

# PFAS Signature® : A Forensic Tool to Differentiate AFFF and Non-AFFF PFAS Sources

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# Agenda

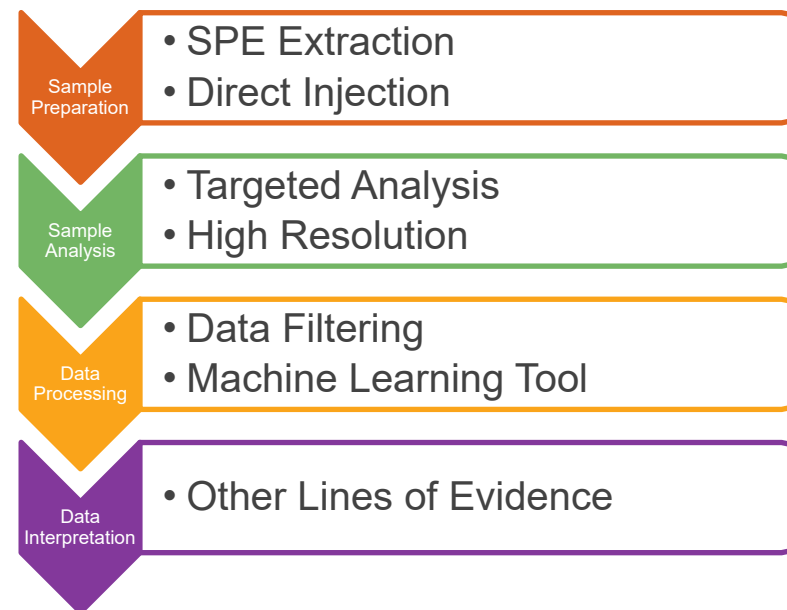
- Overview of Signature®
- High Resolution Mass Spectrometry
  - Targeted vs Suspect Forensics
- Machine Learning Insights
- Site Applications

# Overview of Signature®

# PFAS Signature®

- Source discrimination through the combination of analytical chemistry and data analytics
- High-resolution mass spectrometry extends the list from 40 to **520 PFAS analytes**
- PFAS-focused data filtering tools allows for informed and efficient data reduction
- Trained artificial intelligence/machine learning (AI/ML) tools allows for the identification and discrimination of PFAS Sources
- Identifies gaps that would not have been revealed by targeted analysis alone

## Workflow



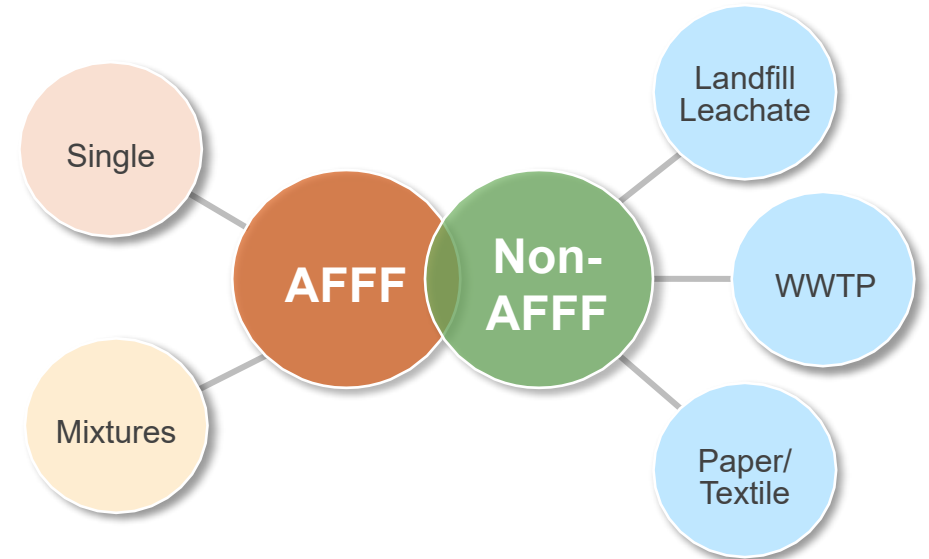
## Tool Components

- Targeted Analysis
- High Resolution MS Analysis
- Data filtering
- PFAS Suspect Screening Library
- PFAS Source Library
- Statistical Analysis

# PFAS Signature®

## Includes Database of Source Specific Signature Library

- AFFF Formulations
  - More than 35 sources of different AFFFs
- AFFF-Impacted Sites (Multiple Matrices)
  - WWTP located within AFFF impacted site
  - AFFF impacted biosolids applied soil
  - AFFF used for emergency response
- Waste Sector
  - Landfill Leachates
  - Municipal WWTP related samples and additives
  - Paper Mill related WWTP samples
  - Compost
- Commercial Products
  - More than 15 commercial products
- Metal Plating



Library is continually populated as more source data is generated

# PFAS Signature® Can Assist in Establishing Background

- From EPA Guidance EPA 540-R-01-003, Background Samples are needed....
  - Gaps in the available data (certain chemicals were excluded from the sample analyses, or certain soil types were not collected).

