

#### The Use of Non-Targeted Analysis for Rapid and Emergency Response: Demonstration Through Mock Scenarios

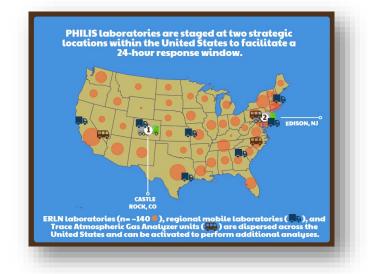
John T. Sloop, Alex Chao, Jennifer Gundersen, Allison L. Phillips, Jon R. Sobus, Elin M. Ulrich, Antony J. Williams, Seth R. Newton



The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency (USEPA)

# Chemical releases into the environment

- In 2021, over 25,000 calls logged by the National Response Center (NRC) reporting environmental discharges
- 30% of an unknown composition
  - Of those of an unknown composition, over 70% reported to penetrate a body of water near the release
- Targeted approach for an unknown chemical is akin to "a shot in the dark"
  - Clear need for systematic approach to elucidating identity of unknown chemicals → NTA!





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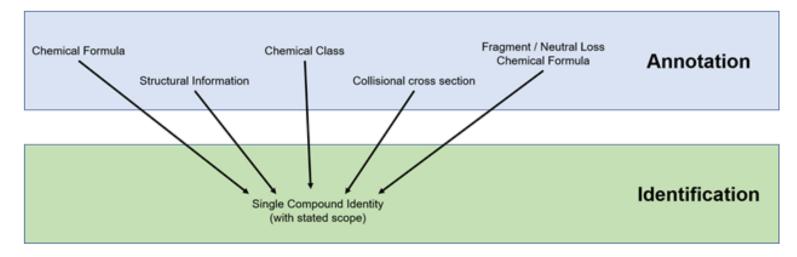
# What is "NTA"?

- Non-targeted analysis
- Mass spectrometry techniques for characterizing the chemical composition of a given sample without the use of *a priori* knowledge regarding the sample's chemical content
  - LC-MS, GC-MS (high resolution mass spectrometry, HRMS)
- No prior knowledge of sample's chemical content → no use of chemical standards
  - How is identity determined?



# "Features" in NTA data

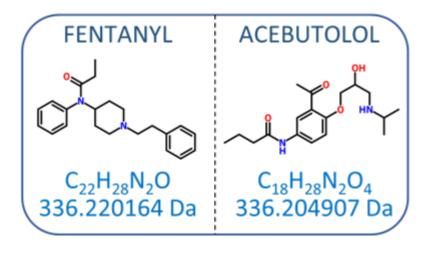
- Molecular feature extracted from data collected during NTA studies
  - Defined by an exact mass at a retention time, associated ions, and intensity of an apparent unknown compound
- Feature annotation vs. feature identification

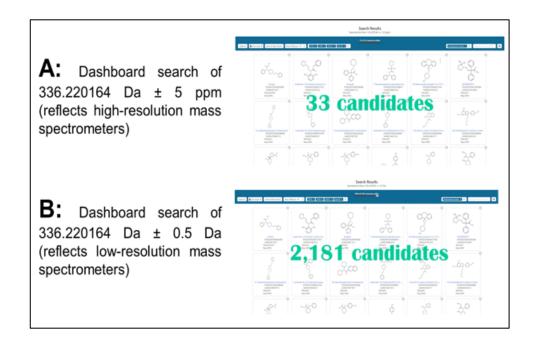


*Figure from: https://nontargetedanalysis.org/reference-content/methods/data-processing-and-analysis/#annotation-and-id* 



# Importance of HRMS





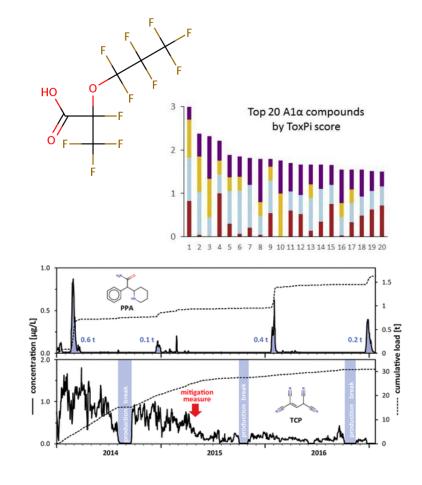
Need instrument with resolving power >> 20,000 to distinguish between the two compounds; not plausible without high-resolution instrumentation

# NTA for Identification of Unknowns

 Identification of novel PFECAs and PFESAs in Cape Fear River Basin (GenX)

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- Identifying high-priority compounds found in drinking water across central NC
- Identifying previously undetected compounds in the Rhine River after major spill events
- Daily screening of potable water sources for detection of potential spills





# NTA in Rapid Response

- Phillips et al. (2021); laid the framework of how NTA could be applied in the field of rapid response
  - High-resolution mass spectrometry (HRMS) vs. traditional, low-resolution instrumentation
  - NTA has been proven as a tool for identifying unknowns
- Logical step after the framework paper was a demonstration

#### Environmental Toxicology and Chemistry

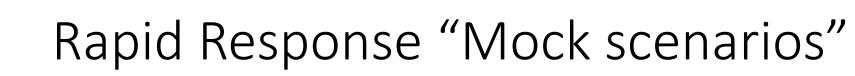
critical perspectives 🛛 🔂 Full Access

A Framework for Utilizing High Resolution Mass Spectrometry and Non-Targeted Analysis (NTA) in Rapid Response and Emergency Situations

Allison L. Phillips, Antony J. Williams, Jon R. Sobus, Elin M. Ulrich, Jennifer Gundersen, Christina Langlois-Miller, Seth R. Newton 🗙

