# Genesis and Study Design for EPA's Non-Targeted Analysis Collaborative Trial (ENTACT)

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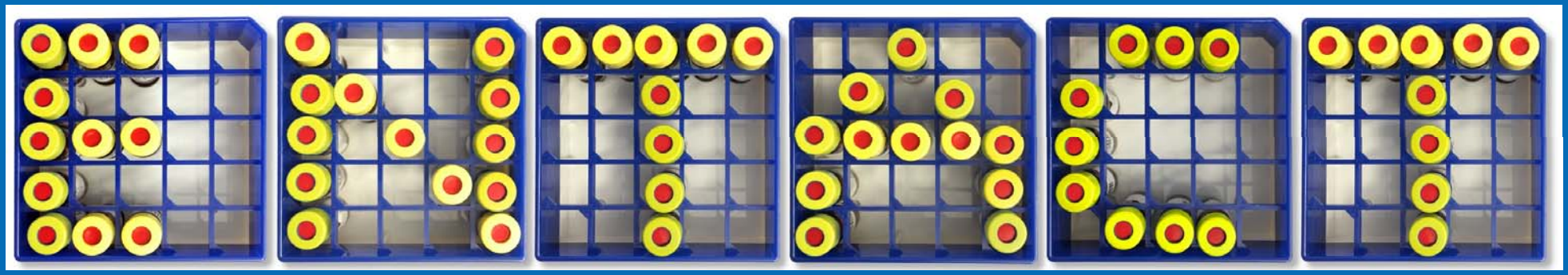

# Drivers for Exposure Science

## 1) Understanding causes of disease

*"…70-90% of disease risks are probably due to differences in environments"*

EPIDEMIOLOGY

**Environment and Disease Risks**

A new paradigm is needed to assess how a lifetime of exposure to environmental factors affects the risk of developing chronic diseases.

Stephen M. Rappaport and Martyn T. Smith

Although the risks of developing chronic diseases are attributed to both genetic and environmental factors, 70 to 90% of disease risks are probably due to differences in environments (*1*–*3*). Yet, epidemiologists increasingly use genome-wide association studies (GWAS) to investigate diseases, while relying on questionnaires to characterize "environmental exposures." This is because GWAS represent the only approach for exploring the totality of any risk factor (genes, in this case) associated with disease prevalence. Moreover, the value of costly genetic information is diminished when inaccurate and imprecise environmental data lead to biased inferences regarding gene-environment interactions (*4*). A more comprehensive and quantitative view of environmental exposure is needed if epidemiologists are to discover the major causes of chronic diseases.

An obstacle to identifying the most important environmental exposures is the fragmentation of epidemiological research along lines defined by different factors. When epidemiologists investigate environmental risks, they tend to concentrate on a particular category of exposures involving air and water pollution, occupation, diet and obesity, stress and behavior, or types of infection. This slicing of the disease pie along parochial lines leads to scientific separation and confuses the definition of "environmental exposures." In fact, all of these exposure categories can contribute to chronic diseases and should be investigated collectively rather than separately.

To develop a more cohesive view of environmental exposure, it is important to recognize that toxic effects are mediated through chemicals that alter critical molecules, cells, and physiological processes inside the body. Thus, it would be reasonable to consider the "environment" as the body's internal chemical environment and "exposures" as the amounts of biologically active chemicals in this internal environment. Under this view, exposures are not restricted to chemicals (toxicants) entering the body from air, water, or food, for example, but also include chemicals produced by inflammation, oxidative stress, lipid peroxidation, infections, gut flora, and other natural processes (*5*, *6*) (see the figure). This internal chemical environment continually fluctuates during life due to changes in external and internal sources, aging, infections, life-style, stress, psychosocial factors, and preexisting diseases.

The term "exposome" refers to the totality of environmental exposures from conception onwards, and has been proposed to be a

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## 2) Ensuring chemical safety and human/ecological health

GIVE A DOG A PHONE
Technology for our furry friends

NewScientist

WEEKLY November 29–December 5, 2014

We've made 150,000 new chemicals

We touch them, we wear them, we eat them

But which ones should we worry about?

SPECIAL REPORT, page 34

THE GOOD FIGHT
Most violence is also virtuous

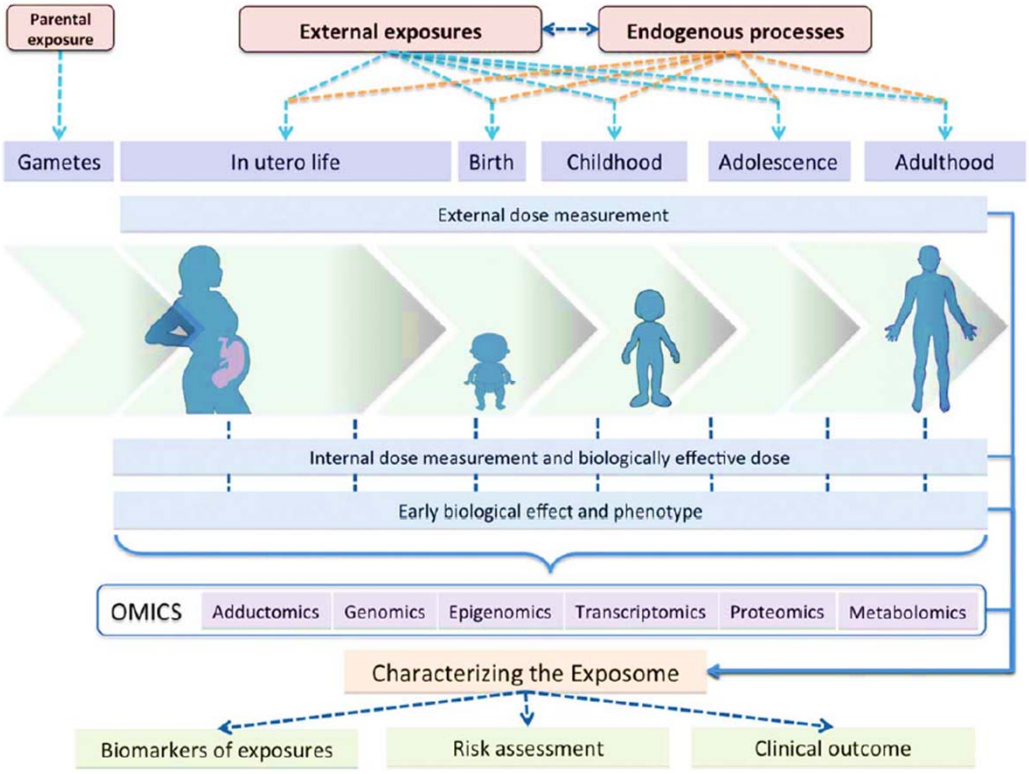
CHAMBER OF SECRETS
The greatest ever find of early human bones

IS IT ALIVE?
Artificial worm could be first digital animal

Content from J. Sobus

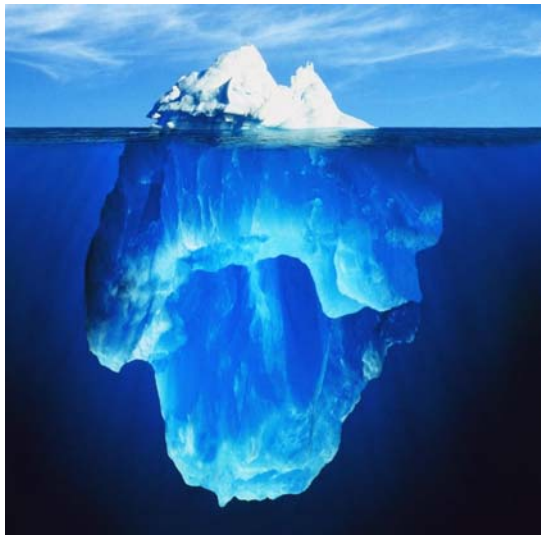
# The Exposome

- First defined by Wild in 2005, the exposome includes chemical and non-chemical stressors, from both internal and external sources across all life-stages
- The exposome is highly variable across time, location, individuals, activities, etc. making measurements difficult
- Exposure categories include:
  - Internal- metabolism, hormones, inflammation
  - Specific external- chemicals, radiation, diet
  - General external- climate, stress, education
- Bottom-up strategy measures all chemicals in sources over time
- Top-down strategy measures all chemicals (transformation products or indicators) in blood




CP Wild, A Scalbert, and Z Herceg, Environ. Mol. Mutagen. 54:480-499, 2013.

