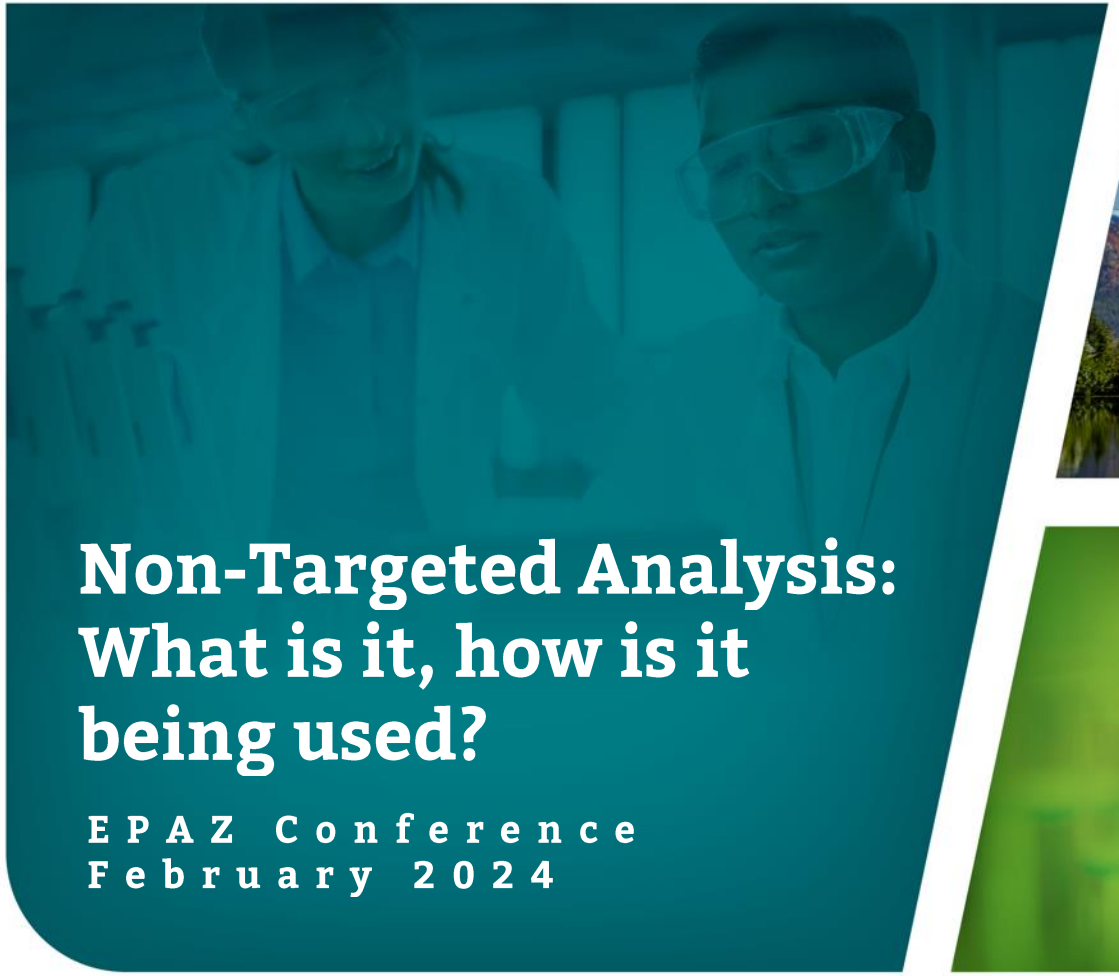

Non-Targeted Analysis: What is it, how is it being used?

EPAZ Conference
February 2024

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Outline

- What is Non-Targeted Analysis (NTA)?
- History, how and why we got here
- High-Resolution Mass Spectroscopy (HRMS)
- Examples with focus on PFAS
- Take-away for Project Managers

Non-Targeted Analysis (NTA)

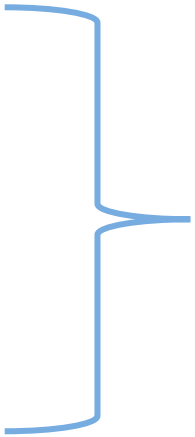
- "… a theoretical concept that can be broadly defined as the characterization of the chemical composition of any given sample without the use of *a priori* knowledge regarding the sample's chemical content."

- Suspect Screening Analysis (SSA) "… the identification of chemicals and/or chemical classes detected by an instrument (*e.g.*, MS) by comparison to a pre-defined user list or library containing known chemicals of interest."