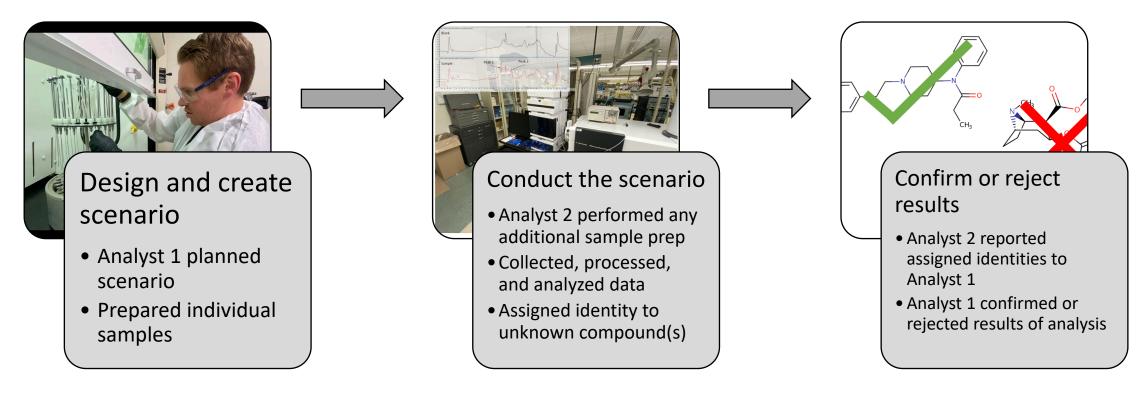
First published: 20 August 2021 | https://doi.org/10.1002/etc.5196

(Submitted 28 June 2021; Returned for Revision 26 July 2021; Accepted 17 August 2021) This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/etc.5196



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 Created samples intended to mimic situations in which a rapid response would be necessary – two analysts for each scenario

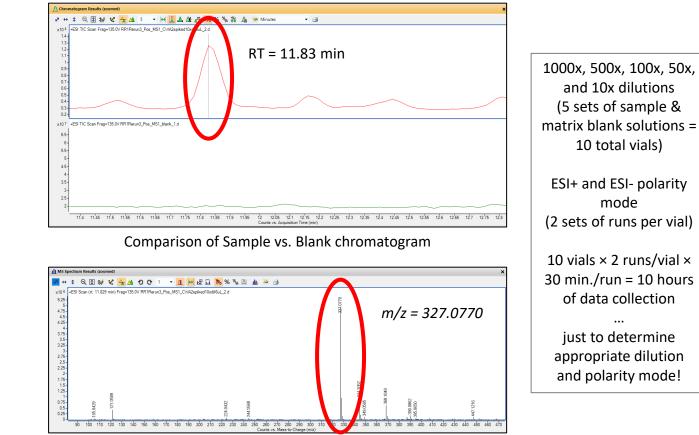




# Method Development via "Mock" mock scenarios

"Mock" mock scenario 1

- Top priority: not contaminating the instrument/saturating the detector
- LC-MS operating in ESI+ and ESI- polarity mode
  - ~ 30 min. run-time
- Sat around all day waiting for dilutions to finish
- Eventually, correct identification: triphenyl phosphate



MS spectrum of sample chromatogram at RT 11.829 min

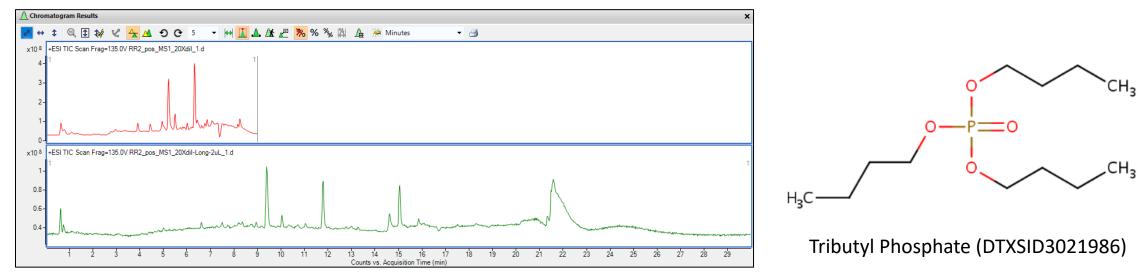
Important takeaway: Need better method for range finding!



# Method Development via "Mock" mock scenarios

- Goals of "2<sup>nd</sup>" mock scenario:
  - Test a rapid range-finding method
  - Start to determine appropriate workflows for MS and MS/MS data

- "Rapid range-finding method"; 9-minute LC-MS method
  - Determine appropriate concentration/dilution factor
  - Determine appropriate polarity (ESI+ or ESI-)
- Tributyl Phosphate