# What is Non-Targeted Analysis?



- **Targeted Analysis- the "known knowns"**
  - Covers <<1% of the exposome
  - Can't solve 21st century public health problems blinded to >99% of exposure data
- **Suspect Screening Analysis (SSA)- the "known unknowns"**
  - Covers ~5-10% of the exposome
  - Need rapid, efficient methods capable of measuring poorly studied compounds
- **Non-Targeted Analysis (NTA)-the "unknown unknowns"**
  - Covers 90-95% of the exposome
  - Need ways to characterize compounds that aren't yet known to exist

# Examples of EPA's NTA Applications

- **Exposure surveillance**
  - What chemicals are in food, products, dust, blood, etc.?


- **Chemical prioritization**
  - What are relevant chemicals & mixtures?


- **Exposure forensics**
  - What are chemical signatures of exposure sources?

- **Effect-directed analysis**
  - What bioactive chemicals are in complex mixtures?

- **Biomarker discovery**
  - What chemicals are predictive of health impairment?

# Introduction to ENTACT

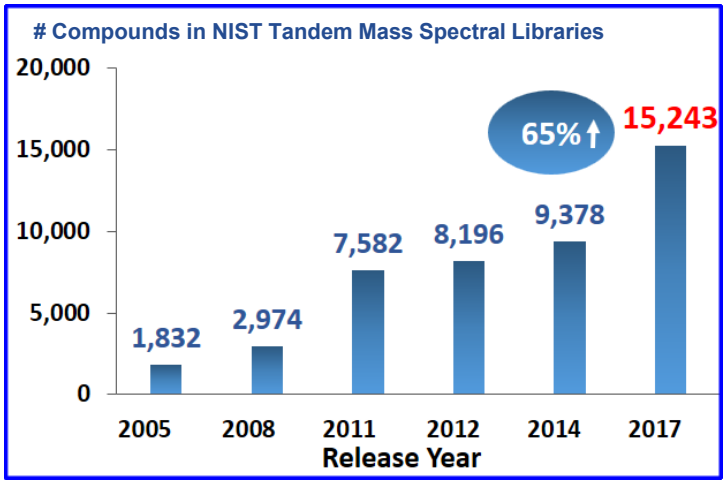
**Background:**

- 2015 workshop at EPA to discuss the state of the science for suspect screening analysis (SSA) and non-targeted analysis (NTA) in the exposure field
- Approximately 200 people attended, ~140 in person, ~60 by webinar
- Sessions on Research and Regulatory Drivers, NTA in environmental media, biological media, emerging techniques, and databases/informatics tools with 11 platform and 15 posters presented on NTA research
- Half-day discussion on how to use EPA's resources from ToxCast– genesis of ENTACT


# ENTACT SSA and NTA 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
- Develop reporting standards for studies using SSA and NTA methods
- Increase compounds/spectra available in reference libraries (with participants and publically available)
- And so much more…

| | October 2017 | June 2018 |
|---|---|---|
| Compounds | 6,810 | 8,081 |
| Spectra | 2,379,547 | 2,763,141 |
| Trees | 10,658 | 12,243 |
| QM Models | 706,758 | 707,074 |




http://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:msms

# 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

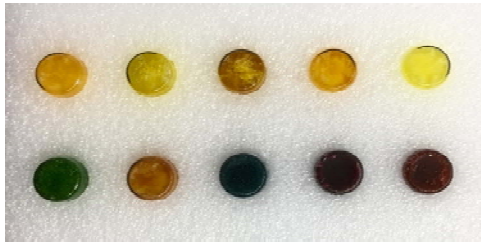
# Resources Provided

- 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 Sample Overview

## Part 1. Ten ToxCast mixtures

95, 185 or 365 substances/mixture



## Part 2. Three standardized exposure relevant extracts

Unaltered

Fortified

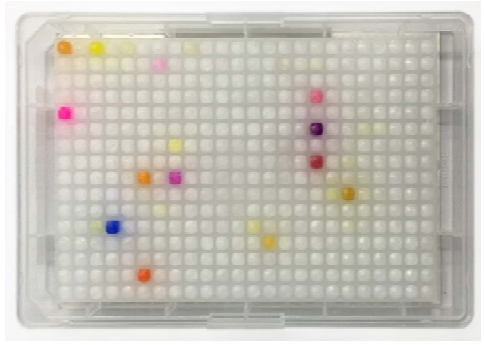
NIST SRM 1957-
Organic Contaminants in Non-fortified Human Serum