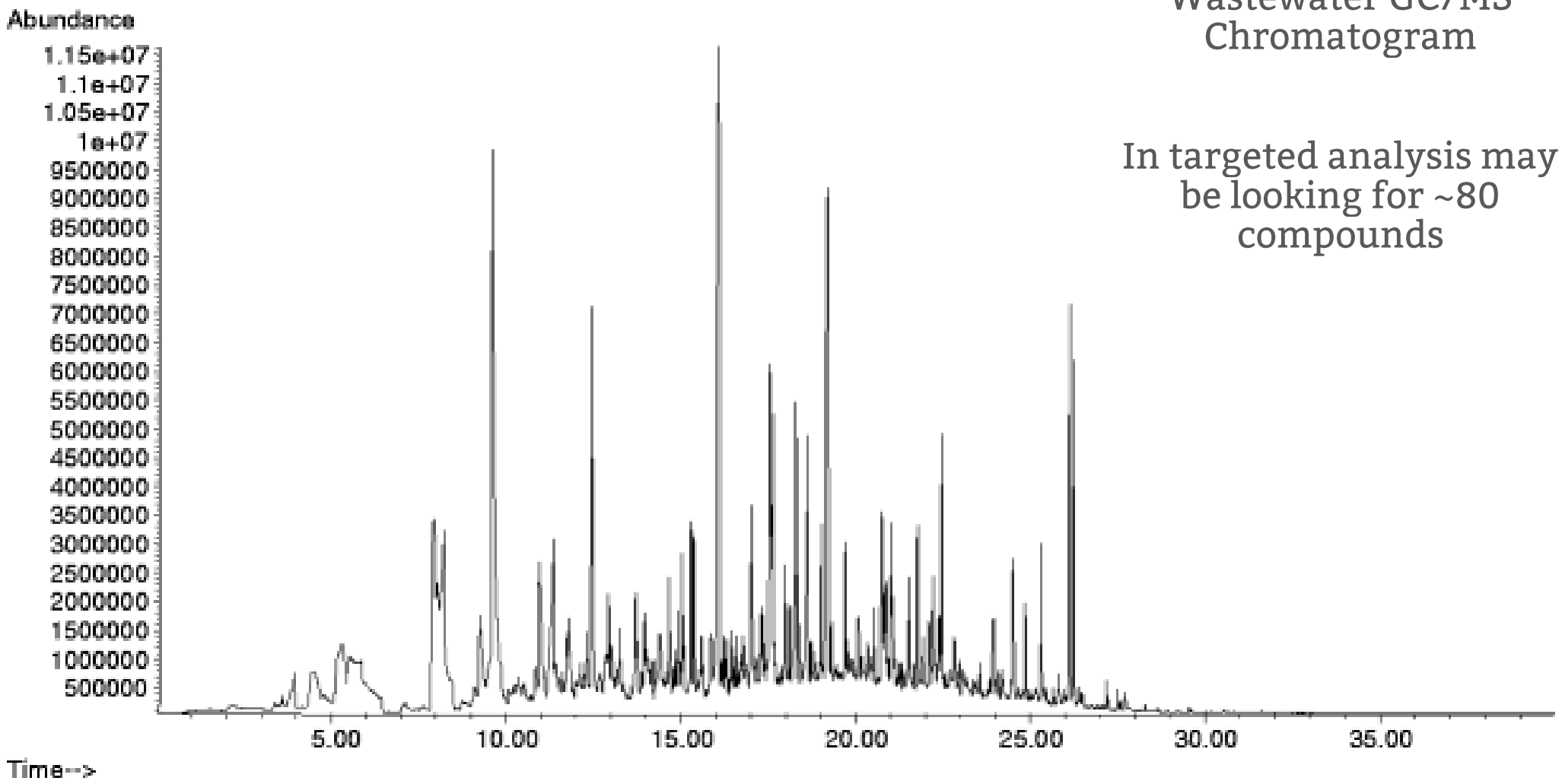
- Not limited to GC or LC + HRMS, but that is my focus here.

History/Background that led us to NTA and SSA

- 1962 – Silent Spring
- 1970-1980s
  - –CWA, SDWA, CAA
    - List of Priority Pollutants
    - Chemical contaminants with MCL/MCLGs
  - Gas Chromatography coupled to Mass Spectrometry (GC/MS)
- 2000s - Enhancement in GC & LC with MS
  - Library development and use with MS – tentatively identified compounds (TICs)
  - HRMS
- Computing Capability and Internet Sharing