"Short" vs. "Long" LC-MS method sample chromatograms

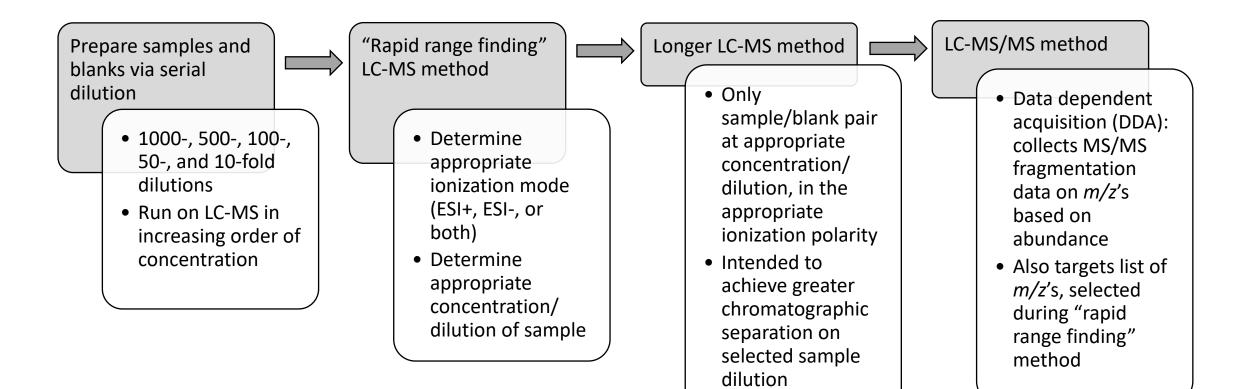


# Method Development Conclusions

- In conclusion, after finishing "method development", we found three areas we should focus on:
  - Data Collection Workflow
  - Data Processing Workflow
  - Metrics for Success