Oregon State University-
Outdoor air exposed silicone wrist-bands

NIST SRM 2585-
Organic Contaminants in House Dust

## Part 3. Individual ToxCast standards

1,269 ENTACT; 4,685 ToxCast all

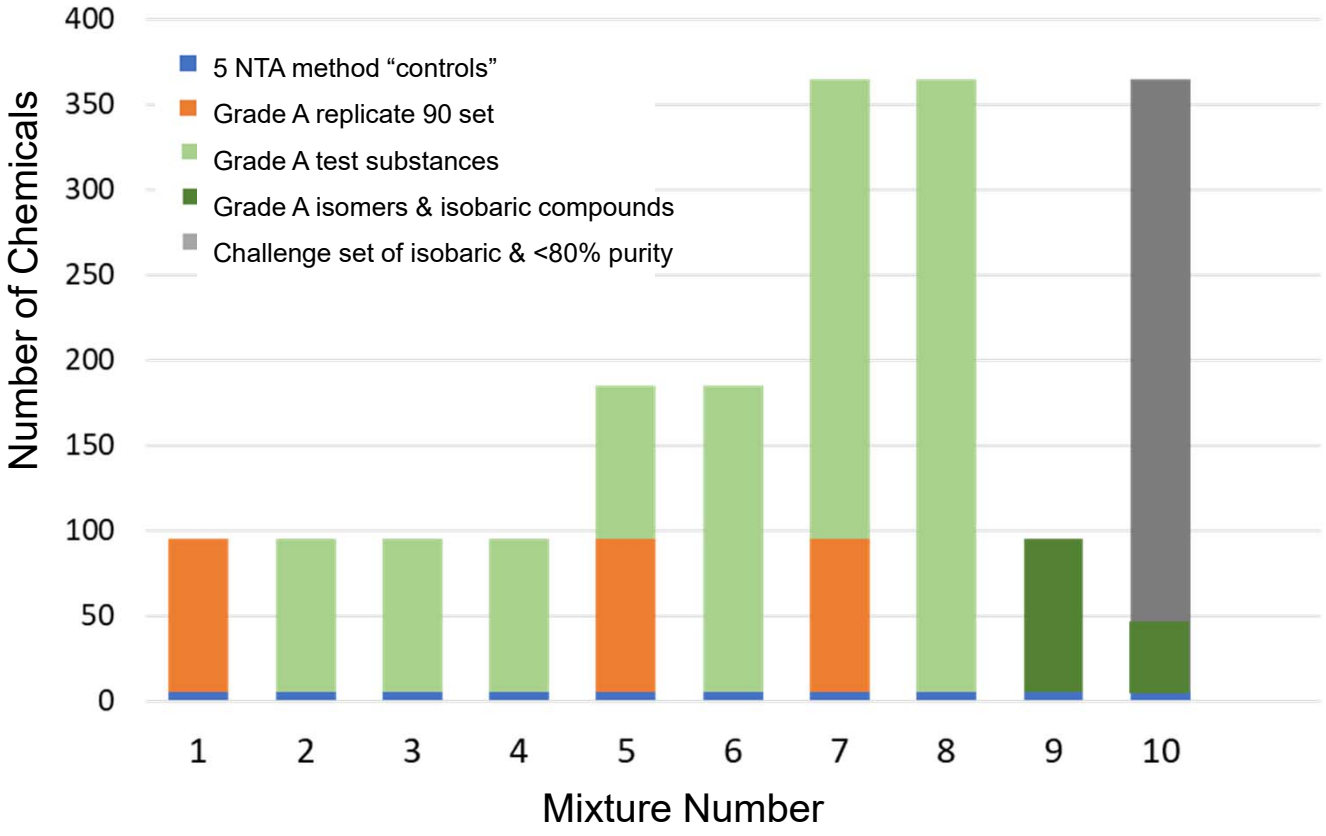


95

185

365

# 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

# ENTACT Participants

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




























# 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 |
| 7b | Direct infusion | NA | Thermo Velos Pro +21T FT-ICR in ESI +/- | NA |

# ENTACT Instrument Methods: LC-ToF

| Lab # | Chromatography | Mobile phase | MS type | MS/MS |
|---|---|---|---|---|
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7a | Direct infusion | NA | Agilent 6560 QToF, nESI +/- & APPI +/- | NA, but drift tube ion mobility spectrometry |
| 8 | 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 |
| 9 | 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 |

# ENTACT Instrument Methods: LC-Orbitrap

| Lab # | Chromatography | Mobile phase | MS type | MS/MS |
|---|---|---|---|---|
| 10a | Direct infusion | NA | Thermo Orbitrap Elite in nESI +/- | NA, but drift tube ion mobility spectrometry |
| 10b | 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 |
| 11 | 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 |
| 12 | 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* |
| 13 | 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 |
| 14 | Dionex LC, Waters Atlantis T3 (150 × 3mm, 3 µm) | Water, methanol, isopropanol, FA | Thermo Q Exactive Plus in ESI +/- | Data Independent, varies 15-120; Dependent 20, 50, 90 |
| 15 | Waters LC, Thermo Hypersil Gold aQ C18 Polar Endcapped (200 ×2.1mm, 1.9µm) | Water, methanol, FA, AF | Thermo Q Exactive Plus in HESI +/-, APCI +/- | NA |

# ENTACT Initial Results: Mixtures

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

<75%

>75 to <125%

>125%

# ENTACT Initial Results: Exposure Samples

| | Dust | Fort. Dust | Serum | Fort. Serum | Band | Fort. Band |
|---|---|---|---|---|---|---|
| Actual | ? | 365 | ? | 95 | ? | 185 |
| Lab # | | | | | | |
| 1 | - | - | - | - | - | - |
| 2 | - | - | - | - | - | - |
| 3 | - | - | - | - | - | - |
| 4 | - | - | - | - | - | - |
| 5 | - | - | - | - | - | - |
| 6 | 87 | 236 | 31 | 92 | 46 | 124 |
| 7 | 277 | 259 | 206 | 222 | 243 | 313 |
| 8 | 150 | 270 | 31 | 54 | 58 | 101 |
| 9 | - | - | - | - | - | - |
| 10a | 917 | 1009 | 638 | 614 | - | - |
| 10b | 772 | 861 | 94 | 145 | 298 | 557 |
| 11 | 120 | 124 | 41 | 52 | 24 | 76 |
| 12 | 188 | 389 | 90 | 178 | 100 | 88 |
| 13 | - | - | - | - | - | - |
| 14 | - | - | - | - | - | - |
| 15 | - | - | - | - | - | - |