Chemical Space

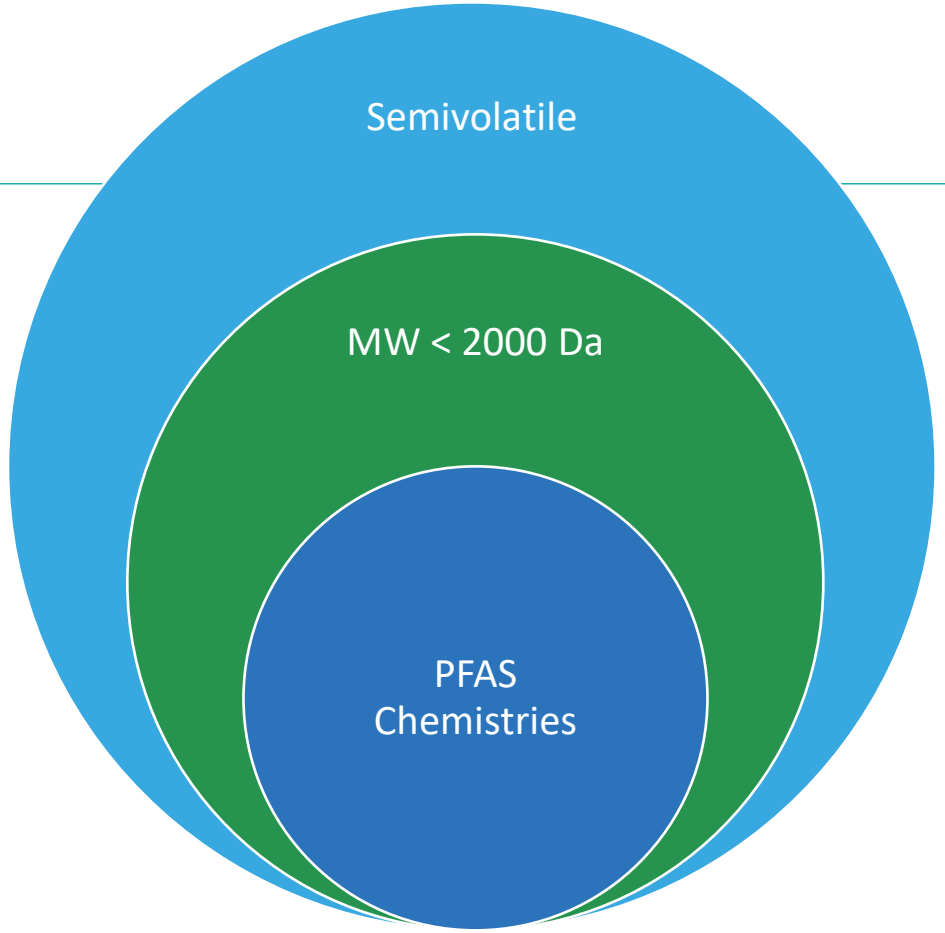


Wastewater GC/MS Chromatogram

In targeted analysis may be looking for ~80 compounds

Chemical Space

- Anthropogenic Organic Compounds
  - 100 million CAS entries
  - TSCA – 85,000 (1462 PFAS)
  - 30,000 – 70,000 chemicals in our households
  - SDWA lists 126 priority pollutants
- PFAS Speciated Methods
  - Method 1633 – 70 compounds
  - Data suggest they only account for 1-50% of total fluorine content
- Non-Speciated Methods
  - Method 1621 just released
  - Combustion Ion Chromatography (CIC)



PFAS Chemical Space

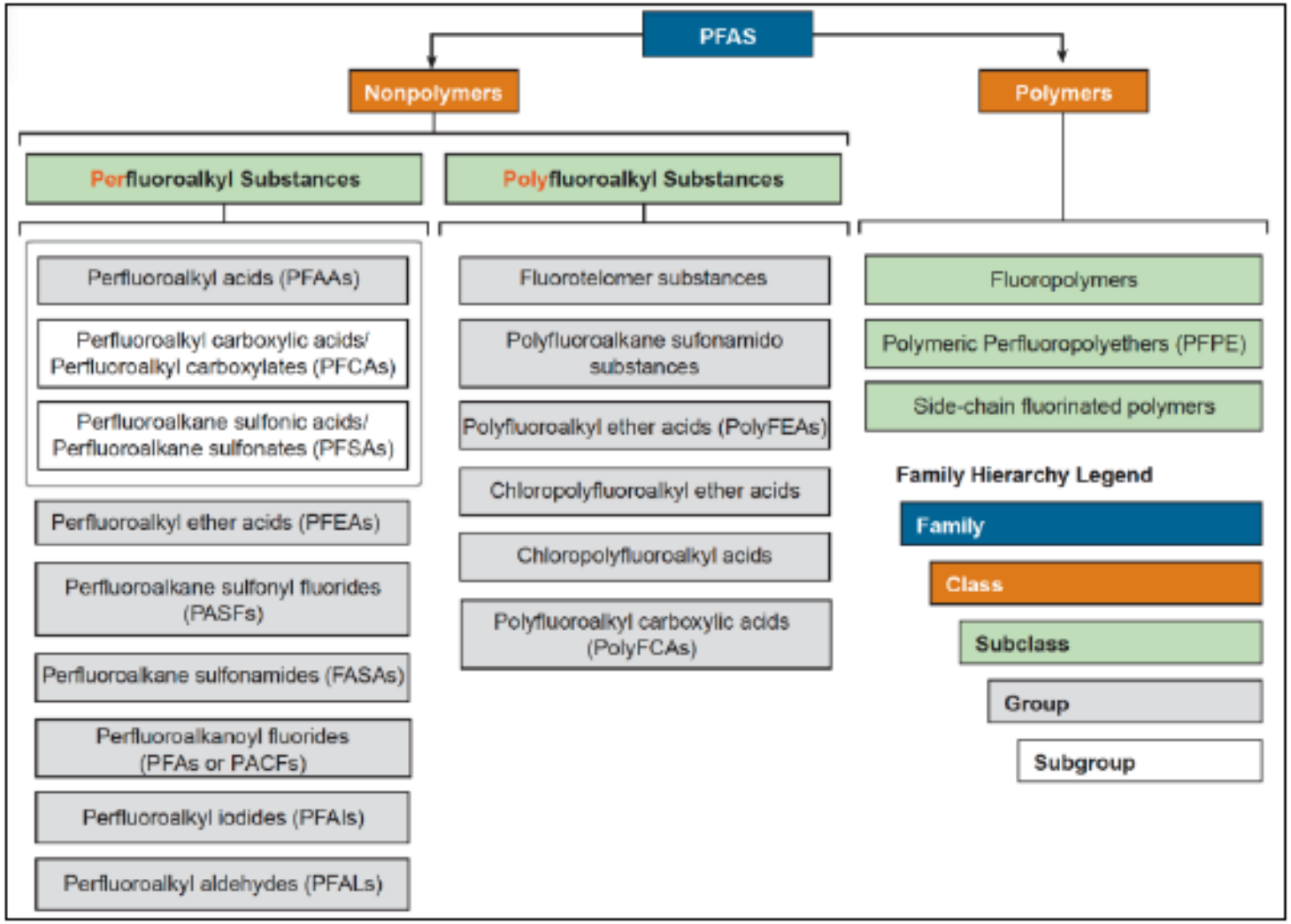


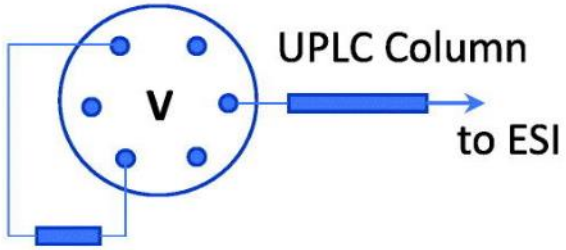
Figure 1. The PFAS Family.