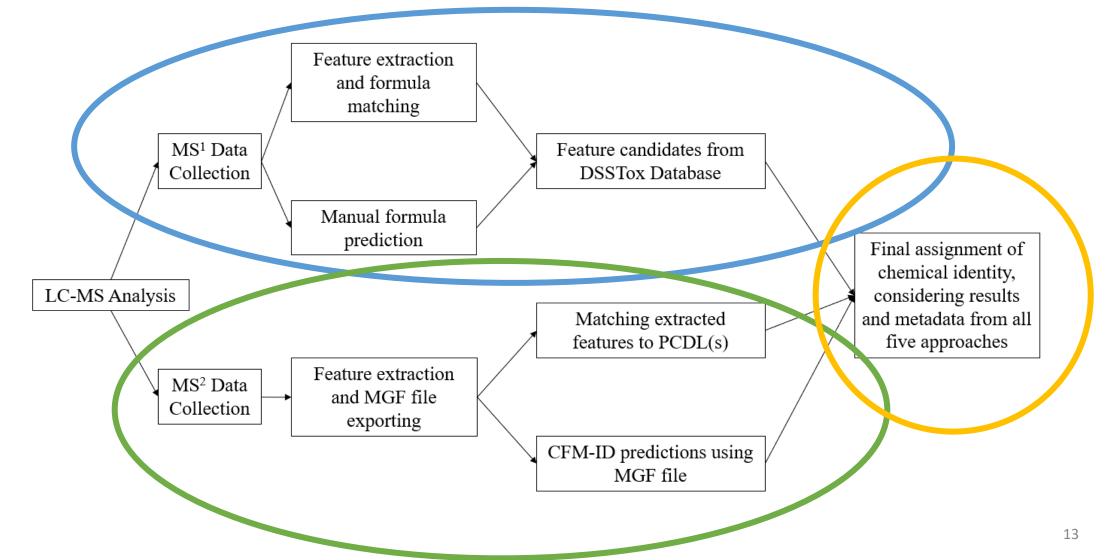


# Data collection workflow





# Data processing workflow





# Data processing workflow

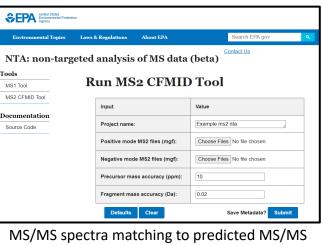
| Project Navigator Ø           | Experie   | ent Name        |              |               |         |                  |          |         |          |                      | Workflow  |                                   | Q.           |
|-------------------------------|-----------|-----------------|--------------|---------------|---------|------------------|----------|---------|----------|----------------------|---|-----------------------------------|--------------|
| Project_Name :                | <         | -               |              |               |         | <br>1            |          |         |          | <br>                 | 1   |                                   | ^            |
| Experiments                   | Compou.   | JTS0805         |              |               | Formula | Ionizatio        | Mass     |         | Retentio | Compou               | Experiment  | nt Setup                          | *            |
| Experiment_Name               | 260.192   |                 | 44676084     |               |         | 2 Esi+           | 260.1929 |         | 6.649    | FindByMo             | <ul> <li>Experim</li> </ul>   | ent Grouping                      |              |
|                               | 218.103   |                 | 43461924     |               |         | Esi+             | 218 1031 |         | 8.392    | FindByMo             | Create 1  | nterpretation                     |              |
| Experiment Name -1+1x         | 325.160   |                 | 20871834     |               |         | Esi+             | 325.1608 |         | 13.334   | Find8yMo             |   |                                   |              |
| Compension in anne            |           |                 | 17424744     |               |         | Esi+             | 183.9778 |         | 21.512   | FindByMo             | Quality Cr  | and and                           | *            |
| Interpretations               | 408.155   |                 | 13195336     |               |         | 2 Esi+           | 408.1552 |         | 9.434    | FindByMo             | deanth cu   | 41104                             | ~            |
| Analyzia                      | 246.157   |                 | 11269487     |               |         | Esi+             | 246.1577 |         | 7.528    | FindBy#to            |   |                                   |              |
| All Entities                  | 268.139   | 10307638        |              |               |         | Esi+             | 268.1397 |         | 7.529    | FindByMo             | Analysis  |                                   | *            |
| C) My Payorites               | 166.005   | 6908511         |              |               |         | 2 Esi+           | 166.0059 |         | 21.657   | FindByMo             |   |                                   |              |
| - Hy ravenes                  | 156.997   | 7634266         | 8727315      |               |         | 2 Esi+           | 156.9972 |         | 0.626    | FindByMo             | Class Pres  | iction                            | 8            |
|                               | 330.036   | 1               | 7416969      |               |         | Esi+             | 330.0364 |         | 10.991   | FindBy410            |   |                                   |              |
|                               | 514.298   |                 | 6158346      |               |         | 2 Esi+           | 514.2989 |         | 7.528    | FindByMo             | The second second   | terpretations                     |              |
|                               | 352.018   | 1               |              |               |         | Esi+             | 352.0188 |         | 10.993   | FindByMo             |   |                                   | ×            |
|                               |           | 2585043         |              |               |         | Esi+             | 519.4291 |         | 20.317   | FindByMo             | Find Similar Entity Lists<br>Export for Recursion<br>IDBrowser Identification |                                   |              |
|                               | 171.11@   | 6554816         |              |               |         | Esi+             | 171.1100 |         | 6.649    | FindByMo<br>FindByMo |   |                                   |              |
|                               |           | 5976257         |              |               |         | Esi+             | 123.0795 |         | 21.50/   |                      |   |                                   |              |
|                               | 424.129.  |                 | 5445514      |               |         | 2 Esi+           | 424.1295 |         | 9.434    | FindByMo             | Eurova 6  |                                   | · · ·        |
| Global Items 0                | 249 958   | 1827425         |              |               |         | Esi+             | 172.0712 |         | 21.657   | FindByMo<br>FindByMo | Legend  | Export fo                         | or identific |
|                               |           |                 |              |               |         |                  | 249.9581 |         |          |                      | Legend  |                                   | v            |
| 8 🛄 My Lists                  | 249.170   | 4885969         |              |               |         | Esi+             | 249.1705 |         | 8.678    | FindByMo             | Legend  | <ul> <li>Spreadsheet (</li> </ul> | Raw)         |
| My Reports                    | 403.199   | 2972270         |              |               |         | ESI+             | 403 1998 |         | 9.454    | FindByMo             |   |                                   |              |
|                               | 216.097   | 1110513         | 4656478      |               |         | E E31+<br>2 E41+ | 347.0633 |         | 1 184    | FindByMo<br>FindByMo |   |                                   |              |
|                               | 142 034   | 3703354         |              |               |         | ESI+             | 142.0348 |         | 0.626    | FindByMo             |   |                                   |              |
|                               | 278.152   | 3430170         |              |               |         | Esi+             | 278.1521 |         | 13.042   | FindByMo             |   |                                   |              |
|                               | 220.027   | 3308028         |              |               |         | Esi+             | 220.0276 |         | 21,532   | FindByMo             |   |                                   |              |
|                               | 312.136   | 3227929         |              |               |         | Esi+             | 312.1365 |         | 12.808   | FindByMo             |   |                                   |              |
|                               | 312.136   | 3227929         |              |               |         | ESI+             | 312.1365 |         | 7.529    | FindByMo             |   |                                   |              |
|                               | 273.267   | 2708925         |              |               |         | Esi+             | 273.2670 |         | 8.321    | FindByMo.            |   |                                   |              |
|                               | 217.951   |                 | 2878732      |               |         | ESI+             | 217.9516 |         | 0.521    | FindByMo             |   |                                   |              |
|                               | 225 005   |                 | 2625573      |               |         | Frie             | 225.0050 |         | 21.512   | FindB Mo             |   |                                   |              |
|                               | 316.020   | 2728433         |              |               |         | Esi+             | 316.0205 |         | 4 803    | FindByMo             |   |                                   |              |
|                               | 284 114   | 2702247         |              |               |         | Fu+              | 284.1140 |         | 7.530    | FindBMo              |   |                                   |              |
|                               | 436.186   |                 | 2566943      |               |         | Esi+             | 436.1868 |         | 10.346   | FindByMo             |   |                                   |              |
|                               | 367 990   | 1952915         |              |               |         | Esi+             | 367.9902 |         | 10.992   | FindB/Mo             |   |                                   |              |
|                               | 101 008   |                 | 2442969      |               |         | Esi+             | 101.0087 |         | 0.626    |                      | v   |                                   |              |
|                               |           |                 |              |               |         | <br>12314        | 101.008/ | 1102.04 | 9.929    | <br>Pailog Wild      | -   |                                   |              |
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Profinder and MPP

|                          | Generate Forr  |   |   |          |
|--------------------------|--|---|---|----------|
| Method Automation A      | Allowed Species  | Limits Charge S   | tate Fragment For                                 | nulas    |
| Chromatograms            | Charge carrier to b<br>Positive ions:                                | e assumed if not kno  |   |          |
| Spectra                  | -electron  |   | egative ions:<br>+electron                        | A        |
| - Identification         | ₩ +H   |   | ⊠-H<br>□+CI                                       |          |
| Identification Workflow  | +K   | _   | +Br   |          |
| Database Search Settings | +C2H5  |   | +CH3C00   |          |
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|                          |  |   |   |          |
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| enerate Formulas 🔺       | Group hits with<br>Bements and limits                                | ate: allow bot  | different charge car                              |          |
| nerate Formulas 🔺        | Group hits with<br>Bements and limits<br>Element                     | ate: allow bot<br>h same formula (but<br>s<br>Minimum                     | different charge car<br>Maximum                   | riers)   |
| A Pormulas               | Group hits with<br>Elements and limits<br>Element<br>C               | allow bot<br>h same formula (but<br>s<br>Minimum<br>3                     | different charge car<br>Maximum<br>30             |          |
| enerate Formulas 🔺       | Group hits with<br>Elements and limits<br>Element<br>C<br>H          | ate: allow bot<br>h same formula (but<br>s<br>Minimum<br>3<br>0           | different charge car<br>Maximum<br>30<br>60       | <b>A</b> |
| enerate Formulas 🔺       | Group hits with<br>Elements and limits<br>Element<br>C<br>H<br>O     | ate: allow bot<br>h same formula (but<br>s<br>Minimum<br>3<br>0<br>0<br>0 | different charge car<br>Maximum<br>30<br>60<br>10 |          |
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| enerate Formulas 🔺       | Group hits with<br>Bements and limits<br>Element<br>C<br>H<br>O<br>N | ate: allow bot<br>h same formula (but<br>s<br>Minimum<br>3<br>0<br>0<br>0 | Maximum<br>30<br>60<br>10<br>10                   | <b>A</b> |