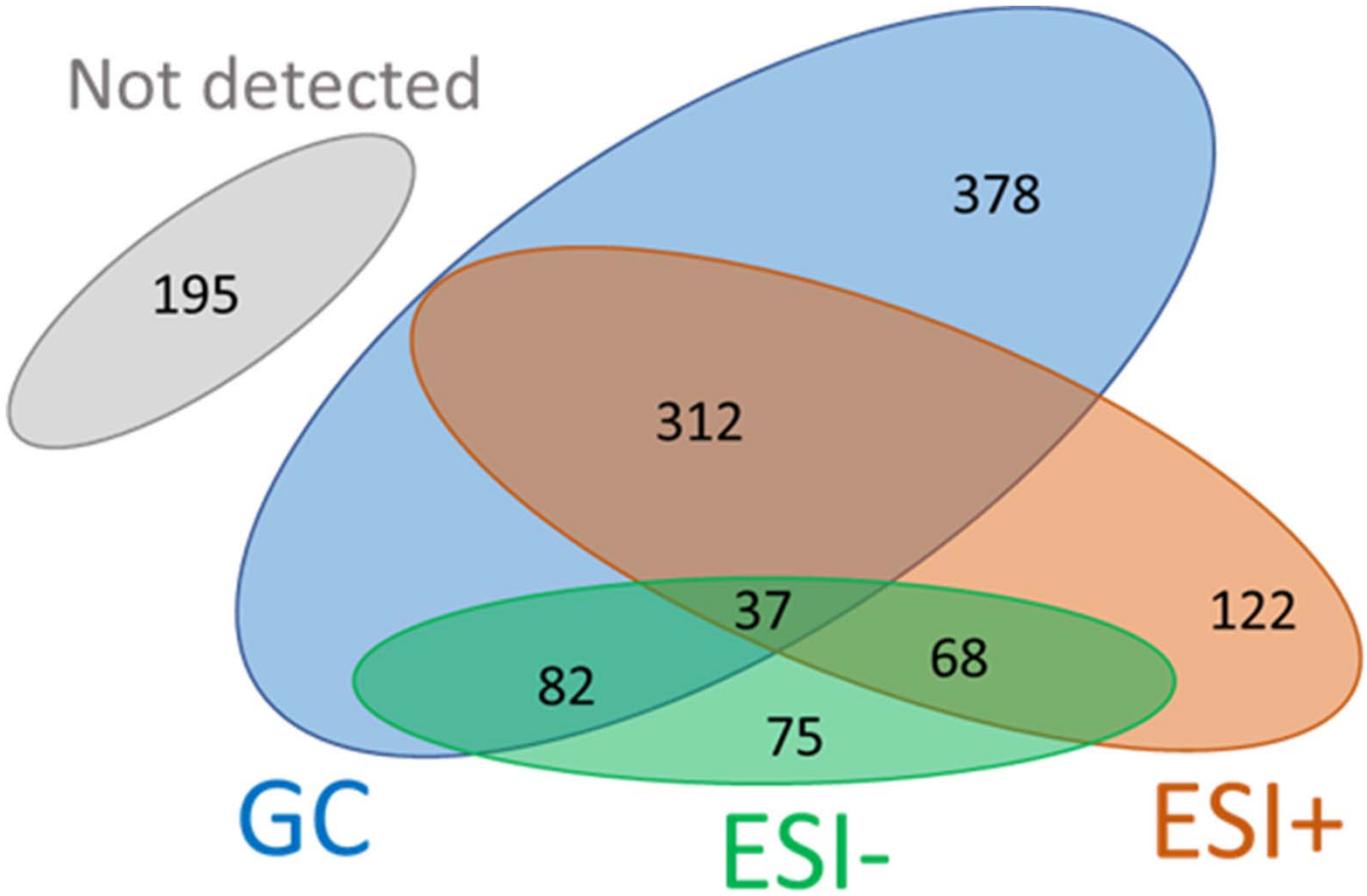
<75%

>75 to <125%

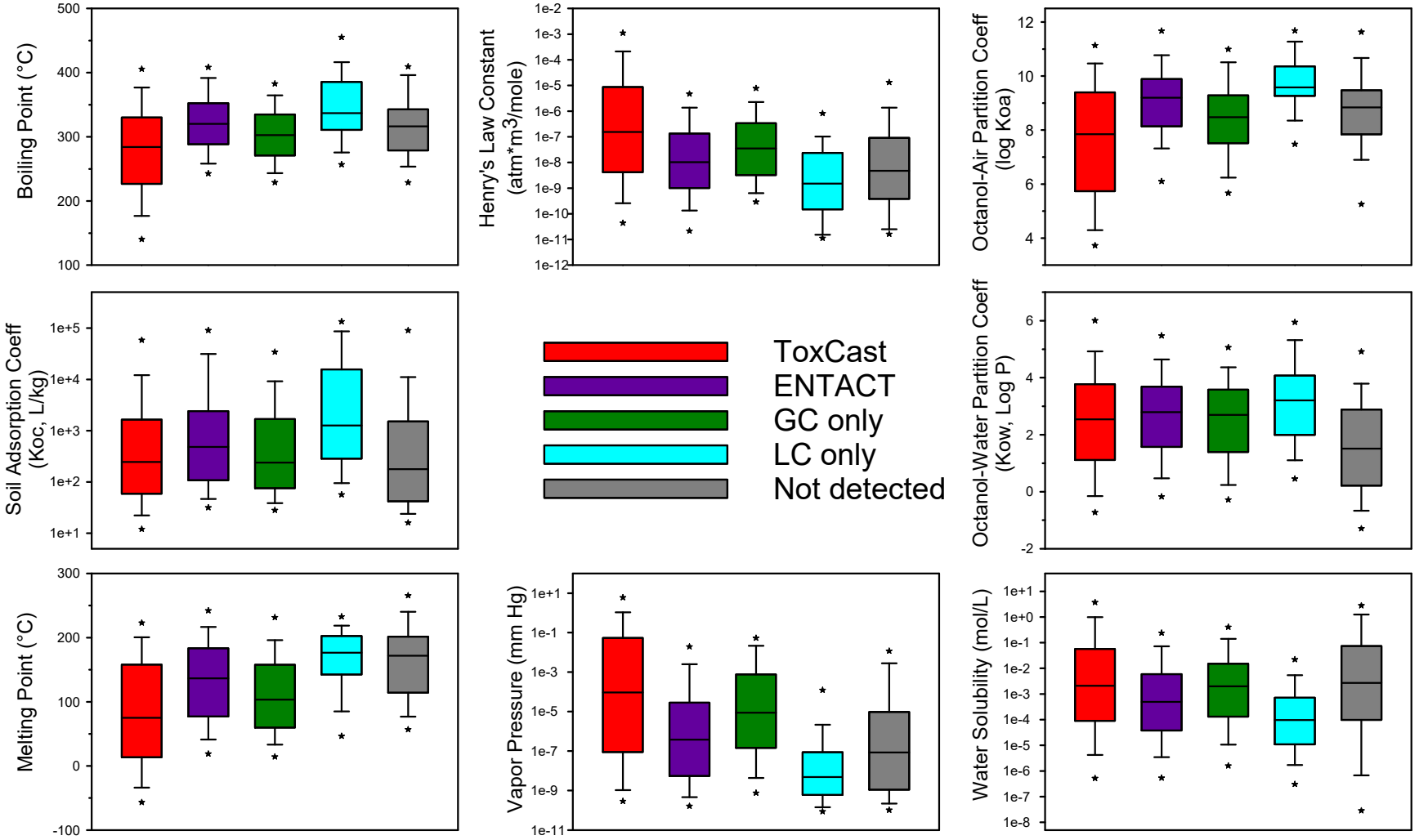
>125%

- not reported

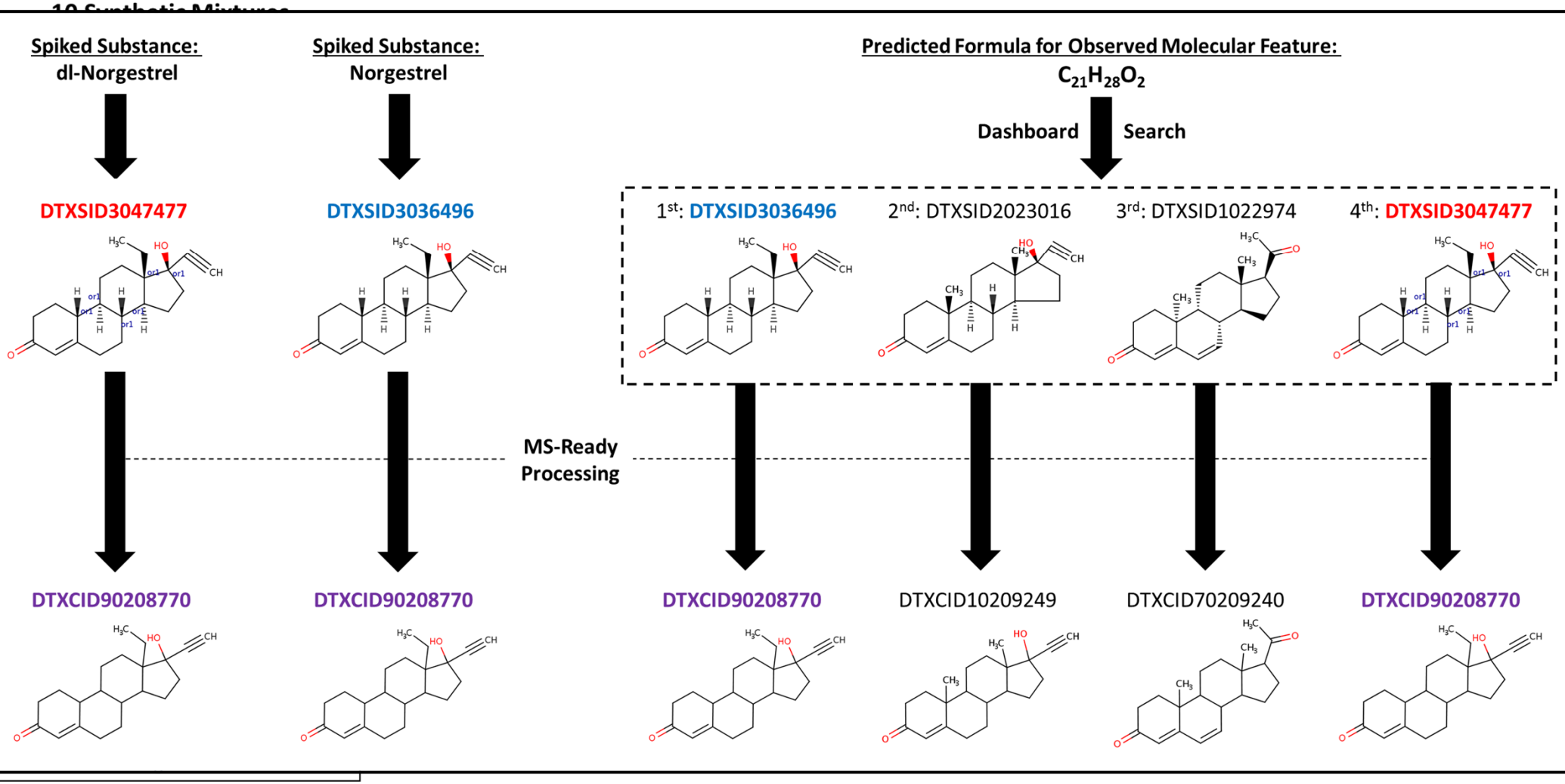
# ENTACT Initial Results: Method Coverage