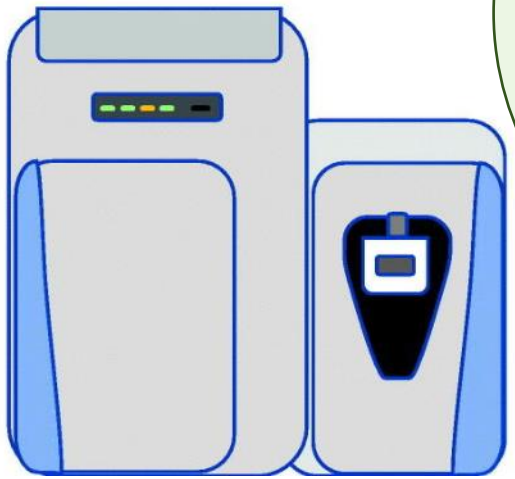
Analysis Workflow

- Sample collection.
  - Volatility, suspended solids, preservation.
- Sample preparation, instrumentation, and search databases affect what can be seen and identified.
- In the PFAS space, there are also variations in preparation and instrumentation type and parameters that impact PFAS types.
  - Work with consulting chemists and the laboratory chemists to develop objectives and analytical path.
- Following data acquisition, preprocessing steps by laboratory, with your input, to reduce data quantity and complexity.
  - Prioritize what to look at and for; evaluate/subtract blanks.

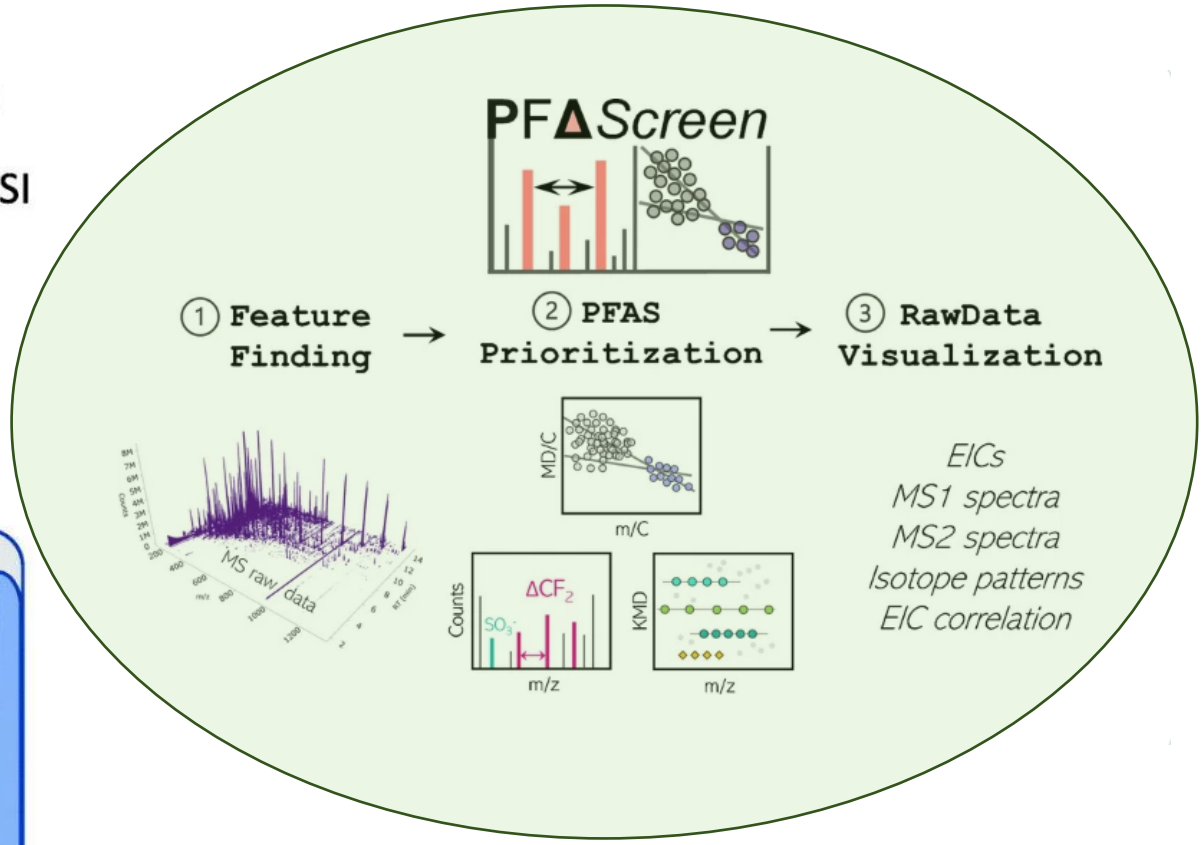
Analysis Workflow (Cont.)

SPE





LC-MS (HRMS)



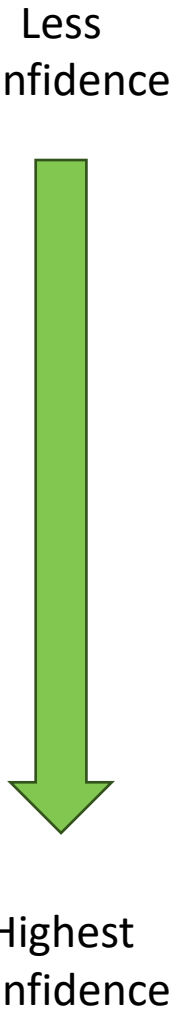
Data Analysis – “Magical Black Box” for this Presentation

- Full-scan *versus* MS/MS fragmentation
- Data dependent (DDA) or data independent acquisition (DIA)
- MS^n
- Mass Defect Identification
- Homologous Pattern Recognition
- Characteristic In-source Fragment Identification
- Characteristic Fragment Ion Flagging
- Fragment Mass Difference Measurements.

Reporting -Identification/Confidence

- Non-targeted, typically no standards used, or available.
- Rely upon Levels that imply confidence.
  - **Level 5** – Exact masses of interest.
  - **Level 4** – Unequivocal molecular formula, insufficient information to propose possible structure(s).
  - **Level 3** – Candidate Structure. Possible, but gray, positional isomers. Class and possibly functional groups.
  - **Level 2** – Probable structure. Library match or diagnostic evidence. No other structure fits, but no standard or literature to support exact.
  - **Level 1** – Indistinguishable from reference standard. Ideal case, confirmation with a reference standard and matches in MS and chromatography.