Suspect screening library for up to 520 PFAS

- Identifies chemicals that would not have been identified by the targeted analysis
- Supports development of the conceptual site model to validate assumptions
- Identifies contributing sources that are not the 'known' or expected source(s)



[Guidance for Comparing Background and Chemical Concentrations in Soil for CERCLA Sites \(epa.gov\)](https://www.epa.gov/epaosopr/ocert/540r01003.pdf)

# PFAS Signature® Applications



**Source identification, screening, and delineation**



**Establishing Background**



**Filling data gaps**



**Understanding PFAS transport**



**On/off site migration**



**Mass balance evaluations**

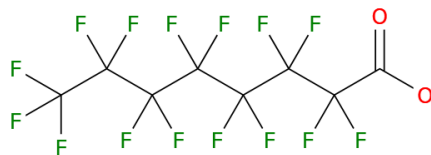
# High Resolution Mass Spectrometry



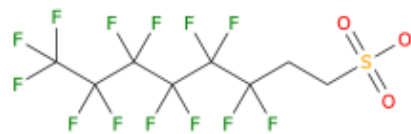
# Targeted vs Suspect Screening

## Targeted Analytes

- PFAAs



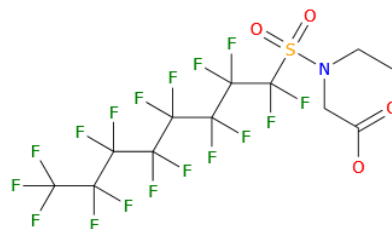
- FTSs



- FASAs

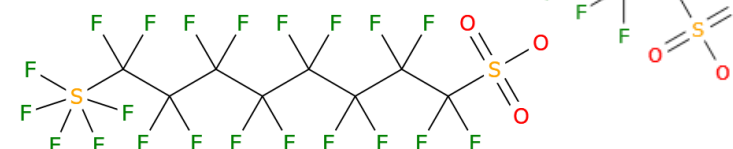
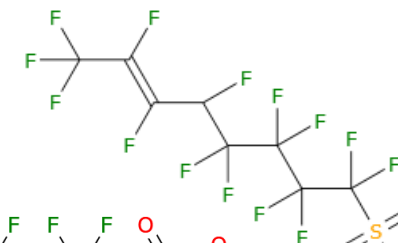
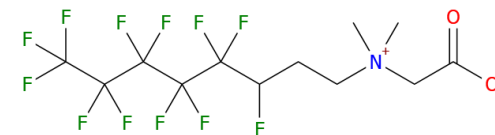
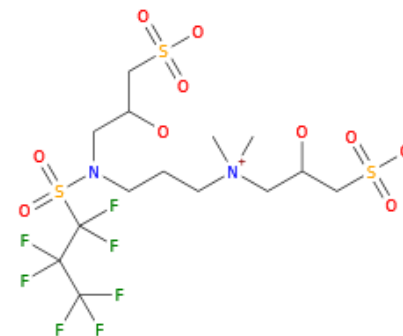


- FASAAs



## Suspect Analytes

- ECF Sulfonamides
- Fluorotelomer Betaines
- Phosphorus Compounds
- H-substituted / Unsaturated PFAAs
- Other Substitutions



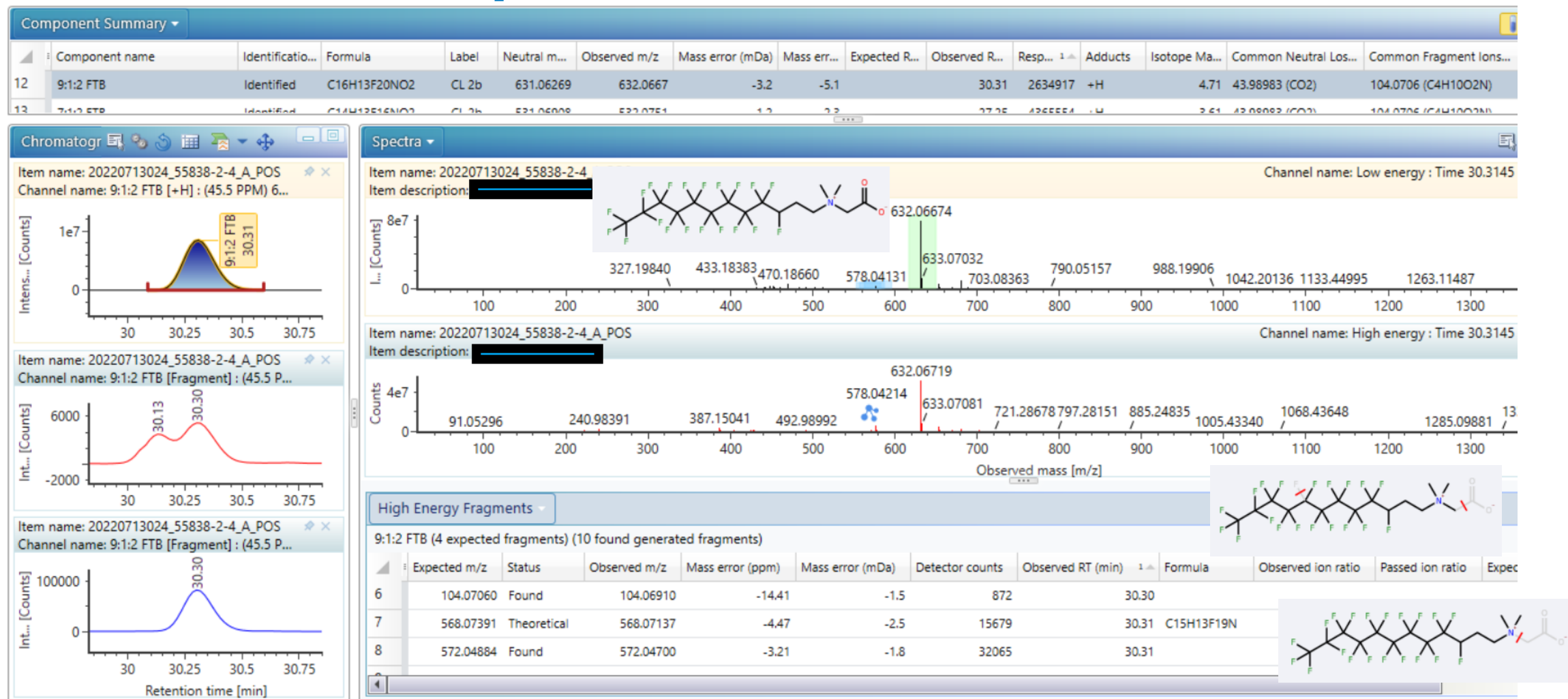
# HRMS Data Filtering and Peak Confirmation