| United States<br>Environmental Protect       | zion                             |                  |  |  |  |  |  |  |  |
|--|----------------------------------|------------------|--|--|--|--|--|--|--|
| <b>Environmental Topics</b>                  | Laws & Regulations About EPA     | Search EPA.gov Q |  |  |  |  |  |  |  |
| NTA: non-targeted analysis of MS data (beta) |                                  |                  |  |  |  |  |  |  |  |
| Tools<br>MS1 Tool                            | Run NTA MS1 Too                  | ol               |  |  |  |  |  |  |  |
| Run MS1 Tool MS1 Tool Algorithms             | Input                            | Value            |  |  |  |  |  |  |  |
| MS1 Tool QA/QC<br>MS1 Tool References        | Project name:                    | Example nta      |  |  |  |  |  |  |  |
| MS2 CFMID Tool                               | Run test files only (debugging): | no v             |  |  |  |  |  |  |  |
| Generatir                                    | ng candidate lists via V         | VebApp MS1 Tool  |  |  |  |  |  |  |  |

Method Editor: Database Search Setting 🔊 🔹 🐑 🔹 Search Library / DB for All Compounds 🔹 🟠 🌽 🖬 🌃 Search Criteria Peak Limits 🛕 Positive Ions 🛕 Negative Ions Search Results Charge carriers Neutral losses Chrom Target/Su Sample Purit Compound + 🗙 + × Identificatio Charge states, if not know Aggregate entification Workflow e.g., [2M+H]+ Dimers Charge state range 1-2 tabase Search Sett Trimers e.g., [3M+H]+ brary Search Setting DB ion type search mod enerate Formulas Neutrals Cations (this type is not applicable to CSV databases



MS/MS spectra matching to predicted MS/MS spectra via WebApp MS2 Tool

Final assignment of chemical identity, considering results and metadata from all 5 approaches

Molecular formula generation via Qual

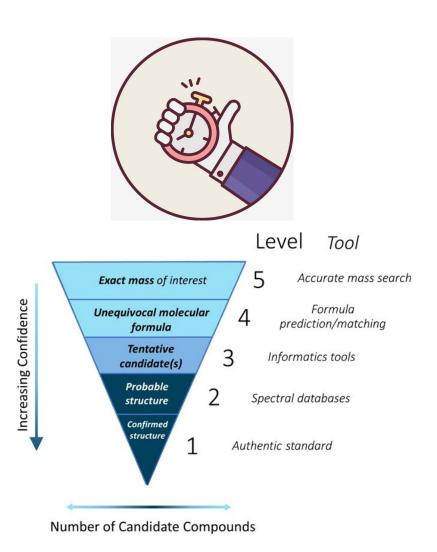


1. Speed of analysis

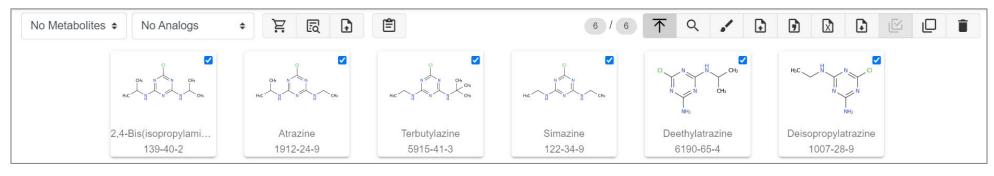
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- 2. Confidence in the eventual chemical identifications
- 3. Degree of hazard assessment that can be performed
- 4. Transferability of the designed NTA method/workflow



# Hazard Comparison Module (HCM)



 Proof-of-concept, web-based implementation of original work of Vegosen and Martin

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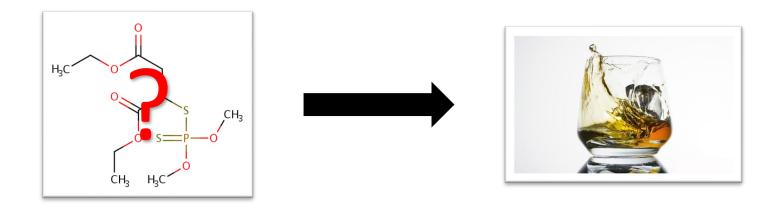
Agency

- Chemicals searched by CAS RNs, chemical names, DTXSIDs, SMILES
- Hazard information converted into scores of low, medium, high, or very high (L, M, H, VH)