Schymanski et al. 2014, Charbonnet et al. 2022



EPA Region 5/MI EGLE Report #2 Attachment 3 February 19, 2020

Table 2. PFAS Tentatively Identified in Fume Suppressants and Effluent at Chrome Plating Facilities by UPLC-MS.

Chem. Ref. #	Tentatively Identified Compound Name	CASRN	Formula	DTXCID	Monoisotopic Mass (g/mol)	RT	Confidence
1	6:2 Fluorotelomer sulfonic acid (6:2 FTS)	27619-97-2	C8 H5 F13 O3 S	DTXCID7037711	427.9758	6.54	1
2	6:4 Fluorotelomer Sulfonic Acid (6:4 FTS)		C10 H9 F13 O3 S		456.0071	7.08	2b
3	4:2 Fluorotelomer sulfonic acid (4:2 FTS)	757124-72-4	C6 H5 F9 O3 S	DTXSID30891564	327.9814	4.14	1
4	8:2 Fluorotelomer sulfonic acid (8:2 FTS)	39108-34-4	C10 H5 F17 O3 S	DTXCID10114844	527.9697	7.73	1
5	Perfluorobutane sulfonic acid (PFBS)	375-73-5	C4 H F9 O3 S	DTXCID3010030	299.9502	2.83	1
6	Perfluorohexanesulfonic acid (PFHxS) - Linear	355-46-4	C6 H F13 O3 S	DTXCID2011859	399.9444	6.01	1
7	Perfluorohexanesulfonic acid (PFHxS) - Branched Isomer	355-46-4	C6 H F13 O3 S	DTXCID2011859	399.9444	5.81	1
8	Perfluoroheptanesulfonic acid (PFHpS)	375-92-8	C7 H F15 O3 S	DTXCID1039347	449.9406	6.74	1
9	Perfluorooctane sulfonic acid (PFOS) - Linear	1763-23-1	C8 H F17 O3 S	DTXCID1011864	499.9386	7.30	1
10	Perfluorooctane sulfonic acid (PFOS) - Branched Isomer	1763-23-1	C8 H F17 O3 S	DTXCID1011864	499.9387	6.90	1
11	Perfluorooctane sulfonic acid (PFOS) - Branched Isomer	1763-23-1	C8 H F17 O3 S	DTXCID1011864	499.9387	7.10	1
12	Perfluorooctane sulfonic acid (PFOS) - Branched Isomer	1763-23-1	C8 H F17 O3 S	DTXCID1011864	499.9386	7.15	1
13	Perfluoropentanoic acid (PFPeA)	2706-90-3	C5 H F9 O2	DTXCID9037612	263.9834	2.15	1
14	Perfluoropentanesulfonic acid (PFPeS)	2706-91-4	C5 H F11 O3 S	DTXCID4037613	349.9470	4.89	1
15	Perfluorocyclohexyl sulfonic acid	2106-55-0	C6 H F11 O3 S	DTXCID10197556	361.9471	4.87	3
16	Perfluoro-4-(perfluoroethyl)cyclohexylsulfonic acid	646-83-3	C8 H F15 O3 S	DTXCID70227328	461.9412	6.60	3
17	Perfluoro-4-(perfluoroethyl)cyclohexylsulfonic acid	646-83-3	C8 H F15 O3 S	DTXCID70227328	461.9412	6.39	3
18	Perfluoro-4-(perfluoroethyl)cyclohexylsulfonic acid	646-83-3	C8 H F15 O3 S	DTXCID70227328	461.9412	6.49	3
19	Perfluoro-4-(perfluoroethyl)cyclohexylsulfonic acid	646-83-3	C8 H F15 O3 S	DTXCID70227328	461.9412	6.72	3
20	1,2,2,3,3,4,5,5,6,6-Decafluoro-4-(trifluoromethyl)cyclohexanesulfonic acid	742-73-4	C7 H F13 O3 S	DTXCID20815698	411.9442	5.87	3
21	6:2 Fluorotelomer phosphate monoester	57678-01-0	C8 H6 F13 O4 P	DTXCID00200745	443.9800	6.38	2a
22	Chlorinated 6:2 Fluorotelomer sulfonic acid		C8 H4 Cl F13 O3 S		461.9367	6.96	2b
23	1,1,2,2,3,3,4,4-Octafluorobutane-1-sulphonic acid	70259-86-8	C4 H2 F8 O3 S	DTXCID801022285	281.9598	1.02	3

Confidence:

1= Confirmed by comparison with reference chemical

2a= Likely structure based on computerized spectrum match

2b= Likely structure based on manual interpretation of MS/MS spectrum

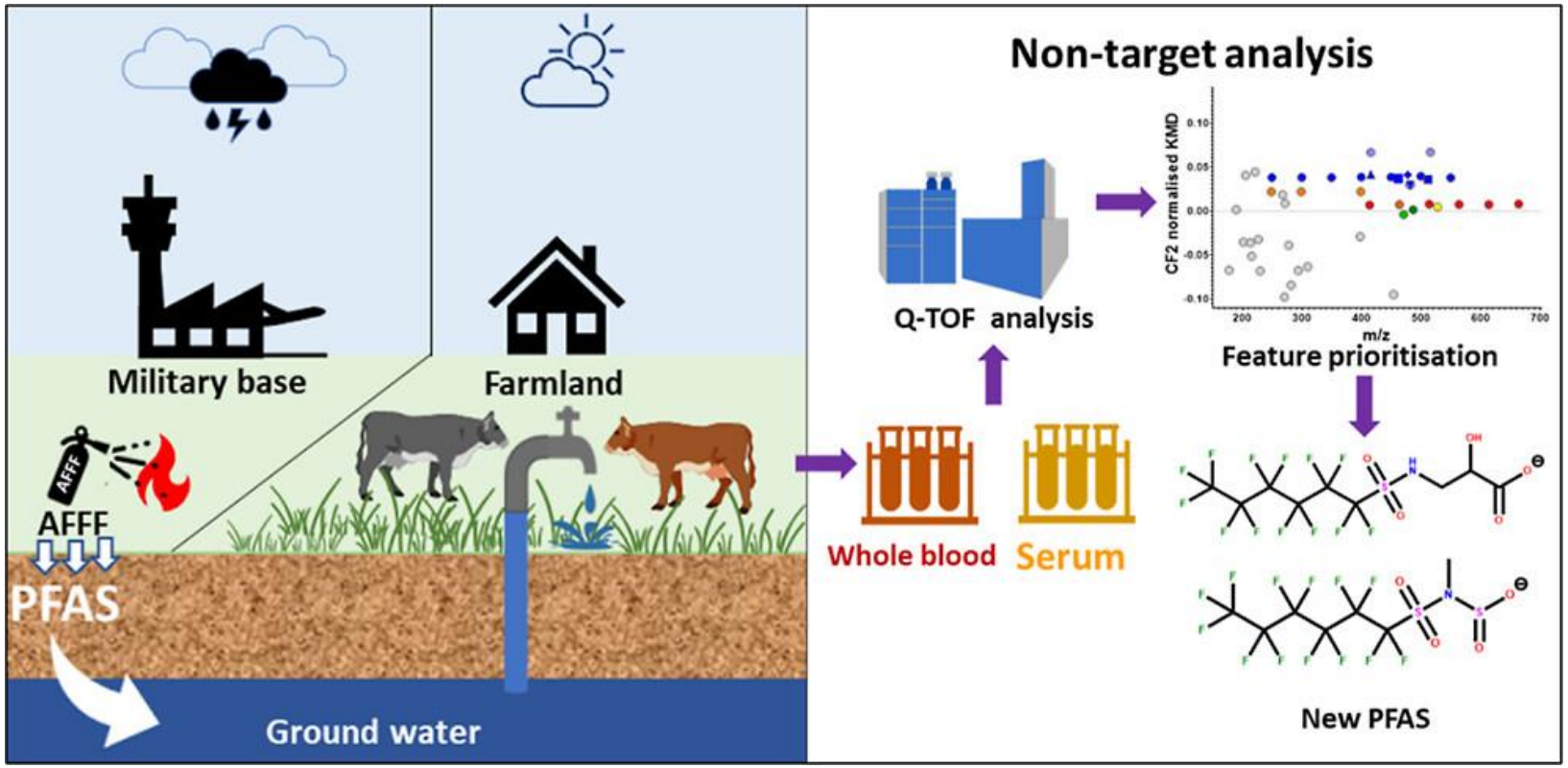
3= Tentative candidate or MS data insufficient for unequivocal identification beyond class (i.e. PFAS chemical)

4 = Formula level identification only (MS/MS unavailable)

5= Exact mass level identification only (no predicted formula)

STUDY RESULTS: PFAS IN FUME SUPPRESSANT PRODUCTS AT CHROME PLATING FACILITIES EGLE June 2020

PFAS Example - AFFF

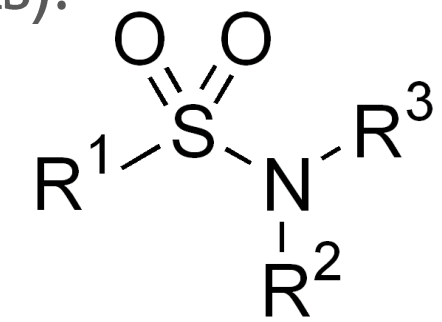


- AFFF contaminated GW and farmlands from firefighting training.
- Measured via NTA in whole blood, and serum from cattle exposed to GW.

Dewapriya, Pradeep et al 2023. https://doi.org/10.1021/acs.est.3c03852

PFAS Example – AFFF (Cont.)

- AFFF used since 1960s, 3-6% by weight of PFAS chemistries.
- Studies using extractable organofluorine technique indicate ~50% of total mass not identified by target analysis.
- Prior studies of farm animal plasma via targeted analysis indicated sulfonic acids (PFSAs) > carboxylic acids (PFCAs).
- This NTA Study Identified:
- 30 non-targeted PFAS chemistries with various confidence identified, including three novel compounds across two different groups:
    - Sulfonamido, sulfuramidous acids



PFAS Example – WWTP

- Influent, effluent, downstream water samples.
- Negative and positive ionization.
- Blank contaminants electronically subtracted from samples.
- Suspect screening and NTA
  - Looked for target analytes first.
- Compared influent and effluent ratios (decrease or increase?).

http://pubs.acs.org/action/showCitFormats?doi=10.1021/acs.est.8b03030
Wang et al., 2018

PFAS Example – WWTP (Cont.)

- 90 PFAS chemistries identified with varying levels of confidence.
- 15 “classes” of PFAS with Level 3 or greater confidence identified.
  - 12 “Legacy” PFAS compounds identified (PFOS, *etc.*).
  - 41 previously reported, covering 7 classes
  - 37 new compounds, covering 6 classes
- Only 1 compound appeared to have been removed by WWTP.
- 4 PFAS classes increased in WWTP – precursors?
- Chlorine-substituted perfluoroalkyl carboxylates(Cl-PFCAs) appear to be produced in the WWTP.