- PFAS homolog data filtering, followed by peak confirmations by HRMS analyst.
- Source analysis using PFAS Signature<sup>®</sup> methodology.

Analyte identification confidence level followed Schymanski et al. (2014) criteria  
cf. Charbonnet et al, 2022

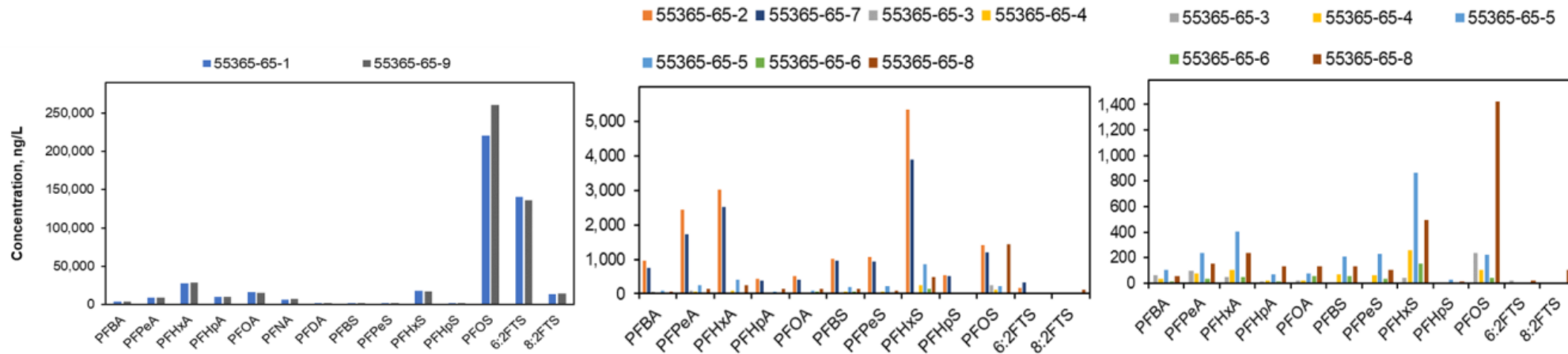
<b>Level 1</b> Confirmed Structure	<ul style="list-style-type: none"><li>• Reference Standard (MS, MS<sup>2</sup>, RT)</li></ul>
<b>Level 2</b> Probable Structure	<ul style="list-style-type: none"><li>• <i>Level 2a.</i> Library Spectrum Match (MS, MS<sup>2</sup>, Experimental Data)</li><li>• <i>Level 2b.</i> Diagnostic Evidence (MS, MS<sup>2</sup>, Experimental Data)</li></ul>
<b>Level 3</b> Tentative Candidates	<ul style="list-style-type: none"><li>• Structure, Substituent, Class (MS, MS<sup>2</sup>, Experimental Data)</li></ul>
<b>Level 4</b> Unequivocal Molecular Formula	<ul style="list-style-type: none"><li>• MS isotope/adduct, fragment</li></ul>
<b>Level 5</b> Exact Mass	<ul style="list-style-type: none"><li>• m/z (MS)</li></ul>