| eminformatics Modules<br>sion: DEV, build: 2022-05-04 16:47:03 UTC                    |               |       | (                         | 🖗 HAZARE       | 0 🚯 AL                    | .erts 🔒 pred                          | ICT 1.0 🖩 PREDICT 2                 | .0 😴               | SEARCH<br>Hazard asses     | STAN           | DARDIZE                | TOX | PRINTS | : |
|---|---------------|-------|---------------------------|----------------|---------------------------|---------------------------------------|-------------------------------------|--------------------|----------------------------|----------------|------------------------|-----|--------|---|
| <b>↑</b>  |               |       |                           |                |                           |                                       |                                     | (                  | Emergency Full             | Response       | • V                    | 3   |        | Ō |
| Chemicals: 32   |               |       | Toxicity: \               | /H - Very High | H - High M                | - Medium L - Low I - In               | conclusive N/A - Not Applicable     | Author             | Custom                     |                | QSAR Model             | )   |        |   |
|   |               |       |                           |                |                           | Human Health Ef                       | fects                               |                    | Emergency<br>Site-Specific |                | Ecotoxicity            | 8   |        |   |
| Skipped (0)<br>Unlikely (0)<br>Filters (0)<br>Sorting (0)<br>Structure<br>CAS<br>Name |               | Acute | Mammalian T<br>Iuhalation | Dermal         | Genotoxicity Mutagenicity | Neurotoxicity<br>ensodx<br>albus<br>S | Systemic Toxicity<br>ansodx<br>a ab | Skin Sensitization | Skin Irritation            | Eye Irritation | Acute Aquatic Toxicity |     |        |   |
| 139-40-2<br>2,4-Bis(isopropyl   | IGBTM<br>1.00 | М     | М                         | L              | L                         | 1                                     | 1                                   | 1                  | М                          | н              | VH                     |     |        |   |
| 1912-24-9<br>Atrazine   | AIGBT         | м     | н                         | L              | L                         | М                                     |                                     | н                  | L                          | м              | VH                     |     |        |   |
| 5915-41-3<br>Terbutylazine  | GВТМ<br>0.94  | м     | 1                         | L              | VH                        |                                       |                                     | н                  | н                          |                | νн                     |     |        |   |
| 122-34-9<br>Simazine  | IGBTM<br>0.91 | м     | н                         | L              | L                         |                                       | М                                   | T                  | н                          | н              | νн                     |     |        |   |
| 6190-65-4<br>Deethylatrazine  | GBTM<br>1.00  | М     | 1                         | 1              | L                         | 1                                     | I                                   | 1                  | 1                          | I              | н                      |     |        |   |
| 1007-28-9<br>Deisopropylatrazine  | GВТМ<br>0.91  | М     |                           |                | L                         |                                       |                                     |                    |                            |                | М                      |     |        |   |



#### Mock scenario 1: Nerve agent spiked into beverage

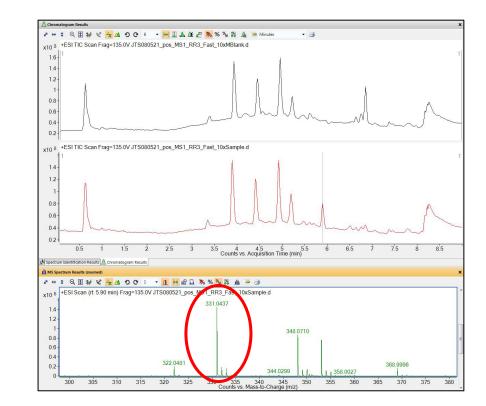


- Scenario: chemical warfare agent (CWA) spiked into alcoholic beverage, intended to poison an individual
- Surrogate of chemical warfare agent, similar to Novichok nerve agents, spiked into pure ethanol by Analyst 1
- Analyst 2 proceeded with data collection workflow



# Mock scenario 1: Results

- Formula matching to MS-Ready formula
  - C<sub>10</sub>H<sub>19</sub>O<sub>6</sub>PS<sub>2</sub>, scored 89.2
- Formula prediction using Molecular formula generator (MFG) tool
  - Top hit =  $C_{10}H_{19}O_6PS_2$ , score of 99.11
- NTA WebApp MS1 tool
  - N=250, Malathion  $(C_{10}H_{19}O_6PS_2)$
  - N=33, Isomalathion  $(C_{10}H_{19}O_6PS_2)$
  - N=17, Becampanel  $(C_{10}H_{11}N_4O_7P)$
- MS/MS matching to PCDLs
  - No good matches (very low scores)
- NTA WebApp MS2 tool
  - Multiple potential matches, malathion one of them (low scoring)



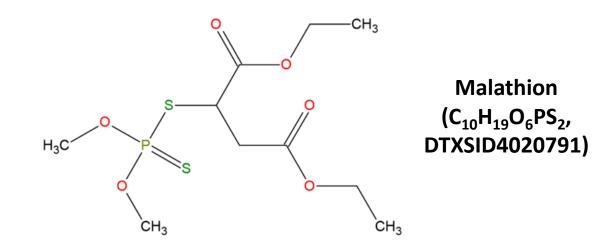
"Rapid range finding" chromatogram and MS spectrum of 10x dilution at RT = 5.90 min; m/z of interest is 331.0437

# Mock scenario 1: Metrics for Success

1. Speed of analysis

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- 13 "active" hours
- 2. Confidence in identification
  - Level 2 (structural assignment)
- 3. Hazard assessment provided
- 4. Transferability of the approach
  - N/A



|  |     |       |             |         |                     | Human Health Effe | ects              |                    |                 |                | Ecotoxicity            |
|--|-----|-------|-------------|---------|---------------------|-------------------|-------------------|--------------------|-----------------|----------------|------------------------|
| Skipped (0)  |     | Acute | Mammalian T | oxicity | enicit              | Neurotoxicity     | Systemic Toxicity |                    |                 |                | A‡                     |
| Unlikely (2)<br>Filters (0)<br>Sorting (0)<br>Structure<br>CAS<br>Name |     | Oral  | Inhalation  | Dermal  | Genotoxicity Mutage | Single Exposure   | Single Exposure   | Skin Sensitization | Skin Irritation | Eye Irritation | Acute Aquatic Toxicity |
| 121-75-5<br>Malathion  |     | н     | VH          | L       | н                   | н                 |                   | н                  | м               | н              | VH                     |
| 64-17-5<br>Ethanol LI  | GBT | VH    | VH          | L       | L                   |                   | М                 | I                  | L               | н              | м                      |

