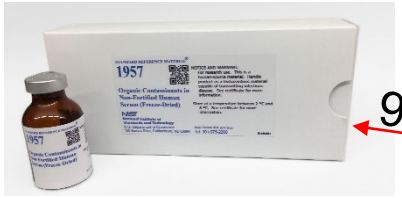


Part 2. Three standard exposure relevant extracts

Unaltered



Fortified



NIST SRM 1957-
Organic Contaminants in Non-fortified Human Serum

Oregon State University-
Outdoor air exposed silicone wrist-bands

NIST SRM 2585-
Organic Contaminants in House Dust

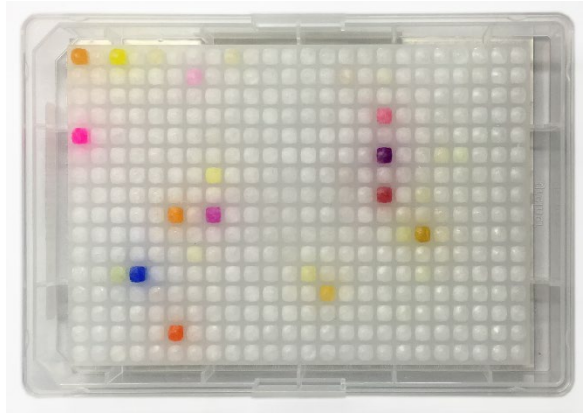
95

185

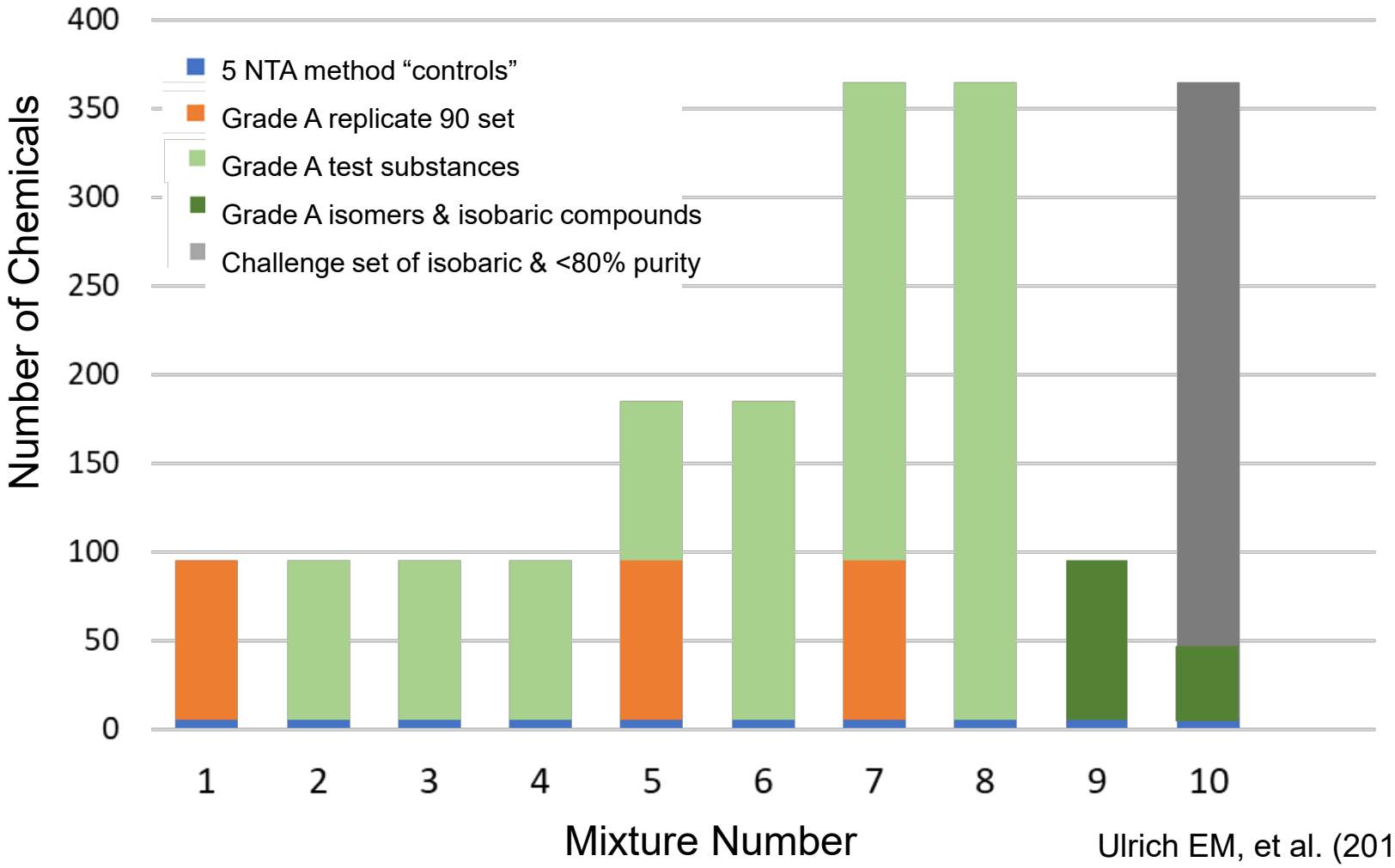
365

Part 3. Individual ToxCast standards

1,269 ENTACT; 4,685 ToxCast all



ENTACT Mixtures- Brainchild of C. Grulke



10 Prepared Mixtures:

1,939 total spiked substances

1,269 unique substances:

1 → spiked 11 times

4 → spiked 10 times

57 → spiked 4 times

33 → spiked 3 times

388 → spiked 2 times

786 → spiked 1 time

Ulrich EM, et al. (2019) *ABC* 411:853-866. doi:10.1007/s00216-018-1435-6

ENTACT Instrument Methods: GC + Other

Lab #	Chromatography	Mobile phase	MS type	MS/MS
1	Agilent GC×GC, Restek Rxi-5ms (30 m × 0.25 mm × 0.25 µm) + Restek Rxi-17Sil MS (0.6 m × 0.25 mm × 0.25 µm)	Helium	Leco HRT+ ToF in EI and CI for confirmation	NA
2	Agilent GC, Agilent J&W VF-5MS (30 m × 0.25 mm × 0.25 µm)	Helium	Agilent Triple Quad in EI	NA
3	Agilent GC, Agilent HP-5ms Ultra Inert (30 m × 0.25 mm × 0.25 µm)	Helium	Agilent ToF in EI	NA
4	Agilent GC×GC, Restek Rtx-5MS (35 m × 0.25 mm × 0.25 µm)+Restek Rxi-17 (0.79 m × 0.1 mm × 0.1 µm)	Helium	Leco Pegasus 4D	NA
9b	Direct infusion	NA	Thermo Velos Pro +21T FT-ICR in ESI +/-	NA

Ulrich EM, et al. (2019) *ABC* 411:853-866. doi:10.1007/s00216-018-1435-6

ENTACT Instrument Methods: LC-ToF

Lab #	Chromatography	Mobile phase	MS type	MS/MS
5	Agilent LC, Agilent Zorbax Eclipse Plus C8 (50 × 2.1 mm, 1.8 µm)	Water, methanol, ammonium formate (AF)	Agilent 6530 QToF, ESI +/-	Data dependent 10, 20, 40 collision
6	Agilent LC, ZORBAX Eclipse Plus C18 (100 × 2.1 mm, 1.8 µm)	Water, methanol, ammonium acetate, acetic acid	Agilent 6530 QToF, ESI +/-	Data dependent 10, 20, 40 collision
7	Agilent LC, Agilent Zorbax RRHD Eclipse Plus C18 (150 × 2.1 mm, 1.8 µm)	Water, acetonitrile, formic acid (FA)	Agilent 6550 QToF, ESI +/-	Data dependent 10, 20, 40 collision
8	Agilent LC, Agilent Zorbax Eclipse Plus C18 (100 × 2.1 mm, 1.8 µm)	Water, methanol, ammonium acetate	Agilent 6550 QToF, ESI +/-	Data dependent 10, 20, 40 collision
9a	Direct infusion	NA	Agilent 6560 QToF, nESI +/- & APPI +/-	NA, but drift tube ion mobility spectrometry
10	Dionex LC, Waters XSelect HSS T3 (150 × 3 mm, 3.5 µm)	Water, acetonitrile, FA	Bruker maXis II TOF, ESI+, Bruker maXis II UHR QToF in ESI+	Data dependent 35 collision energy
11	Waters LC, ACQUITY UPLC BEH C18 (50 × 2.1 mm,1.7 µm)	Water, methanol, AF	Waters Xevo G2-XS QToF in ESI +/-	Data independent, Low = 4, High = ramp 10-45

Ulrich EM, et al. (2019) *ABC* 411:853-866. doi:10.1007/s00216-018-1435-6

ENTACT Instrument Methods: LC-Orbitrap

Lab #	Chromatography	Mobile phase	MS type	MS/MS
12a	Direct infusion	NA	Thermo Orbitrap Elite in nESI +/-	NA
12b	Thermo LC, Thermo Accucore C30 (150 × 2.1 mm, 2.6 µm)	Water, acetonitrile, isopropanol, AF, FA	Thermo Q Exactive in HESI +/-	Data dependent 30, 60 collision
13	Dionex LC, MAC-MOD Analytical ACE Excel C18-PFP (125 × 3 mm, 2 µm)	Water, acetonitrile, FA or ammonium hydroxide	Thermo Q Exactive in ESI +/-, APCI +/-	Data dependent 15, 30, 45 collision
14	Dionex LC, Waters XBridge BEH C18 (50 ×2.1 mm, 3.5 µm)	Water, methanol, FA	Thermo Q Exactive in HESI +/-	Data dependent, 50 collision varied by *m/z*
15	Thermo LC, Thermo Hypersil GOLD aQ C18 Polar Endcapped (100 × 2.1 mm, 1.9 µm)	Water, acetonitrile, FA	Thermo Q Exactive in HESI +/-	Data independent, stepped 30
16	Dionex LC, Waters Atlantis T3 (150 × 3 mm, 3 µm)	Water, methanol, isopropanol, FA	Thermo Q Exactive Plus in ESI +/-	Data Independent, varies 15-120; Dependent 20, 50, 90
17	Waters LC, Thermo Hypersil Gold aQ C18 Polar Endcapped (200 ×2.1 mm, 1.9 µm)	Water, methanol, FA, AF	Thermo Q Exactive Plus in HESI +/-, APCI +/-	NA

ENTACT Initial Results: Mixtures

Actual	499 Mix 1 95	500 Mix 2 95	501 Mix 3 95	502 Mix 4 95	503 Mix 5 185	504 Mix 6 185	505 Mix 7 365	506 Mix 8 365	507 Mix 9 95	508 Mix 10 365
1	128	148	166	187	292	269	318	470	177	410
2	142	154	102	129	250	242	401	399	105	452
3	48	40	48	59	110	101	97	130	37	109
4	72	71	63	70	136	125	273	313	49	265
5	301	130	375	341	408	404	719	687	198	327
6	65	66	74	72	105	118	193	215	54	162
7	587	552	596	554	798	846	1327	1274	509	1176
8	93	114	116	106	182	201	360	374	73	330
9	337	372	303	365	321	363	466	505	510	463
10	135	130	125	154	188	195	284	295	100	153
11	70	57	64	66	105	115	176	125	35	159
12a	595	486	571	630	746	669	899	910	588	792
12b	66	170	51	41	272	116	214	101	163	404
13	51	37	35	39	74	59	124	109	42	105
14	137	65	45	74	68	234	413	408	120	317
15	215	249	212	249	207	275	245	254	140	253
16	1298	1258	1304	1209	1651	1641	2520	2538	1202	2193
17	153	217	221	199	254	321	523	651	496	396