# An HRMS Example



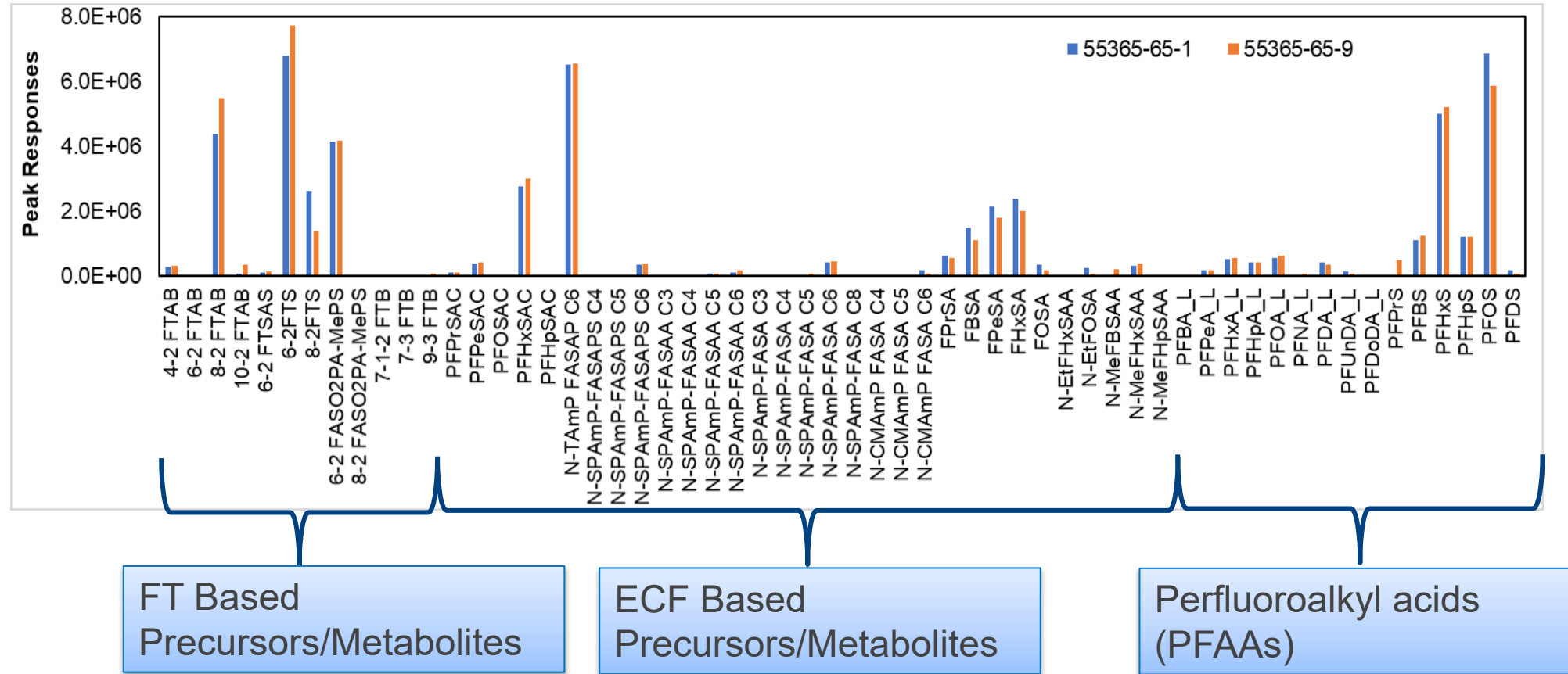
# Targeted vs Suspect Forensics

# PFAS Signature® provides information that cannot be obtained from targeted analysis alone



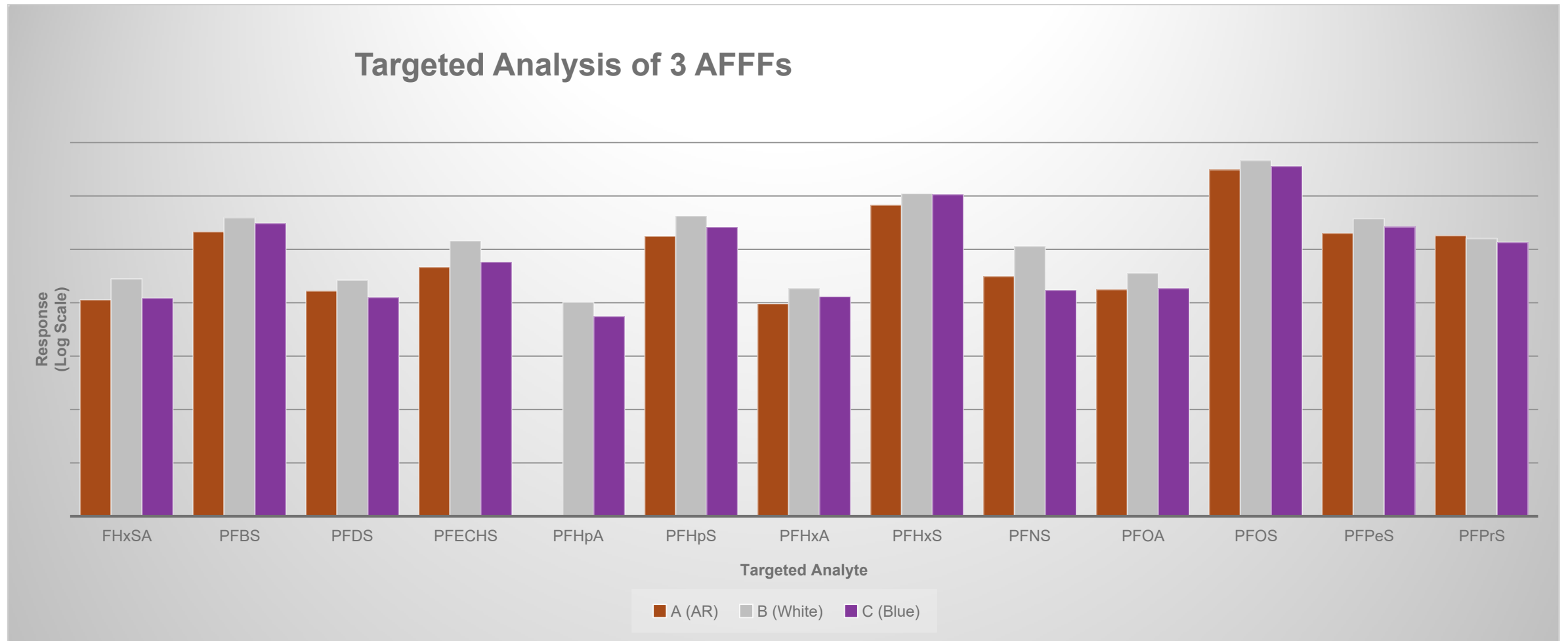
- Targeted analysis provides very limited information
- Only information on PFAAs which are commonly found associated with many sources
- Not enough information to identify or discriminate PFAS sources