# Mock scenario 2: Raid on "drug house"

- Scenario: drug house raided under suspicion of fentanyl processing with another illicit drug
  - Some illicit drug being "cut" with fentanyl or fentanyl-analog
- Two surrogates (of illicit drug and Fentanyl) spiked onto:
  - Dusty area of benchtop in lab (surface wipe, "traditional sampling")
  - Carpet sample (extraction of porous material, "non-traditional sampling")



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# Mock scenario 2: Results

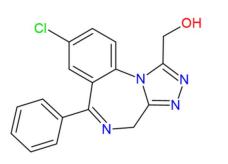
- Existence of multiple peaks made it unrealistic to choose individual peaks of interest by visual inspection alone
  - Sort by abundance after blank subtraction following data collection
- Feature 1 (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>O at 324.0783 Da):
  - MS-Ready formula and MFG formula agreed with top hit from WebApp MS1 tool:  $\alpha$ -hydroxy alprazolam
  - Using WebApp MS2 tool,  $\alpha$ -hydroxy alprazolam ranked 2<sup>nd</sup> highest
- Feature 2 (C<sub>23</sub>H<sub>36</sub>N<sub>2</sub>O<sub>2</sub> at 372.2718 Da):
  - MS-Ready formula and MFG formula agreed with top hit from WebApp MS1 tool: finasteride
  - MS/MS match via PCDLs for finasteride

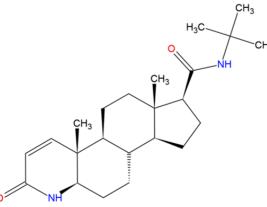
# Mock scenario 2: Metrics for Success

1. Speed of analysis

vironmental Protection

- 30 "active" hours
- 2. Confidence in identifications
  - Level 2 (structural assignments)
- 3. Hazard assessment provided
- 4. Transferability of the approach
  - Different individual (familiar with NTA, but not with specifics of the workflows prior to this scenario) assumed role of "Analyst 2"





 $\alpha$ -hydroxy alprazolam

Finasteride

| Chemicals: 9 Toxicity: VH - Very High H - High M - Medium L - Low I - Inconclusive N/A - Not Applicable Authority: Authoritative O Screening O |        |       |             |          |                           |                  |                   |                    | g 🛈 QSAR Model  |                |                        |
|--|--------|-------|-------------|----------|---------------------------|------------------|-------------------|--------------------|-----------------|----------------|------------------------|
|  |        |       |             |          |                           | Human Health Eff | fects             |                    |                 |                | Ecotoxicity            |
| Skipped (0)  |        | Acute | Mammalian 1 | Toxicity | enicity                   | Neurotoxicity    | Systemic Toxicity |                    |                 |                | 2ţ                     |
| Unlikely (6)<br>Filters (0)<br>Sorting (0)<br>Structure<br>CAS<br>Name   |        | Oral  | Inhalation  | Demal    | Genotoxicity Mutagenicity | Single Exposure  | Single Exposure   | Skin Sensitization | Skin Irritation | Eye Irritation | Acute Aquatic Toxicity |
| 37115-43-8<br>alpha-Hydroxyalp   |        | М     |             |          | L                         |                  |                   |                    |                 |                | VH                     |
| No CAS<br>No Name  | LIKELY | М     |             |          | L                         |                  |                   |                    |                 |                | н                      |
| No CAS<br>No Name  | LIKELY | М     |             |          | L                         |                  |                   |                    |                 |                | н                      |
| 98319-26-7<br>Finasteride  |        | м     |             |          | L                         |                  |                   |                    |                 |                | м                      |
| 75-64-9<br>tert-Butylamine   | GBT    | VH    | н           | L        | L                         | I.               | I                 | I.                 | VH              | н              | м                      |
| No CAS<br>No Name  | LIKELY | L     |             |          | L                         |                  |                   |                    |                 |                | Н                      |

### Mock scenario 3: Industrial spill into surface water

- Scenario: industrial chemical mix (aqueous film forming foam, AFFF) spilled into river/lake
- Commercially available AFFF mixture (Solberg Type 6) spiked into surface water sample
- Selected a total of 14 features for further investigation, multiple features assigned a structure



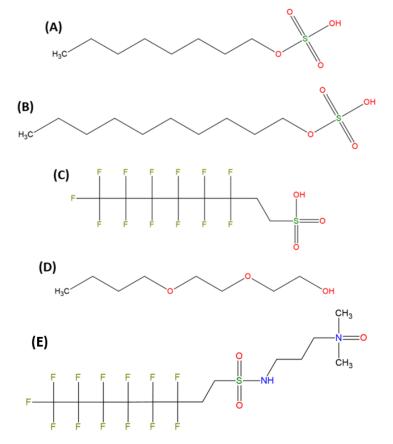
#### Mock scenario 3: Results (Structural identifications)

• 6 features  $\rightarrow$  5 structural assignments

Environmental Protection

Agency

- Chemical (E) present in both ESI+/ESI- data
- For each of these chemicals, molecular formula, WebApp MS1 tool, and one of the MS/MS approaches agreed on identity
- Reported identifications in 68 hours, structure assignments confirmed via literature review



(A) Octyl hydrogen sulfate; (B) Decyl hydrogen sulfate;
(C) 6:2 fluorotelomer sulfonic acid; (D) 2-(2-Butoxyethoxy)ethanol;
(E) N,N-Dimethyl-3-((perfluorohexyl)ethylsulfonyl) aminopropanamine N-oxide