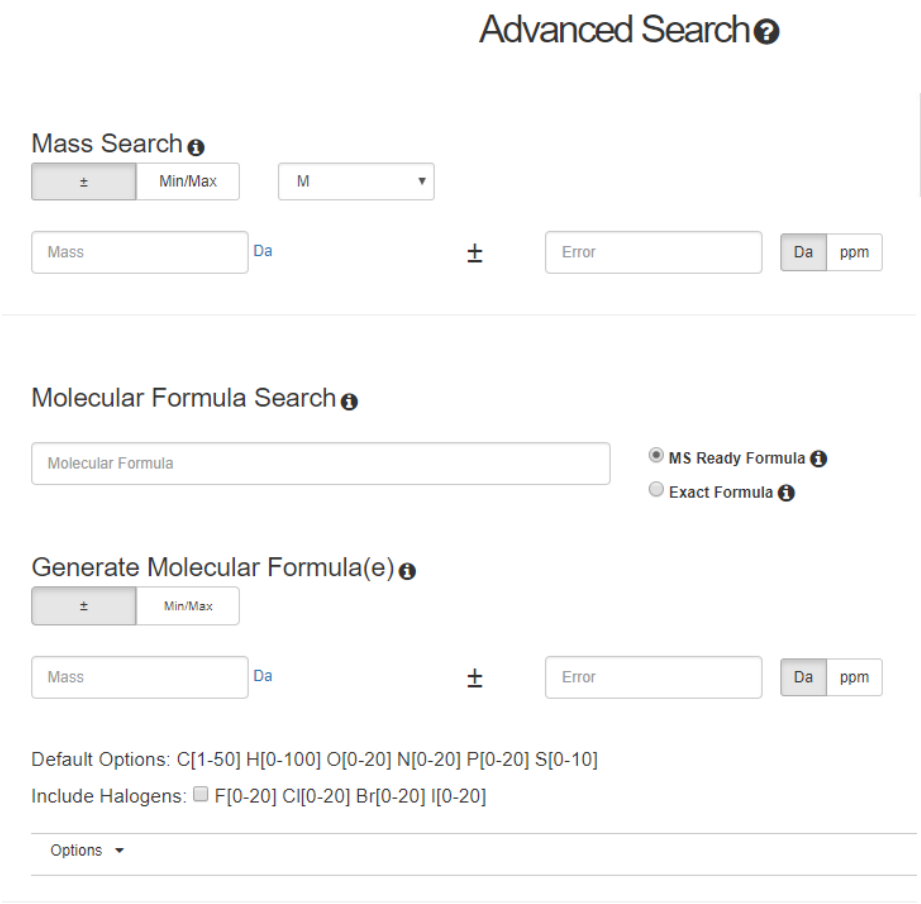
# ENTACT Initial Results: Chemical Space