# PFAS Signature<sup>®</sup> Provides More PFAS Information

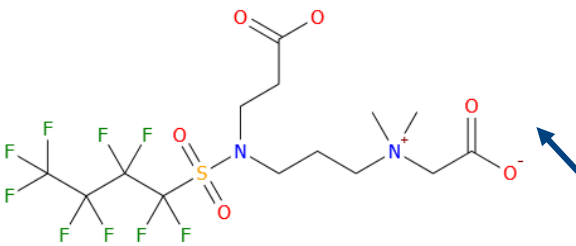
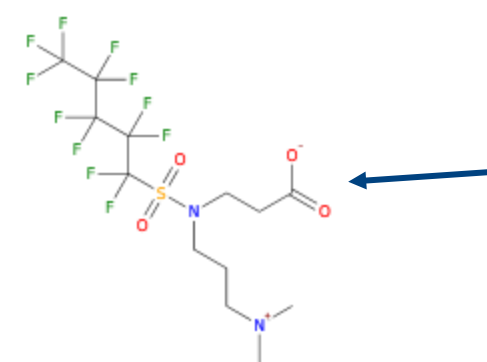
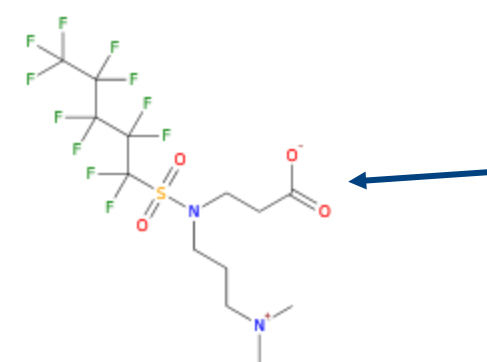


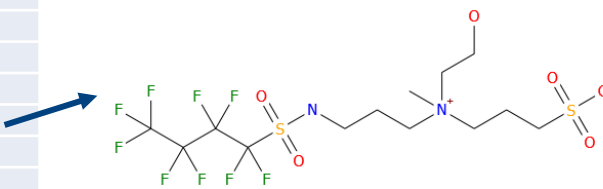
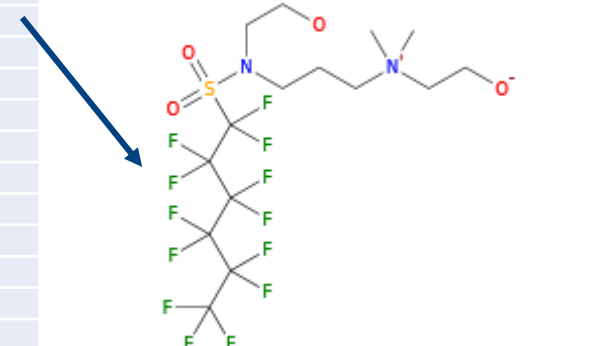
More than 100 analytes detected with high resolution mass spec  
Branched and linear isomers demonstrates mix of both FT and ECF chemistries

# A Source Library Case Study



# PFAS Signature<sup>®</sup> Provides More PFAS Information

A&C, Not B		B, Not A or C	
	4:2 FTSHA (+)	N-AHOB-FASAPS C6 (-)	N-HOEAmP-FASE C6 (+)
	7:1 PFAS (-)	N-DiHOPAmHOB-FASA C3 (-)	N-HOEAmP-FASE C7 (+)
	H-UPFOS (-)	N-DiHOPAmHOB-FASA C4 (-)	N-HOEAmP-FASE C8 (+)
	N-CMAMP FASA C3 (+)	N-DiHOPAmHOB-FASA C5 (-)	N-SHOPAmP-FASA C6 (-)
	N-CMAMP FASA C4 (-/+)	N-DiHOPAmHOB-FASA C6 (-)	N-SHOPAmP-FASAA C5 (+)
	N-CMAMP FASA C5 (-/+)	N-DiHOPAmHOB-FASAPS C3 (-)	N-SPAmP-FASA C3 (+)
	N-CMAMP FASA C6 (-/+)	N-DiHOPAmHOB-FASAPS C4 (-)	N-SPAmP-FASA C4 (-)
	N-CMAMP FASAP C4 (+)	N-DiHOPAmHOB-FASAPS C5 (-)	N-SPAmP-FASA C4 (+)
	N-CMAMP FASAP C5 (+)	N-DiHOPAmHOB-FASAPS C6 (-)	N-SPAmP-FASA C5 (-)
	N-CMAMP FASAP C6 (+)	n-F5S-PFHpS (-)	N-SPAmP-FASA C5 (+)
	N-MeFHxSAA (-)	N-HOEAmHOP-FASA C4 (+)	N-SPAmP-FASA C6 (-)
	N-OxAmP-FASA C4 (+)	N-HOEAmHOP-FASA C5 (+)	N-SPAmP-FASA C6 (+)
	N-TAmP FASAP C3 (+)	N-HOEAmHOP-FASA C6 (+)	N-SPAmP-FASA C8 (-)
	N-TAmP FASAP C4 (+)	N-HOEAmP FASAPS C2 (+)	N-SPAmP-FASA C8 (+)
	PFBSaAm (-)	N-HOEAmP FASAPS C3 (+)	N-SPAmP-FASAPS C3 (-/+)
	PFBSAC (+)	N-HOEAmP FASAPS C4 (+)	N-SPAmP-FASAPS C4 (-/+)
	PFHpSaAm (-)	N-HOEAmP FASAPS C5 (+)	N-SPAmP-FASAPS C5 (-/+)
	PFHpSAC (+)	N-HOEAmP FASAPS C6 (+)	N-SPAmP-FASAPS C6 (-/+)
	PFHxSAC (+)	N-HOEAmP FASAPS C7 (+)	N-SPAmP-FASAPS C8 (-)
	PFPeSaAm (-)	N-HOEAmP FASAPS C8 (+)	N-SPAmP-MeFASA C3 (+)
	PFPeSAC (+)	N-HOEAmP-FASA C2 (+)	N-SPAmP-MeFASA C5 (+)
	PFPrSaAm (-/+)	N-HOEAmP-FASA C3 (+)	N-SPHOEAmP-FASA C4 (+)
	PFPrSAC (+)	N-HOEAmP-FASA C4 (+)	N-SPHOEAmP-FASA C5 (+)
		N-HOEAmP-FASA C5 (+)	N-TAmP-FASA C3 (+)
		N-HOEAmP-FASA C6 (+)	PFDODS (-)
		N-HOEAmP-FASA C8 (+)	U-PFDS (-)
		N-HOEAmP-FASE C3 (+)	U-PFNS (-)
		N-HOEAmP-FASE C4 (+)	U-PFUnDS (-)
		N-HOEAmP-FASE C5 (+)	
23 analytes		57 analytes	