#### Mock scenario 3: Results (All other assignments)

| Feature ID | Polarity<br>(ESI+/ESI-) | Measured accurate<br>mass (Da) | RT (min) | Final Identification Level   |
|------------|-------------------------|--------------------------------|----------|--|
| 7          | ESI+                    | 208.9575                       | 8.449    | Level 4 (C <sub>5</sub> H <sub>5</sub> Cl <sub>2</sub> N <sub>3</sub> S) |
| 8          | ESI+                    | 162.9899                       | 6.275    | Level 4 (C <sub>4</sub> H <sub>3</sub> ClN <sub>2</sub> O <sub>3</sub> ) |
| 9          | ESI+                    | 99.9837                        | 6.325    | Level 5  |
| 10         | ESI+                    | 184.1077                       | 6.255    | Level 5  |
| 11         | ESI-                    | 135.9952                       | 6.256    | Level 5  |
| 12         | ESI-                    | 257.9545                       | 6.320    | Level 5  |
| 13         | ESI-                    | 307.9910                       | 8.447    | Level 5  |
| 14         | ESI-                    | 335.9635                       | 8.447    | Level 5  |

# Mock scenario 3: Metrics for Success

1. Speed of analysis

ntal Protectior

- 68 "active" hours
- 2. Confidence in identifications
  - Structures assigned for 5 chemicals, confirmed postanalysis
- 3. Hazard assessment provided
- 4. Transferability of the approach
  - N/A; same Analyst 2 as scenario 1

| Chemicals: 5   |       | Toxicity:   | VH - Very High | H - High M                | - Medium L - Low I - In | conclusive N/A - Not Applicable | e Authority: A     | uthoritative    | U Screening    | g 🕛 QSAR Model         |
|--|-------|-------------|----------------|---------------------------|-------------------------|---------------------------------|--------------------|-----------------|----------------|------------------------|
|  |       |             |                |                           | Human Health Eff        | fects                           |                    |                 |                | Ecotoxicity            |
| Skipped (0)  | Acute | e Mammalian | Toxicity       | enicit                    | Neurotoxicity           | Systemic Toxicity               |                    |                 |                | Ę1                     |
| Unlikely (8)<br>Filters (0)<br>Sorting (0)<br>Structure<br>CAS<br>Name | Oral  | Inhalation  | Dermal         | Genotoxicity Mutagenicit. | Single Exposure         | Single Exposure                 | Skin Sensitization | Skin Irritation | Eye Irritation | Acute Aquatic Toxicity |
| 110-11-2<br>Octyl hydrogen s…  | М     |             |                | VH                        |                         |                                 |                    | Н               |                | М                      |
| 142-98-3<br>Decyl hydrogen s…  | М     |             |                | н                         |                         |                                 |                    | н               |                | н                      |
| 112-34-5 GB<br>2-(2-Butoxyethox  | M     | 1           | L              | L                         |                         |                                 | 1                  | М               | н              | L                      |
| 80475-32-7 G<br>N,N-Dimethyl-3-((                                      | BT /  |             |                | 1                         |                         |                                 |                    |                 |                | 1                      |
| 29765-95-5<br>3,3,4,4,5,5,6,6,7, LIKE                                  | м     |             |                | T                         |                         |                                 |                    |                 |                | L                      |

# Metrics for Success for All scenarios

1. Speed of analysis

ental Protection

- All chemical assignments provided to Analyst 1 within 72-hour window
- 2. Confidence in the eventual chemical identifications
  - Majority of chemicals were assigned a structure; all structure assignments were confirmed post-analysis
- 3. Degree of hazard assessment that can be performed
  - Utilized the Hazard Comparison Module to aggregate relevant measured and predicted toxicity values for chemicals assigned a structure
- 4. Transferability of the designed NTA method/workflow
  - Different individual assumed the role of "Analyst 2" for mock scenario 2 than the other scenarios; method and workflow could ultimately be transferred to regional, state, and other labs with minimal training to incorporate NTA

# Current Limitations and Future Work

• Current limitations/Future work:

ntal Protectior

- All scenarios included "known" chemicals

   → continue to improve workflows for
   narrowing down lists of tentative
   candidates
- Qualitative, not quantitative → incorporating quantitative NTA approaches for concentration estimates, improving hazard assessment
- Only used LC-MS → Exploring GC-MS NTA methods for rapid response scenarios

|                          | Medium/High<br>Concentration   | Trace Concentration  |
|--------------------------|--|--|
| Known Chemical           | <b>Easy</b> - chemicals of interest can be identified using rapid range finding                                    | <b>Easy</b> - if information<br>about chemical(s) of<br>interest are available<br>(e.g., the masses of the<br>compounds) |
| Undocumented<br>Chemical | Medium Difficulty<br>- focus can be placed on<br>selected features; correct<br>identification is not<br>guaranteed | <b>Difficult</b> – situational<br>information is needed;<br>chances of identification<br>are lower                       |

"Known Unknowns" vs. "Unknown Unknowns"

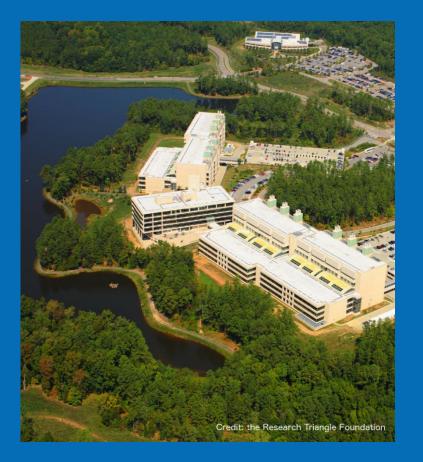


# Conclusions

- Situations where traditional, targeted methods cannot elucidate the identity of an unknown → NTA is a useful, additional analytical tool
- The three mock scenarios presented showcase the applicability of NTA approaches
- The success of each mock scenario against the identified metrics for success was discussed
  - Level of success increases as complexity of specific scenario decreases



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