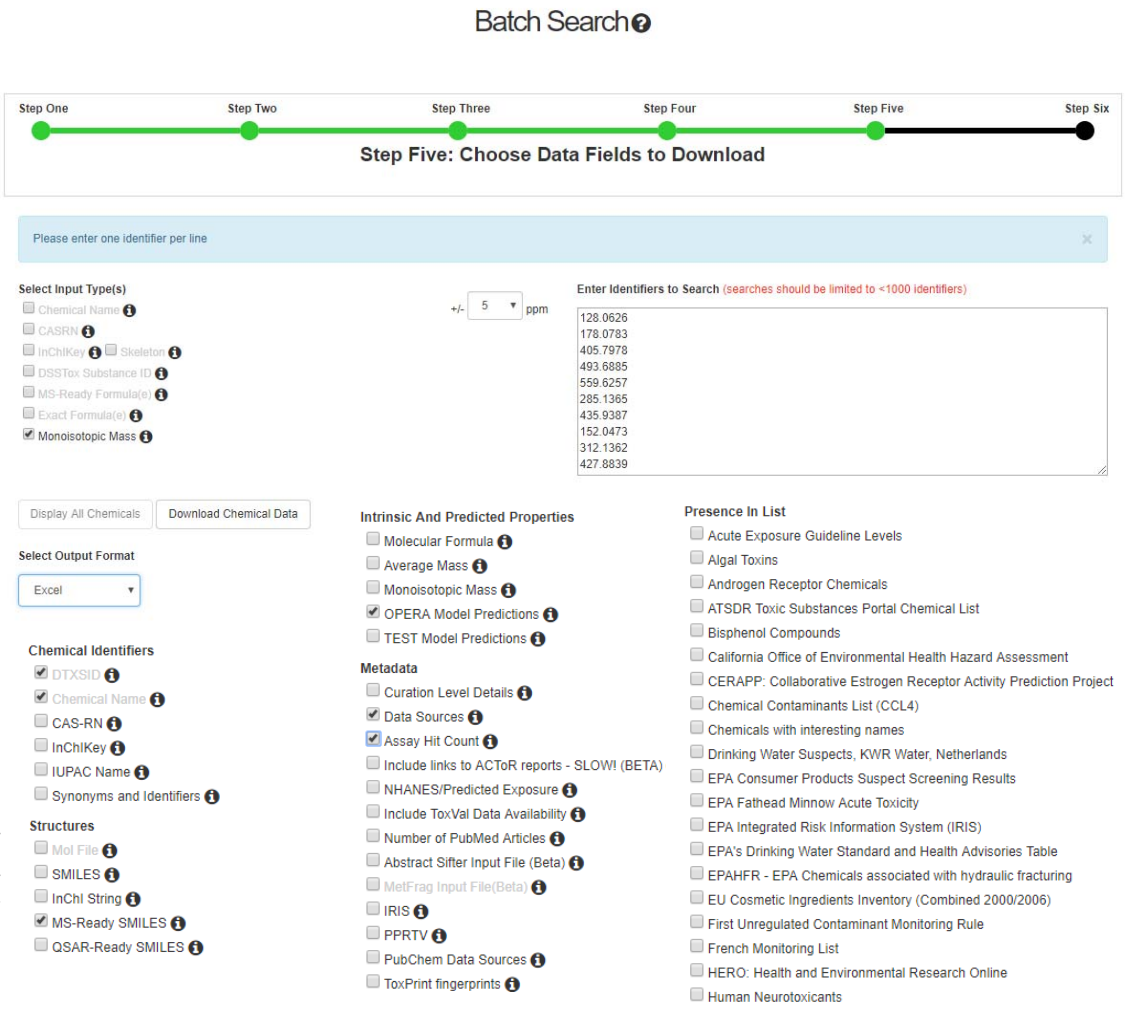


# EPA's Blinded Analysis Workflow



# Chemistry Dashboard: MS Searches




https://comptox.epa.gov/dashboard

# Chemistry Dashboard: MS Searches

| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | INPUT | MASS_DIFFER | DTXCID_INDIVIDU | MONOISOTOPIC | SMILES_INDIVID | DTXSID | PREFERRED_NAME | CASRN | DATA_SOURCE | TOXCAST_% | TOXCAST_# | MS_READY_SMILES |
| 2 | 405.7978 | 2.82E-05 | DTXCID0028117 | 405.7977718 | ClC1CC2C(C1Cl)( | DTXSID7020267 | Chlordane | 57-74-9 | 85 | 32.12 | 185/576 | ClC1CC2C(C1Cl)C1(Cl)C(Cl)=C(Cl)C2(Cl)C1(Cl)Cl |
| 3 | 405.7978 | 2.82E-05 | DTXCID0028117 | 405.7977718 | ClC1CC2C(C1Cl)( | DTXSID6038326 | cis-Chlordane | 5103-71-9 | 40 | 0.0 | 0/163 | ClC1CC2C(C1Cl)C1(Cl)C(Cl)=C(Cl)C2(Cl)C1(Cl)Cl |
| 4 | 405.7978 | 2.82E-05 | DTXCID0028117 | 405.7977718 | ClC1CC2C(C1Cl)( | DTXSID4038346 | trans-Chlordane | 5103-74-2 | 39 | 0.61 | 1/163 | ClC1CC2C(C1Cl)C1(Cl)C(Cl)=C(Cl)C2(Cl)C1(Cl)Cl |
| 5 | 405.7978 | 2.82E-05 | DTXCID10811528 | 405.7977718 | ClC1=C(Cl)C2(Cl)( | DTXSID10858938 | 2,2,4,5,6,7,8,8-Octachloro-2,3,3a,4 | 5566-34-7 | 17 | - | - | ClC1=C(Cl)C2(Cl)C3CC(Cl)(Cl)CC3C1(Cl)C2(Cl)Cl |
| 6 | 405.7978 | 2.82E-05 | DTXCID501012273 | 405.7977718 | ClC1C(Cl)C2C(C1( | DTXSID80874140 | Octachlordane isomer | IOCAS_87414 | 2 | - | - | ClC1C(Cl)C2C(C1Cl)C1(Cl)C(Cl)=CC2(Cl)C1(Cl)Cl |
| 7 | 405.7978 | 2.82E-05 | DTXCID201012274 | 405.7977718 | ClC1CC2C3C1C( | DTXSID40874141 | Compound K | 64250-96-0 | 2 | - | - | ClC1CC2C3C1C(Cl)=C(Cl)C(Cl)(C2(Cl)Cl)C3(Cl)Cl |
| 8 | 405.7978 | 0.00011 | DTXCID20704159 | 405.79791 | ClC1=NC2=C(NC( | DTXSID70753415 | 2-Chloro-6,8-diiodo-7H-purine | 89379-13-5 | 1 | - | - | ClC1=NC2=C(NC(I)=N2)C(I)=N1 |
| 9 | 128.0626 | 2.576E-07 | DTXCID00913 | 128.0626003 | C1=CC2=CC=CC | DTXSID8020913 | Naphthalene | 91-20-3 | 128 | 3.1 | 18/580 | C1=CC2=CC=CC=C2C=C1 |
| 10 | 128.0626 | 2.576E-07 | DTXCID7037686 | 128.0626003 | C1=CC2=CC=CC | DTXSID2059770 | Azulene | 275-51-4 | 34 | - | - | C1=CC2=CC=CC=CC2=C1 |
| 11 | 128.0626 | 2.576E-07 | DTXCID00101940 | 128.0626003 | C=C1C=CC2=CC | DTXSID20179449 | 1-Methylene-1H-indene | 2471-84-3 | 9 | - | - | C=C1C=CC2=CC=CC=C12 |
| 12 | 128.0626 | 2.576E-07 | DTXCID00913 | 128.0626003 | C1=CC2=CC=CC | DTXSID50188347 | Sodium naphthalide | 3481-12-7 | 4 | - | - | C1=CC2=CC=C[C-]=C2C=C1 |
| 13 | 128.0626 | 2.576E-07 | DTXCID50159299 | 128.0626003 | C1=CC23C=CC12 | DTXSID60236808 | (4.2.2)-Propella-2,4,7,9-tetraene | 88090-34-0 | 4 | - | - | C1=CC23C=CC12C=CC=C3 |
| 14 | 493.6885 | 2.7E-05 | DTXCID2027541 | 493.688527 | ClC1=C(Cl)C(Cl)=( | DTXSID5024267 | Polychlorinated biphenyls | 1336-36-3 | 38 | - | - | - |
| 15 | 493.6885 | 2.7E-05 | DTXCID2027541 | 493.688527 | ClC1=C(Cl)C(Cl)=( | DTXSID4047541 | Decachlorobiphenyl | 2051-24-3 | 35 | - | - | ClC1=C(Cl)C(Cl)=C(C(Cl)=C1Cl)C1=C(Cl)C(Cl)=C(Cl)C( |
| 16 | 178.0783 | 4.9678E-05 | DTXCID904254 | 178.0782503 | C1=CC2=C(C=C1 | DTXSID6024254 | Phenanthrene | 85-01-8 | 101 | 2.33 | 13/557 | C1=CC2=C(C=C1)C1=C(C=CC=C1)C=C2 |
| 17 | 178.0783 | 4.9678E-05 | DTXCID203878 | 178.0782503 | C1=CC2=CC3=C( | DTXSID0023878 | Anthracene | 120-12-7 | 99 | 3.89 | 21/540 | C1=CC2=CC3=C(C=CC=C3)C=C2C=C1 |
| 18 | 178.0783 | 4.9678E-05 | DTXCID3040778 | 178.0782503 | C1=CC=C(C=C1)( | DTXSID4060109 | Benzene, 1,1'-(1,2-ethynediyl)bis- | 501-65-5 | 27 | - | - | C1=CC=C(C=C1)C#CC1=CC=CC=C1 |