Reported vs Actual

<75%

>75 to <125%

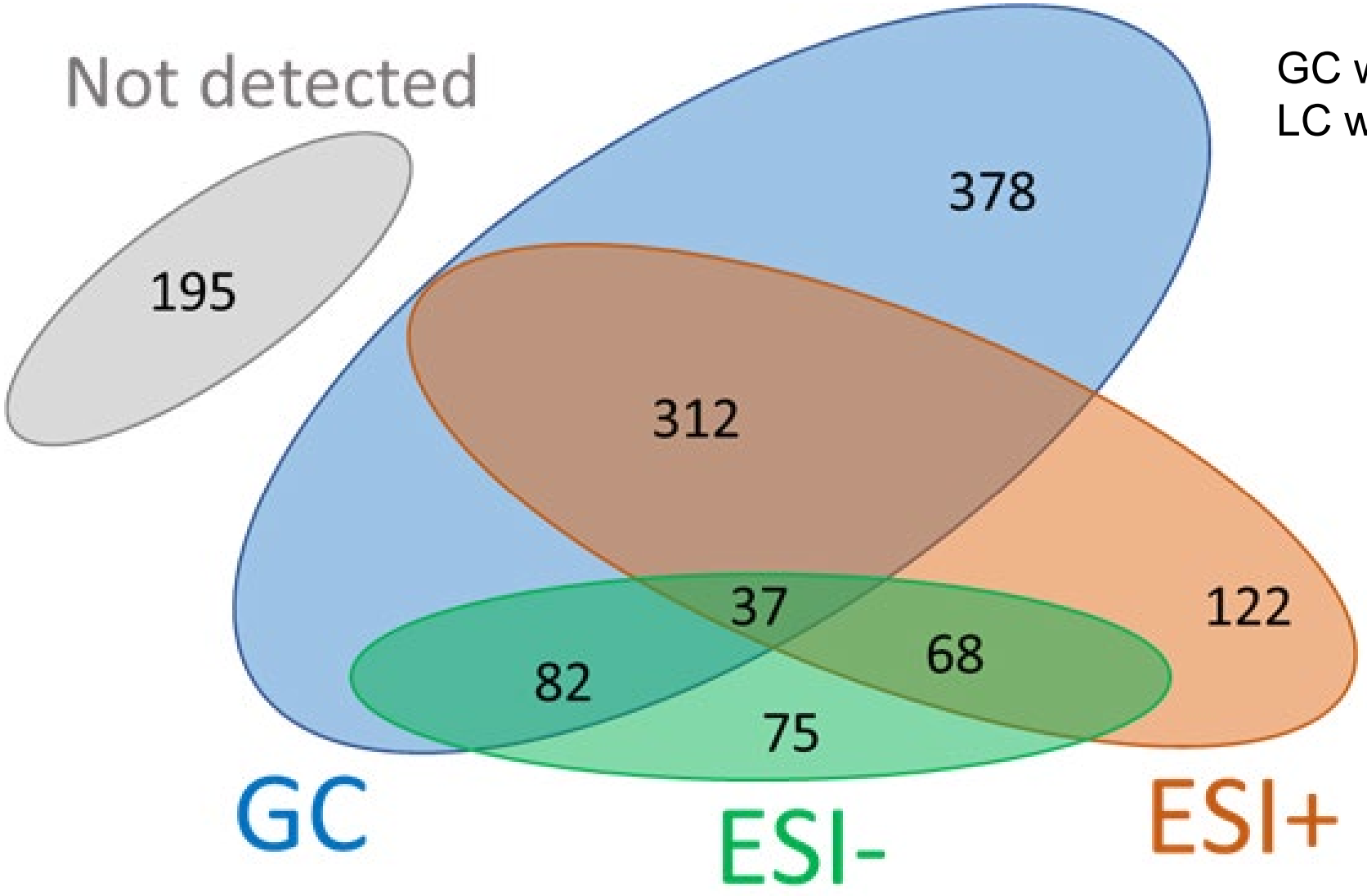
>125%

59/180

34/180

87/180

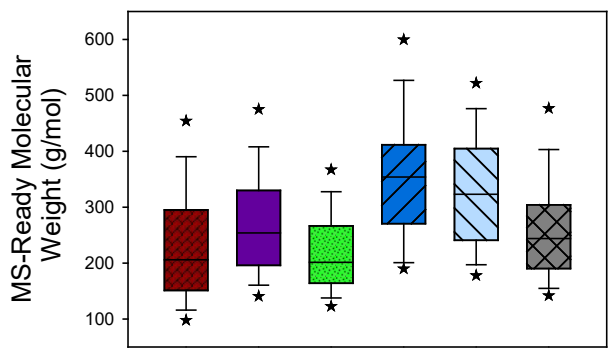
ENTACT Initial Results: Method Coverage

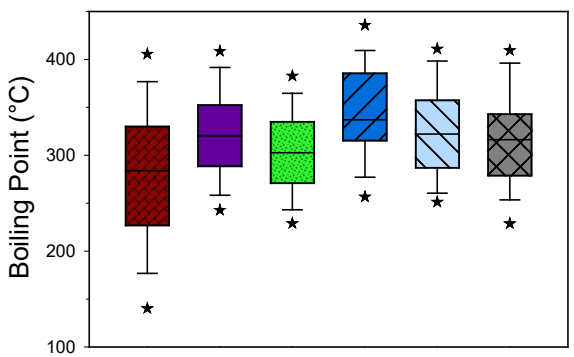


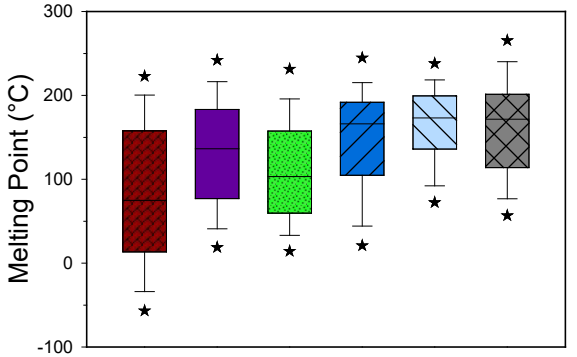
GC with electron impact
LC with electrospray +/-