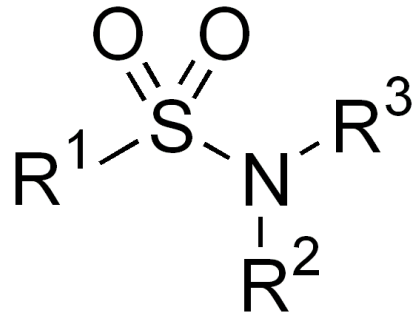
PFAS Example – Semiconductor Associated

- Photolithography construction onto wafers has entailed use of PFAS chemistries.
  - Photoacid generators (PAGs), photosensitizers, antireflective coatings, rinse surfactants.
- In 2017, PFOS was “eliminated” from manufacturing[1], and commitment was made to phase out PFOA and related by 2025.
- Study[2] focused on acidic and neutral PFAS chemistries.
- Ten WW samples from five semiconductor plants 2020-2021.
- Target analysis via isotopic dilution.

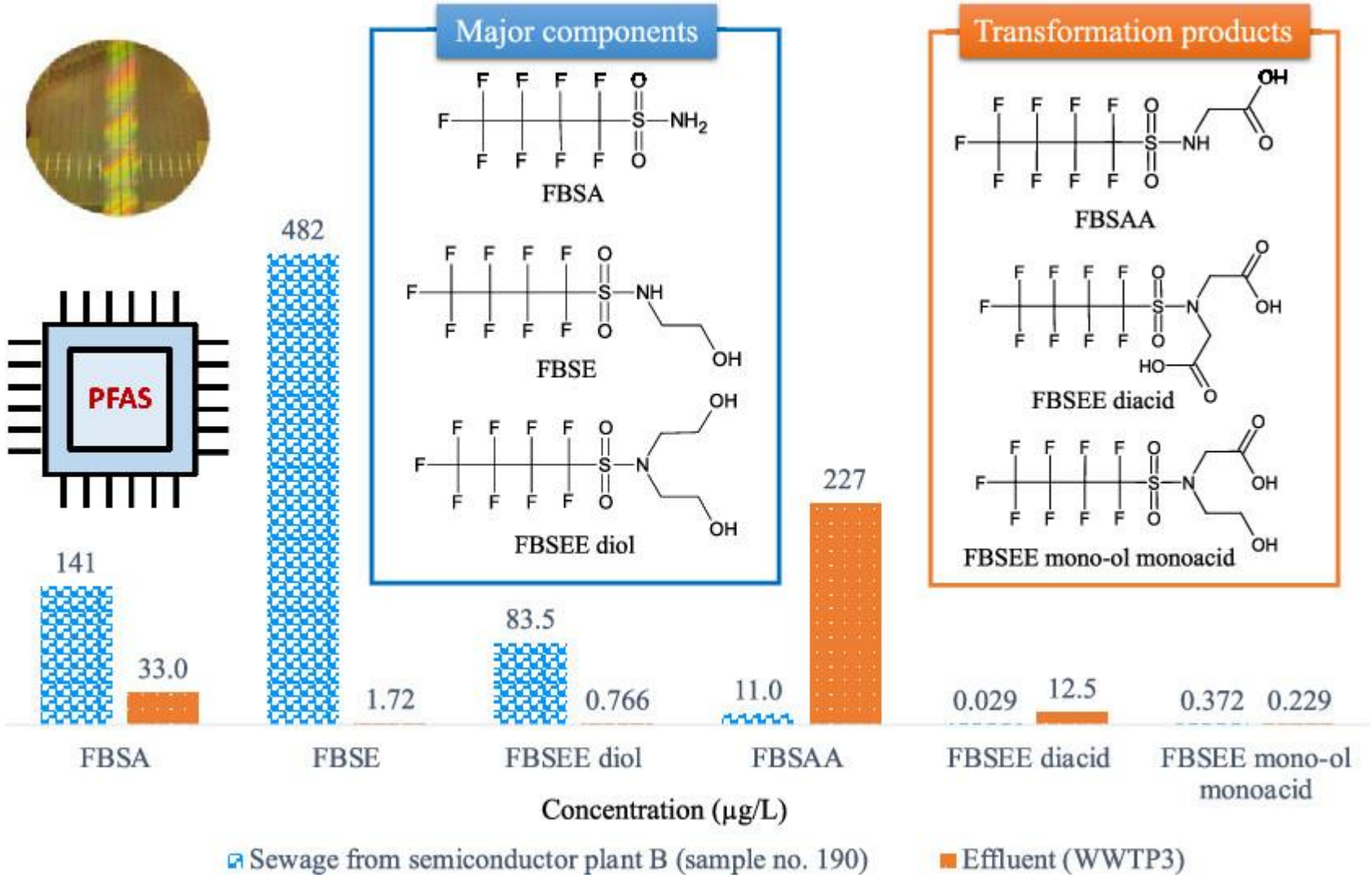
1 – World Semiconductor Council, 2017
2 – https://doi.org/10.1021/acs.est.3c04435?urlappend=%3Fref%3DPDF&jav=VoR&rel=cite-as

PFAS Example – Semiconductor Associated (Cont.)

- 20 PFAS compounds identified, 15 for the first time associated with semiconductors.
- Primarily C_4 sulfonamido derivatives up to 480 µg/L. Three ultra-short chain (C_2-C_4) identified 0.004 – 20 µg/L.
- Propose conversion of alcohols to acids during aerobic treatment.
- Sulfonamido compounds made up ~90% of total, yet prior data focused on carboxylic acids, which was minor component.
- Aerobic degradation can result in C_4 target end products (*e.g.*, PFBS, PFBA).



PFAS Example – Semiconductor Associated (Cont.)



Technical Take-Away

- Allows us to open up the chemical space.
- ~6000 PFAS chemicals *vs* ~100 by targeted analysis.
- Identify novel compounds for risk, remediation, fate and transport.