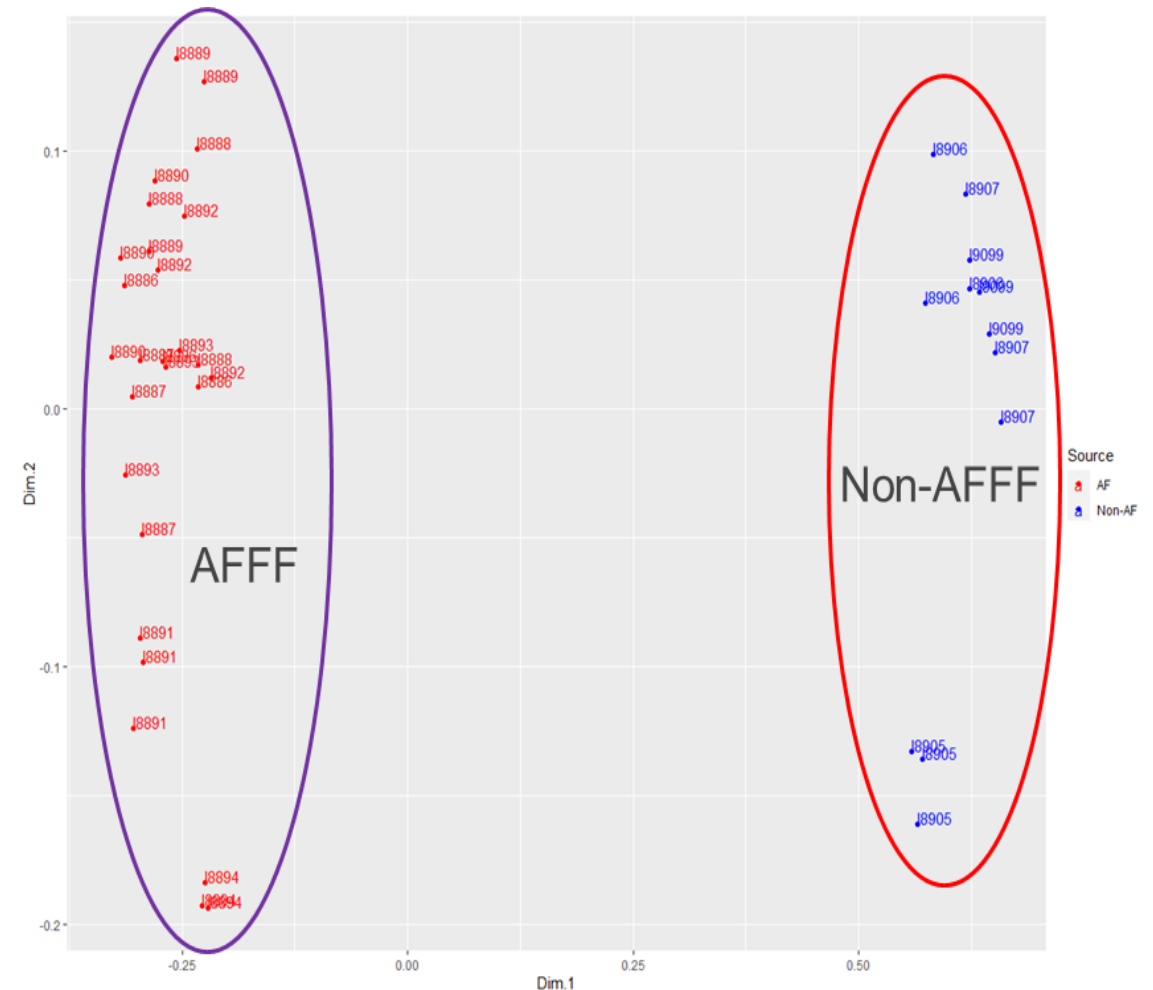




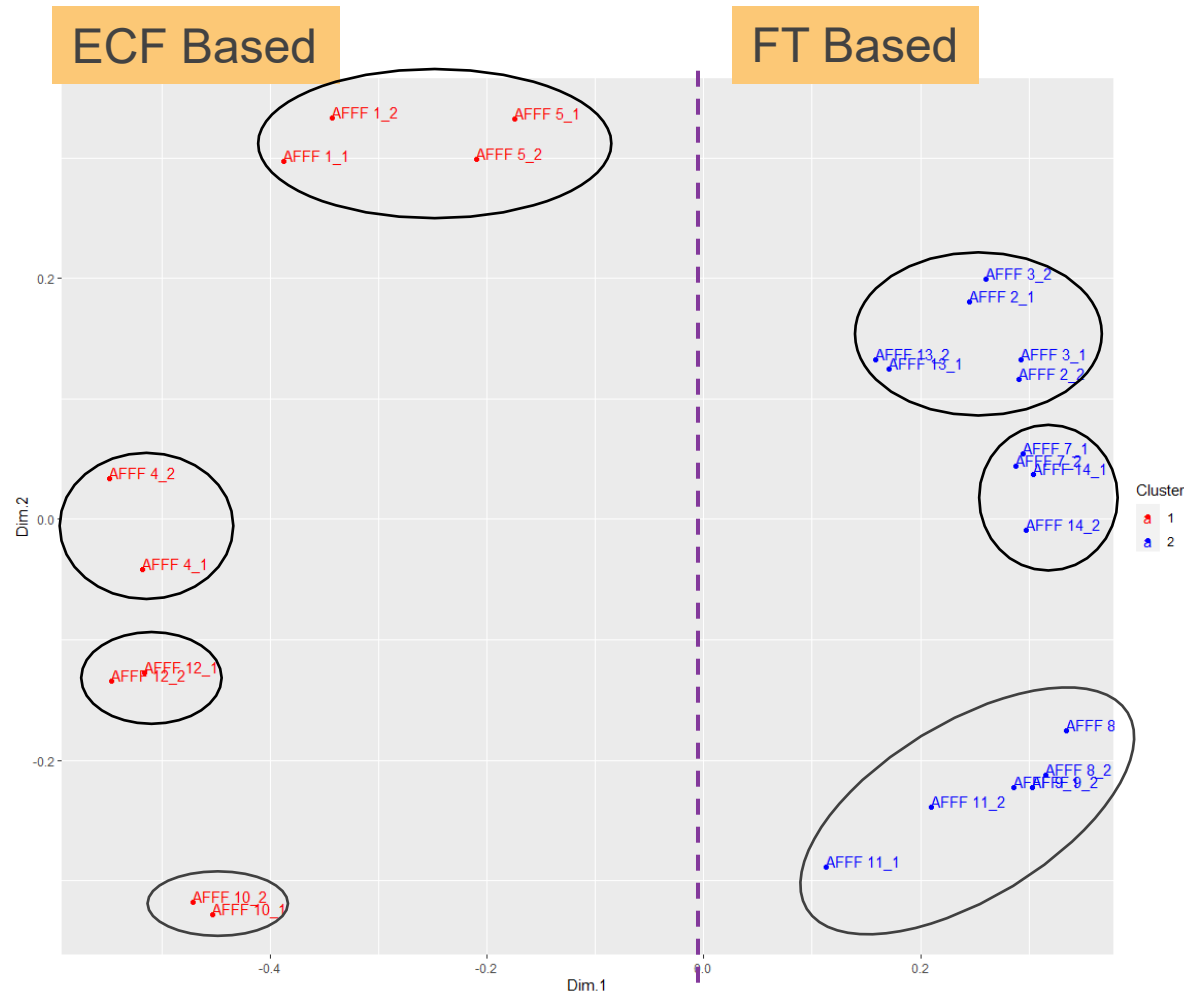
# Machine Learning Insights

# PFAS Signature® Uses AI/ML to Train on, then Distinguish Between, Sources

- Discriminates AFFF chemistry and formulations through development of source libraries with known samples
- Source discrimination of AFFF vs Non-AFFF in environmental samples
- Provides Delineation of Distinct PFAS Sources and Co-Occurrence