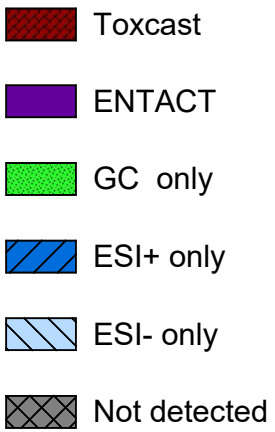
Ulrich EM, et al. (2019) *ABC* 411:853-866. doi:10.1007/s00216-018-1435-6

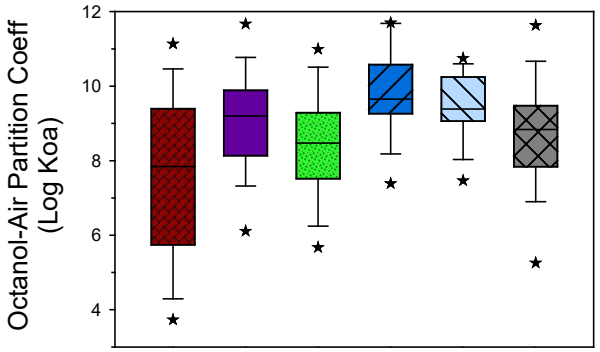
ENTACT Initial Results: Chemical Space

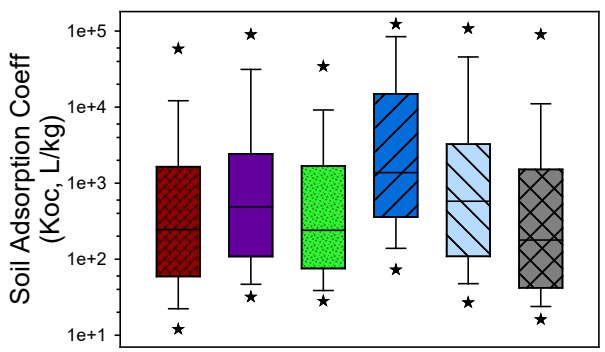


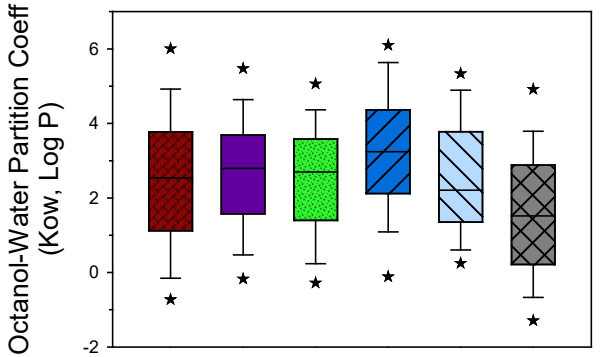


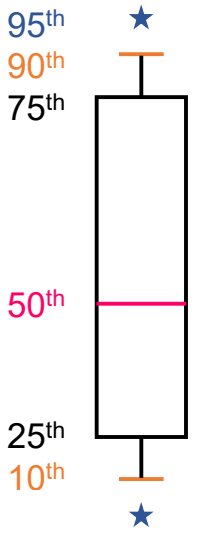


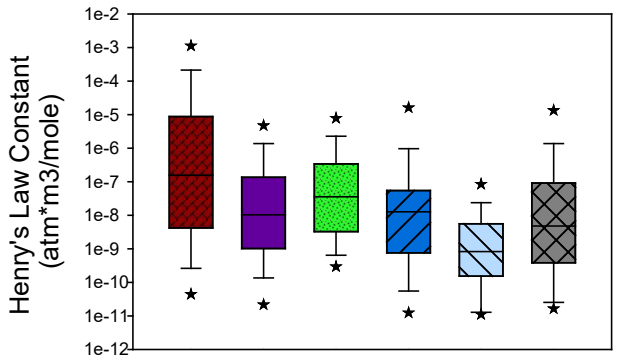


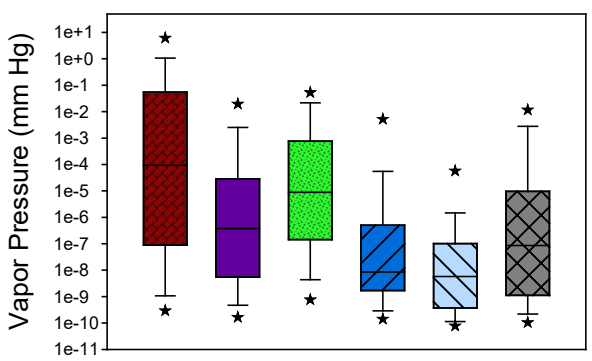


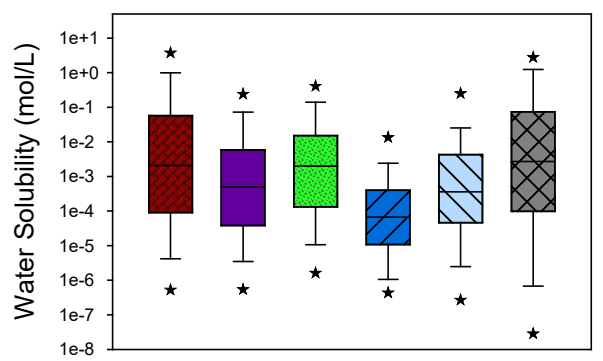












What other questions can be answered with ENTACT data?
What models can be built with ENTACT data?

- What percentage of standard mixture chemicals are correctly identified?
- Does the complexity of the mixture/matrix impact performance?
- Which methods perform better overall? For specific chemical classes?
- Can retention behavior be modeled for multiple conditions?
- What chemical space is being covered by each method? Overlap? Predictive models?
- What can be done to expand coverage?
  - Sensitivity
  - Suspect list
  - Physicochemical parameters
  - Matrix effects
- What unintended components or by-products are in standard mixtures?
  - Impurities
  - Reaction products
  - Degradation products
- In environmental samples, what chemicals do methods agree are present? Does this agree with SRM reported data? Is this predictable?
- How can these data be used to refine exposure estimates?
- Can we use these data to develop higher throughput semi-quantitative methods?