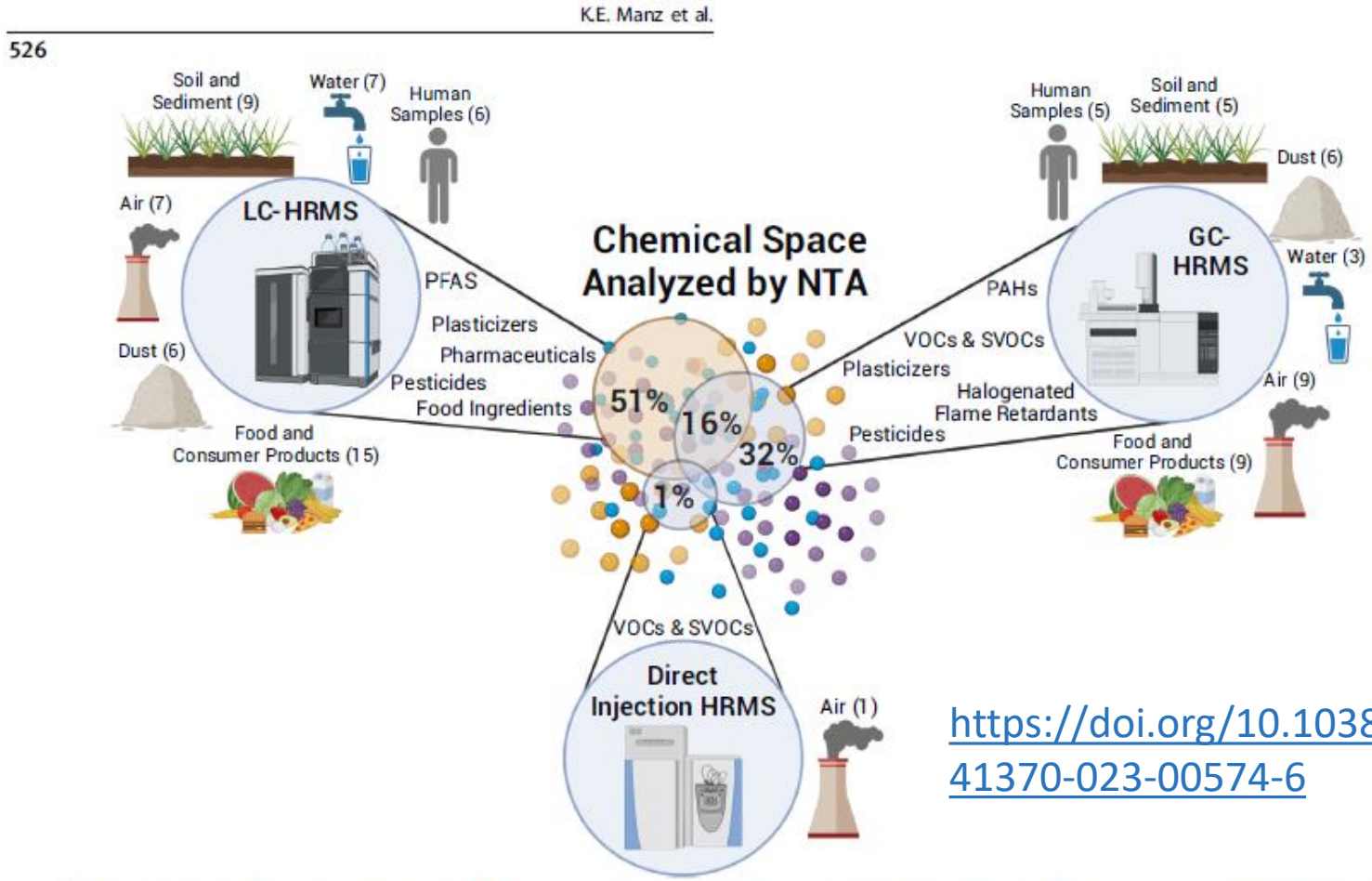


Fig. 1 Schematic diagram of the chemical space analyzed by HRMS using NTA. The number and percentage of studies using liquid chromatography (LC), gas chromatography (GC), or direct injection paired with high-resolution mass spectrometry (HRMS) are shown, including the types of chemicals that were detected and the media that have been characterized using each approach. Figure created with BioRender.com.

https://doi.org/10.1038/s41370-023-00574-6

Contracting and Interpretation Take Away

- Have the end in mind, what you are looking for and why, legal implications.
- Start with indication that target analysis is insufficient – non-speciated (Method 1621) *versus* speciated (targeted) analysis.
- Cost may be unit + time & materials (interpretation).
- Reported data should/will include Confidence Levels of compound identification.
- Non-quantitative, only provides compound types and potential names. Greatly reduces potential for false positives.
- Data files are akin to retaining a sample, can be used later for retrospective analysis and interpretation. Retain files.
- Reproducibility of NTA, due to the multiple data processing options, is challenging. This would hopefully preclude regulations that codify or require the use of NTA.

Thank you.
Questions?

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- Dewapriya, Pradeep et al 2023. Novel Per- and Polyfluoroalkyl Substances Discovered in Cattle Exposed to AFFF-Impacted Groundwater. https://doi.org/10.1021/acs.est.3c03852
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- Chen, Yi-Ju et al 2024. Emerging Perfluorobutane Sulfonamido Derivatives as a New Trend of Surfactants Used in the Semiconductor Industry. https://doi.org/10.1021/acs.est.3c04435
- GitHub - JonZwe/PFAScreen: PFAScreen is an open-source Python based non-target screening software tool.
- Targeted and Nontargeted Analysis of PFAS in Fume Suppressant Products at Chrome Plating Facilities. Michigan EGLE June 2020
- Best Practices for Non-Targeted Analysis. https://nontargetedanalysis.org/