| 19 | 178.0783 | 4.9678E-05 | DTXCID50105817 | 178.0782503 | C#CC1=CC=C(C= | DTXSID40183326 | 4-Ethynylbiphenyl | 29079-00-3 | 10 | - | - | C#CC1=CC=C(C=C1)C1=CC=CC=C1 |
| 20 | 178.0783 | 0.000701127 | DTXCID00116534 | 178.0775989 | NS(=O)(=O)N1CC( | DTXSID90194043 | Sah 41-178 | 4108-89-8 | 9 | - | - | NS(=O)(=O)N1CCCCCC1 |
| 21 | 435.9387 | 6.3E-06 | DTXCID2014609 | 435.9387063 | NC1=C(C(=NN1C | DTXSID4034609 | Fipronil | 120068-37-3 | 87 | 21.44 | 143/667 | NC1=C(C(=NN1C1=C(Cl)C=C(C=C1Cl)C(F)(F)F)C#N)S( |
| 22 | 435.9387 | 0.000837331 | DTXCID70214016 | 435.9395373 | OC1C(O)C(OP(O) | DTXSID20151508 | Inositol 1,4-bisphosphate 5-phosph | 116778-73-5 | 7 | - | - | OC1C(O)C(OP(O)(O)=O)C(OP(O)(O)=S)C(O)C1OP(O)(O |
| 23 | 559.6257 | 3E-05 | DTXCID404246 | 559.62573 | BrC1=CC(Br)=C(C | DTXSID9030048 | 2,2',4,4',5-Pentabromodiphenyl eth | 60348-60-9 | 52 | 2.65 | 3/113 | BrC1=CC(Br)=C(OC2=C(Br)C=C(Br)C(Br)=C2)C=C1 |
| 24 | 559.6257 | 3E-05 | DTXCID404246 | 559.62573 | BrC1=CC(Br)=C(C | DTXSID2024246 | Pentabromodiphenyl ether | 32534-81-9 | 47 | 5.31 | 6/113 | - |
| 25 | 559.6257 | 3E-05 | DTXCID8031287 | 559.62573 | BrC1=CC=C(OC2 | DTXSID4052689 | 2,2',4,4',6-Pentabromodiphenyl eth | 189084-64-8 | 24 | - | - | BrC1=CC=C(OC2=C(Br)C=C(Br)C=C2Br)C(Br)=C1 |
| 26 | 559.6257 | 3E-05 | DTXCID8031283 | 559.62573 | BrC1=CC=C(OC2 | DTXSID4052685 | 2,2',3,4,4'-Pentabromodiphenyl eth | 182346-21-0 | 19 | - | - | BrC1=CC=C(OC2=C(Br)C(Br)=C(Br)C=C2)C(Br)=C1 |
| 27 | 559.6257 | 3E-05 | DTXCID70524263 | 559.62573 | BrC1=CC(OC2=C | DTXSID50573491 | 1,2,3-Tribromo-5-(3,4-dibromophe | 366791-32-4 | 11 | - | - | BrC1=CC(OC2=CC(Br)=C(Br)C=C2)=CC(Br)=C1Br |
| 28 | 559.6257 | 3E-05 | DTXCID404246 | 559.62573 | BrC1=CC(Br)=C(C | DTXSID70107595 | Brominated diphenyl ethers | 90193-67-2 | 9 | - | - | - |
| 29 | 285.1365 | 6.524E-06 | DTXCID00809656 | 285.1364935 | O=C(C=CC=CC1= | DTXSID3021805 | Piperine | 94-62-2 | 58 | 10.14 | 28/276 | O=C(C=CC=CC1=CC2=C(OCO2)C=C1)N1CCCCC1 |
| 30 | 285.1365 | 6.524E-06 | DTXCID60196731 | 285.1364935 | [H]C12CC3=C4C( | DTXSID9023336 | Morphine | 57-27-2 | 50 | - | - | [H]C12CC3=C4C(OC5C(O)C=CC1C45CCN2C)=C(O)C= |
| 31 | 285.1365 | 6.524E-06 | DTXCID30202010 | 285.1364935 | [H]C12CC3=C4C( | DTXSID8023133 | Hydromorphone | 466-99-9 | 30 | - | - | [H]C12CC3=C4C(OC5C(=O)CCC1C45CCN2C)=C(O)C= |
| 32 | 285.1365 | 6.524E-06 | DTXCID90197312 | 285.1364935 | [H]C12CC3=C4C( | DTXSID8046327 | Norcodeine | 467-15-2 | 24 | 3.13 | 2/64 | [H]C12CC3=C4C(OC5C(O)C=CC1C45CCN2)=C(OC)C= |
| 33 | 285.1365 | 6.524E-06 | DTXCID60196731 | 285.1364935 | [H]C12CC3=C4C( | DTXSID1048914 | Morphine sulfate pentahydrate | 6211-15-0 | 20 | 0.0 | 0/109 | [H]C12CC3=C4C(OC5C(O)C=CC1C45CCN2C)=C(O)C= |
| 34 | 427.8839 | 1.22E-05 | DTXCID206261 | 427.8839122 | ClCC(Cl)OP(=O) | DTXSID9026261 | Tris(1,3-dichloro-2-propyl) phospha | 13674-87-8 | 88 | 22.47 | 131/583 | ClCC(Cl)OP(=O)(OC(Cl)Cl)OC(Cl)Cl |
| 35 | 427.8839 | 1.22E-05 | DTXCID903805 | 427.8839122 | ClCC(Cl)COP(=O) | DTXSID7023805 | Tris(2,3-dichloropropyl)phosphate | 78-43-3 | 22 | - | - | ClCC(Cl)COP(=O)(OCC(Cl)Cl)OCC(Cl)Cl |
| 36 | 427.8839 | 1.22E-05 | DTXCID601026700 | 427.8839122 | ClCC(Cl)COP(=O) | DTXSID10887405 | Phosphoric acid, bis[2-chloro-1-(ch | 68460-03-7 | 5 | - | - | ClCC(Cl)COP(=O)(OC(Cl)Cl)OC(Cl)Cl |
| 37 | | | | | | | | | | | | |