ENTACT RP vs. HILIC Comparison

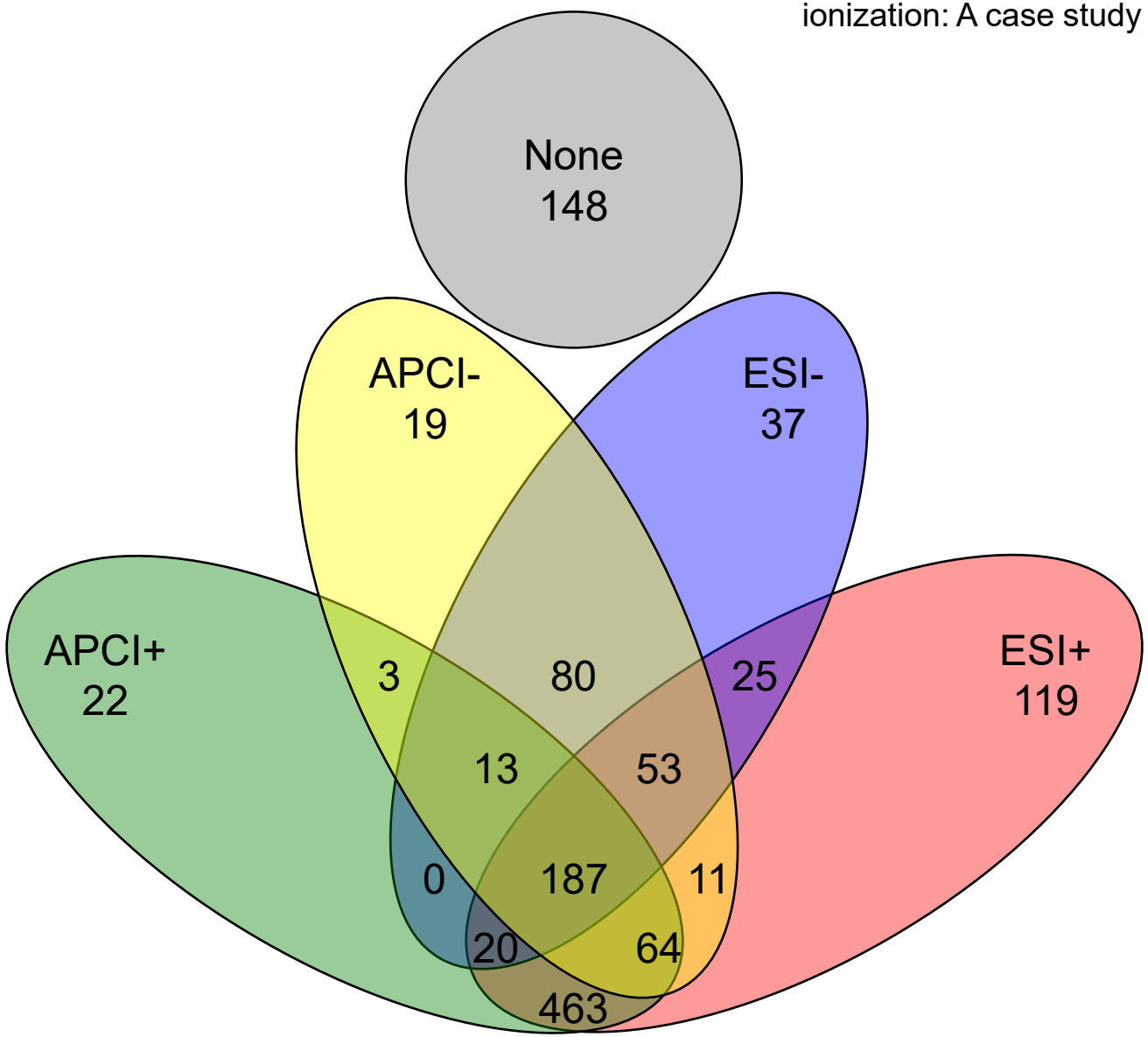
Mixture 505 substance	*Log Kow	RP		HILIC		% signal enhancement
		t_r	peak area	t_r	peak area	
5-Amino-2-methylbenzenesulfonic acid	-1.66	1.32	1.80×10^5	3.90	2.08×10^6	1160
Citric acid	-1.66	ND	ND	17.60	2.75×10^5	NA
3-carboxy-5-sulfobenzoic acid	-0.49	0.97	6.96×10^4	17.10	2.09×10^4	30.0
Panthenol	-1.29	1.10	5.30×10^4	3.94	1.95×10^5	367
Sodium 3-chloro-2-hydroxypropane-1-sulfonic acid	-0.77	0.78	2.57×10^5	4.63	9.77×10^5	380.
6-hydroxynaphthalene-2-sulfonic acid	-0.24	1.32	2.26×10^5	3.95	7.52×10^5	333
4-Ethoxy-4-oxobut-2-enoic acid	-0.047	4.28	8.12×10^4	4.50	1.04×10^6	1280
4-Methylbenzenesulfonic acid	-0.007	1.90	4.77×10^5	3.89	2.94×10^6	617
Amiloride hydrochloride	-0.051	1.15	2.53×10^6	3.16	4.70×10^7	1860
Famotidine	-0.40	1.10	1.29×10^6	3.37	2.69×10^7	2090
Phenformin hydrochloride	-0.30	2.69	8.69×10^6	2.36	6.81×10^7	783
Dodecyltrimethylammonium chloride	-0.26	9.84	1.04×10^7	1.62	1.07×10^8	1030
Acetazolamide	-0.24	1.81	1.02×10^5	ND	ND	NA
Butoxycarboxim	-0.12	2.13	6.93×10^5	1.37	4.53×10^6	655
1,1,3-Trimethyl-2-thiourea	-0.018	1.45	1.31×10^6	ND	ND	NA

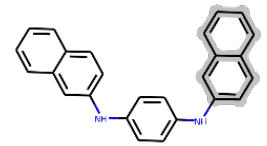
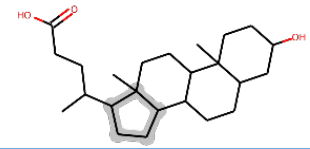
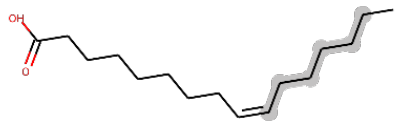
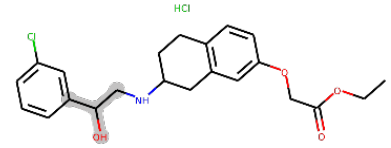
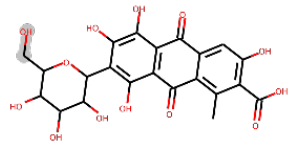
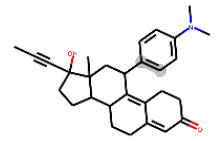
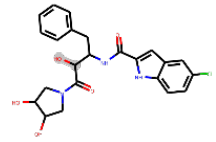
*OPERA Predicted ND: no peak detected NA: not applicable

Singh, R. R. et al., “Exploration of HILIC’s capability to capture polar chemical space in exposomic matrices,” in preparation.

ENTACT LC Ionization Comparison

Singh, R.R. et al., “Expanded coverage of NT-LC-HRMS using atmospheric pressure chemical ionization: A case study with ENTACT mixtures,” submitted, ABC.



ToxPrint Substructure	Odds ratio	Structure with ToxPrint
ring:fused_[6_6]_naphthalene (APCI)	7.607	
chain:alkaneCyclic_pentyl_C5	∞	
chain:alkaneLinear_hexyl_C6 and _octyl_C8	∞	
bond:COH_alcohol_sec-alkyl	8.543	
bond:COH_alcohol_pri-alkyl	6.891	
chain:alkaneCyclic_ethyl_C2_ (connect_noZ)	6.891	
bond:COH_alcohol_generic	3.256	

ENTACT Summary and Future Work

- # features in mixtures >> intentionally added substances
- 195 substances not detected by GC or LC-ESI methods, 37 detected by all
- HILIC improves retention time and signal for negative log Kow compounds
- 148 substances not detected by LC- ESI or APCI
- ToxPrints help predict ionization mode success

- Added GC-Orbitrap and GC-QTOF to cover more volatile chemical space
- Cross laboratory comparison coming soon
- Extraordinary data mining possibilities

NTA Applications at EPA

- **Exposure surveillance**
  - What chemicals are in food, 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?

Exposomics Experimental Design

Name	Example	Purpose
Tracers	Isotopically labeled standards: $^{13}C_3$-Atrazine, D_3-Thiamethoxam, $^{13}C_4$,$^{15}N_2$-Fipronil	Allows tracking of chromatographic performance and mass accuracy
Replication	Triplicate injections of same sample vial	Removes risk of “one hit wonder”
Run order randomization	8, 3, 7, 4, 2, 1, 10, 5, 8, 6, 9, 2, 5, 4, 1, 9, 4, 7, 3, 8, 1, 6, 10, 9, 6, 7, 5, 3, 2, 10	Minimizes/averages out batch or sample order effects (e.g., carryover, temp & instrument drift)
Pooled QC sample	Combine 5 mg/µL from each of 10 samples (total 50 mg/µL) prior to extract to create pooled QC	Separate confirmation of presence with different matrix, MS2 IDs
Blanks	Solvent, method, matrix, double blanks	Allows identification/subtraction/deletion of interferences introduced in lab processes
Multiple lines of evidence for ID	Retention time prediction/matching, Spectral library/prediction matching, Data source ranking, Functional/product uses, Media occurrence	Improves confidence in identification when chemicals standards are unavailable

Benchmarking and Publications for Non-targeted Analysis (BP4NTA)

A diverse working group consisting of representatives from all sectors dedicated to improving NTA through benchmark methods and performance, standardized definitions and publication standards.

Contact Ben Place (benjamin.place@nist.gov) or Elin Ulrich (ulrich.elin@epa.gov) for more information

About BP4NTA

- **Basics**

  Started at ENTACT workshop in 2018; now 77 members: 30 government, 25 industry, 22 academia
  - Monthly conference calls or in-person meetings
  - Collaborative documentation/discussions via Google Drive
  - 9 working groups for definitions

- **Short term goals**

  1. Publish a white paper containing:
     - NTA terms and their definitions;
     - Calculations of performance metrics;
     - Reporting recommendations to promote transparency/reproducibility;
     - Scientific best practices.
  2. Build consensus with like-minded organizations (e.g., NORMAN).
  3. Work with journal editors and NTA researchers to establish guidelines NTA reporting for methods and performance evaluations.

- **Long term goal (~10 yrs)**

  Move the field of NTA toward proficiency testing, using a mechanism like ASTM/ISO Guidance on Performance and Data Reporting Requirements. Define proficiency levels for SSA, NTA, expert, competent, etc.

Chemical Surveillance in 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*[,∥]