https://comptox.epa.gov/dashboard

# True Positive Rates

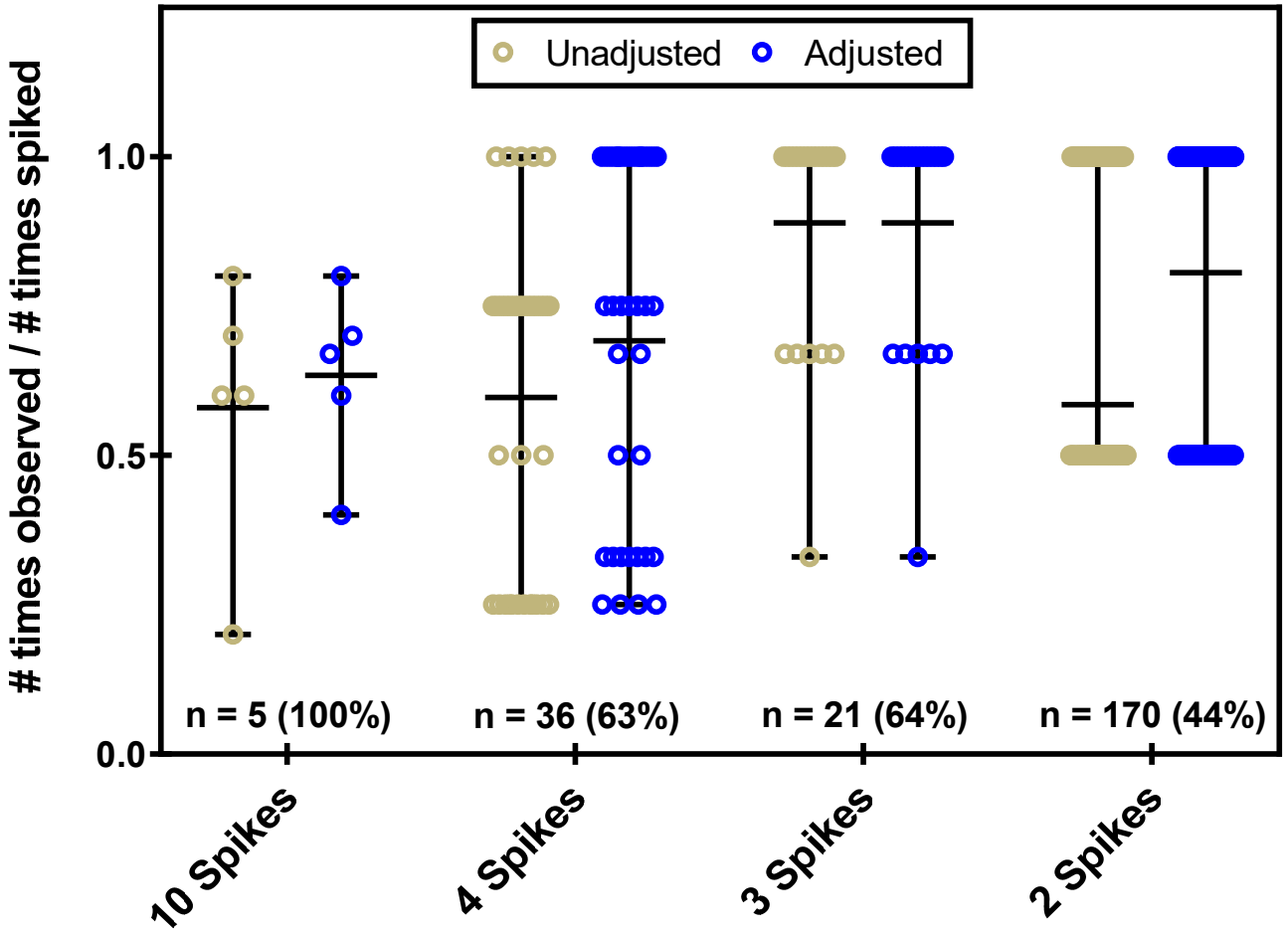
| | | | | Blinded Results | | | Unblinded Results | | |
|---|---|---|---|---|---|---|---|---|---|
| **Mix** | Spiked Substances | Spiked Isomers | Spiked Isobars | True Positives | TPR | Adj. TPR | True Positives | TPR | Adj. TPR |
| **1** | 95 | 2 | 2 | 33 | 0.35 | 0.35 | 46 | 0.48 | 0.49 |
| **2** | 95 | 2 | 2 | 12 (35) | 0.13 (0.37) | 0.13 (0.38) | 19 (53) | 0.20 (0.56) | 0.20 (0.57) |
| **3** | 95 | 0 | 0 | 26 | 0.27 | 0.27 | 47 | 0.49 | 0.49 |
| **4** | 95 | 0 | 0 | 44 (36) | 0.46 (0.38) | 0.46 (0.38) | 58 (58) | 0.61 (0.61) | 0.61 (0.61) |
| **5** | 185 | 2 | 4 | 66 | 0.36 | 0.36 | 103 | 0.56 | 0.56 |
| **6** | 185 | 2 | 2 | 81 | 0.44 | 0.44 | 103 | 0.56 | 0.56 |
| **7** | 365 | 18 | 26 | 156 | 0.43 | 0.45 | 225 | 0.62 | 0.65 |
| **8** | 365 | 2 | 6 | 144 | 0.39 | 0.40 | 195 | 0.53 | 0.54 |
| **9** | 95 | 52 | 67 | 18 | 0.19 | 0.42 | 19 | 0.20 | 0.44 |
| **10** | 364 | 207 | 257 | 19 (80) | 0.05 (0.22) | 0.12 (0.51) | 31 (107) | 0.09 (0.29) | 0.20 (0.68) |

*Note: results in ( ) are based on a 2nd analysis using altered methods (ACN vs. MeOH)*

How good are we?

How good can we be?

# Reproducibility of Results




**# Times Observed / # Times Spiked:**

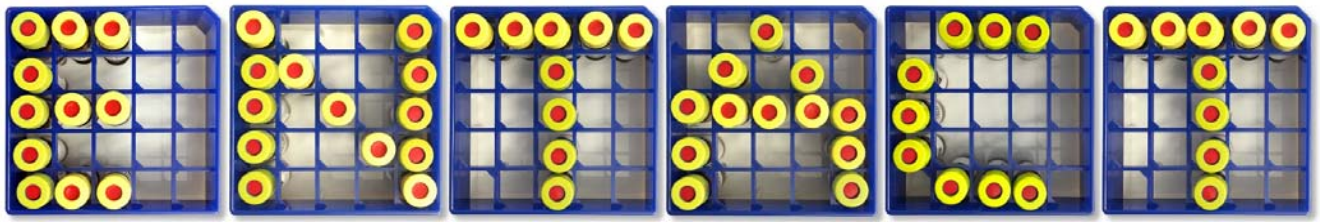
**Global Unadj. = 62%**

**Global Adj. = 75%**

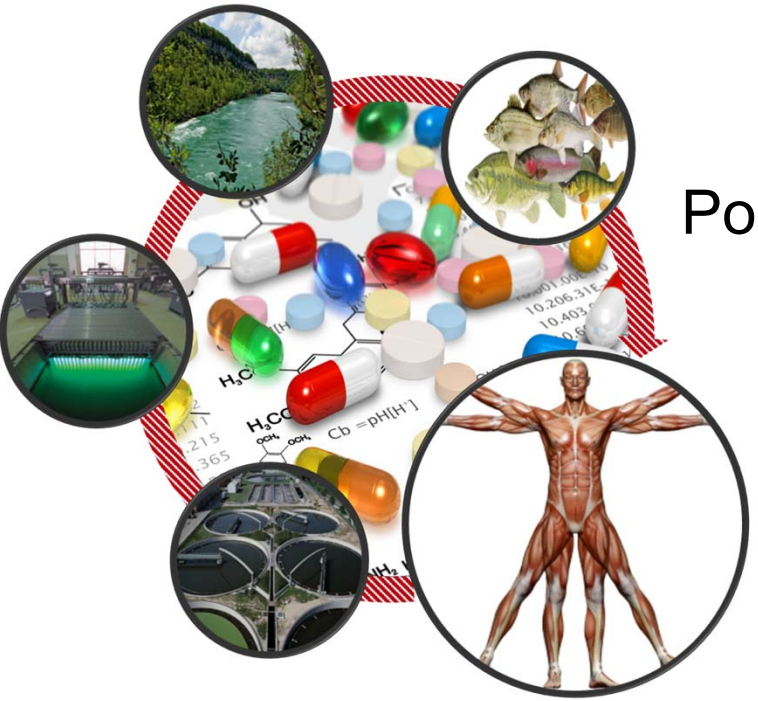
# What other questions can be answered with ENTACT data? What models can be built with ENTACT data?

- 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?

# Next Steps



# Workshop

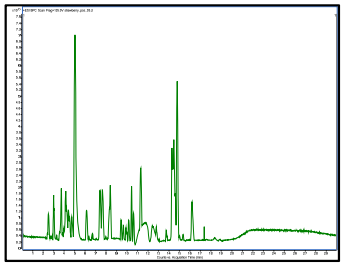


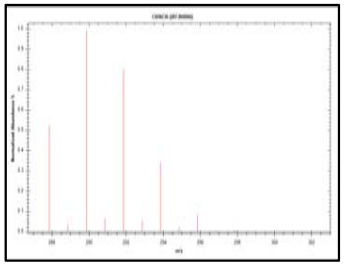
August 13-15, 2018 in RTP, NC

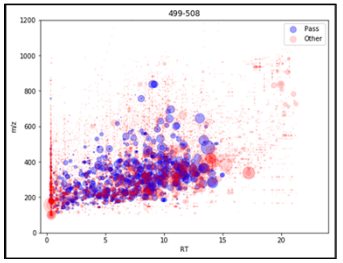
http://bit.ly/Entact2018

Poster abstract deadline: July 13, 2018

Attendance is ***FREE***





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