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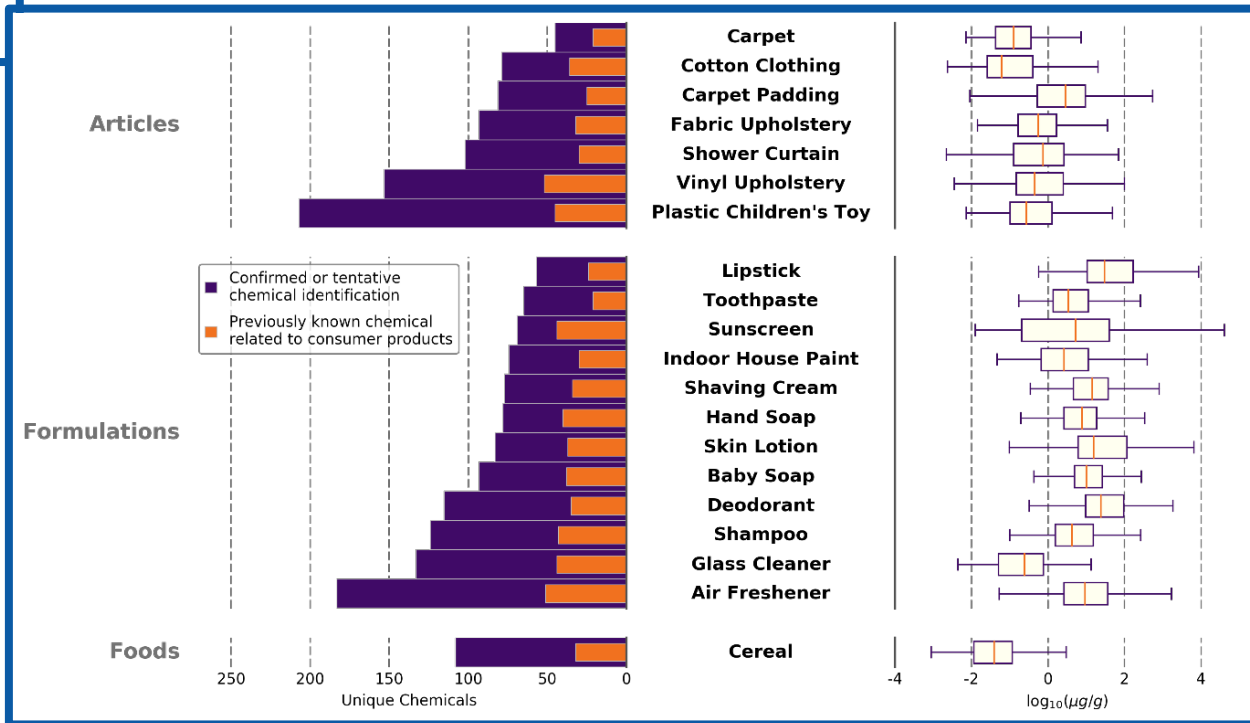
19% of chemicals identified by NTA are on consumer product chemical lists



Identify/Prioritize CECs in 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]

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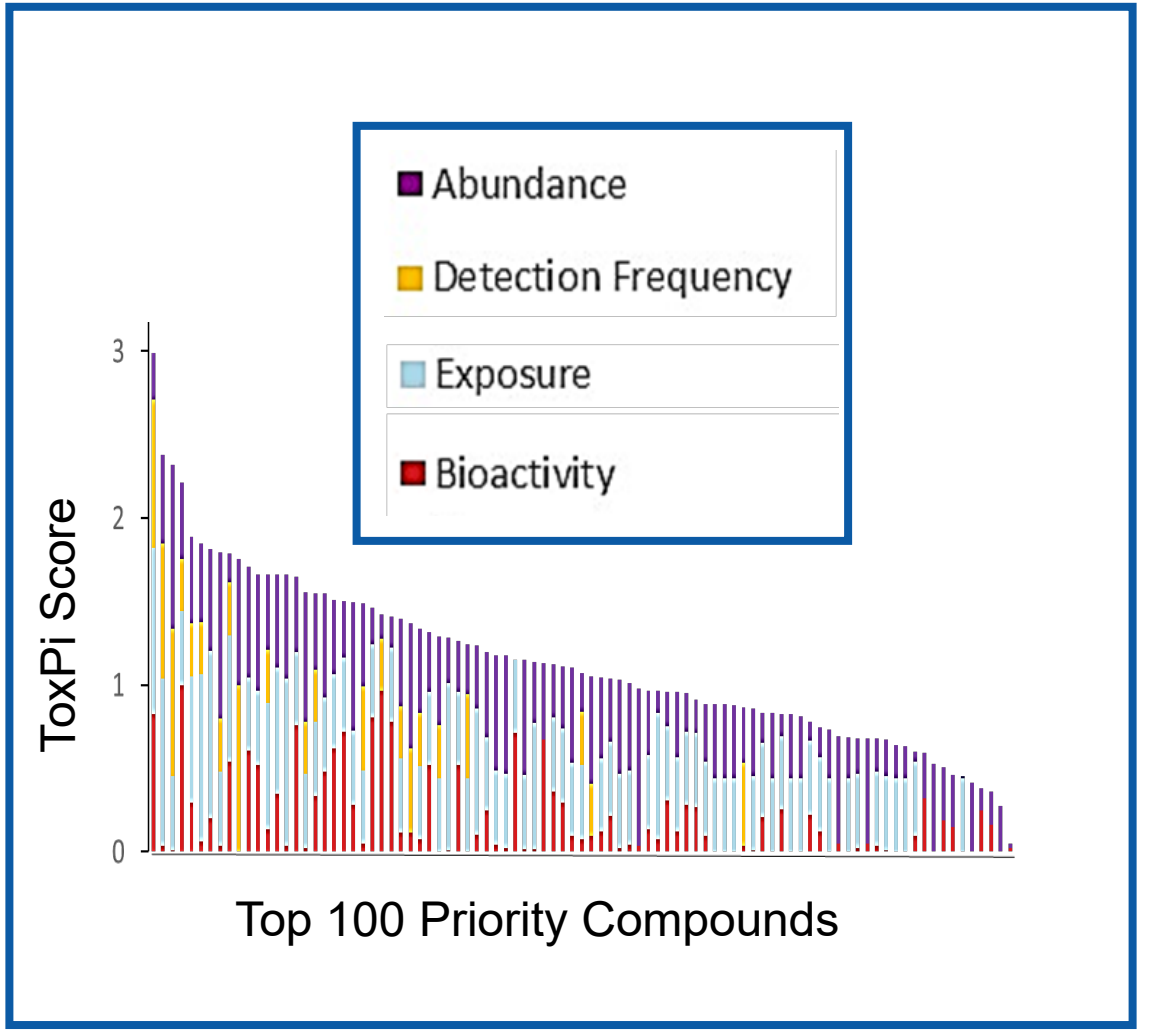
[c] United States Environmental Protection Agency, National Center for Computational Toxicology, Research Triangle Park, NC 27709, United States



Chemical Forensics for Content of Products

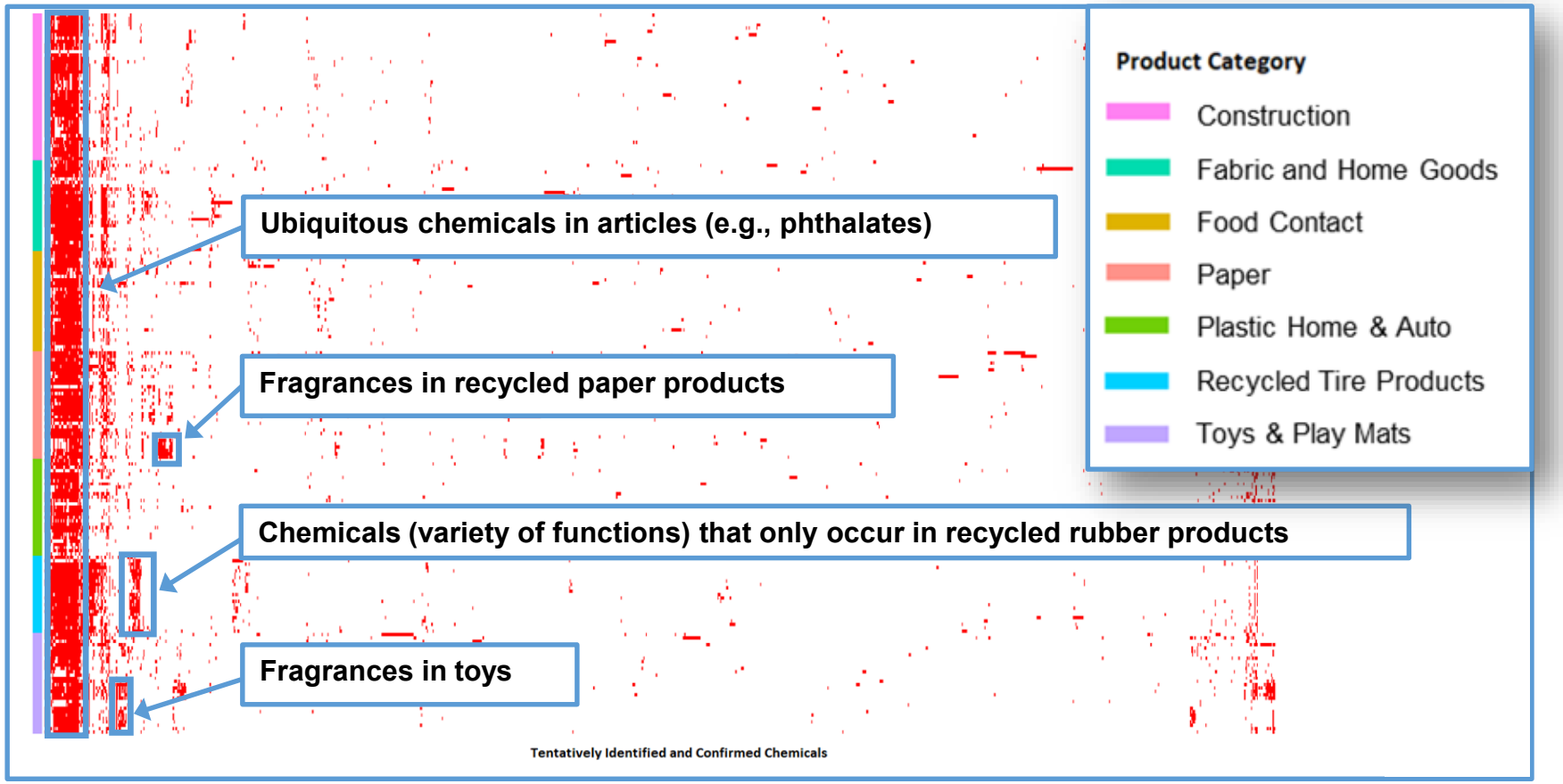


Figure by C. Lowe and K. Isaacs

Biomarker and Metabolite Monitoring

Journal of Pharmacological and Toxicological Methods 92 (2018) 77–94

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

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

Research article

mRNA transfection retrofits cell-based assays with xenobiotic metabolism

Danica E. DeGroot[a], Adam Swank[b], Russell S. Thomas[a], Mark Strynar[c], Mi-Young Lee[d], Paul L. Carmichael[d], Steven O. Simmons[a,*]

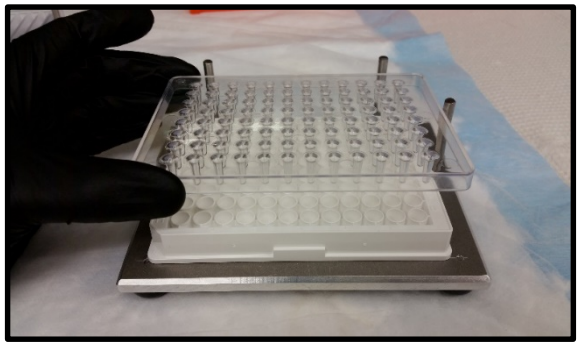
Environmental Science & Technology

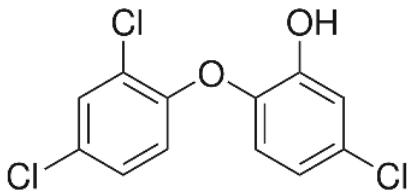
Article

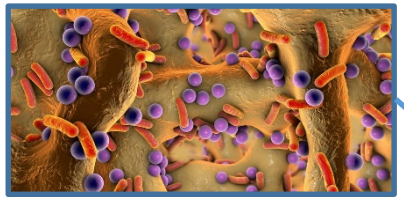
pubs.acs.org/est

Identification of Biomarkers of Exposure to FTOHs and PAPs in Humans Using a Targeted and Nontargeted Analysis Approach

Sonia Dagnino,[*,†,§] Mark J. Strynar,[*,†] Rebecca L. McMahen,[†,§] Christopher S. Lau,[‡] Carol Ball,[∥] Stavros Garantziotis,[⊥] Thomas F. Webster,[#] Michael D. McClean,[#] and Andrew B. Lindstrom[†]







Microbe-free

PK Differences

PD Differences