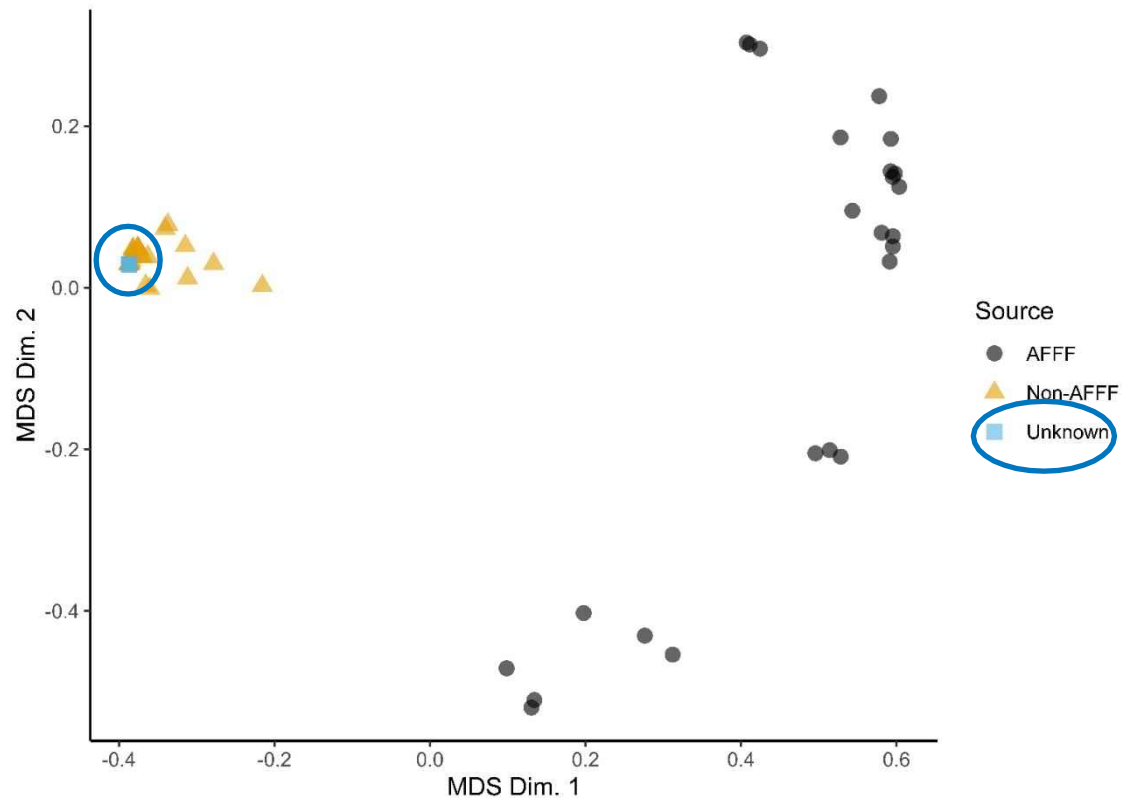


# PFAS Signature<sup>®</sup> Can Differentiate Between AFFF Sources



- Discriminates AFFF chemistry and formulations
- Identification of unknown manufacturing source

# Visualizing a new, unknown sample

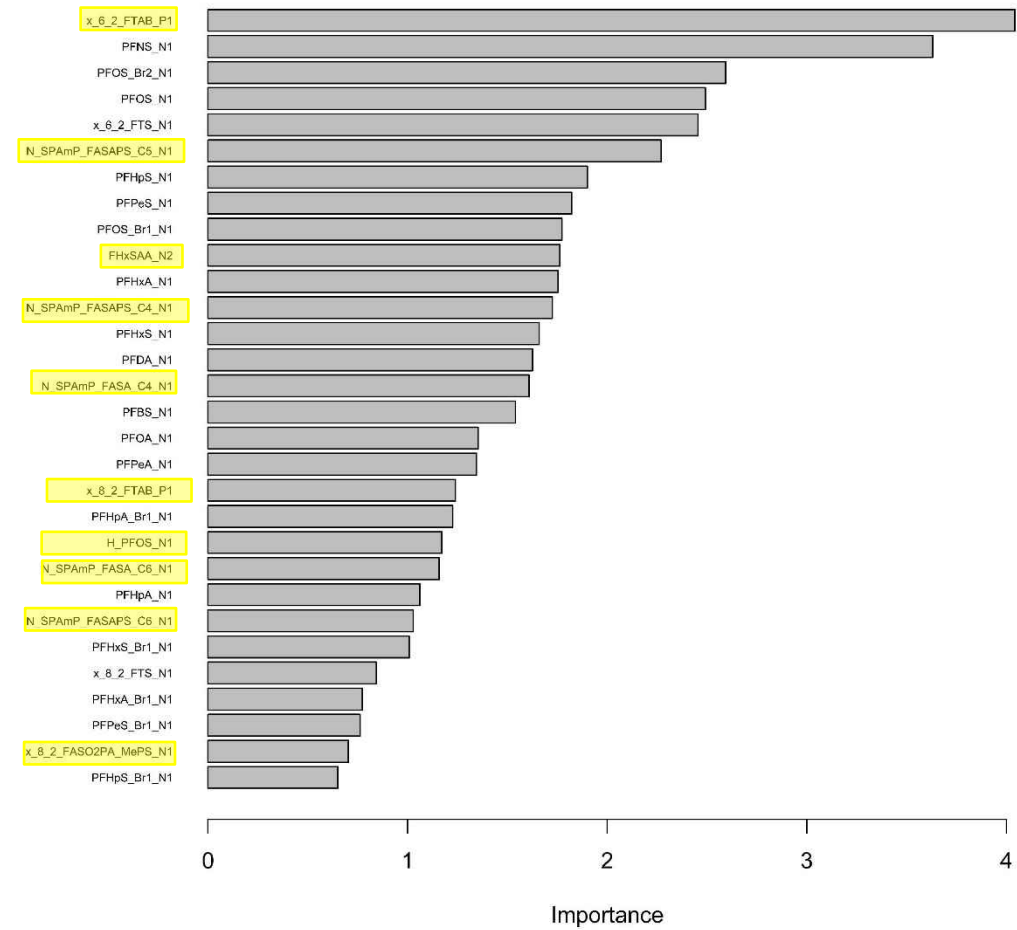


Signature<sup>®</sup> assesses how the unknown sample compares to the training set to understand the similarities and differences between the unknown and known sources

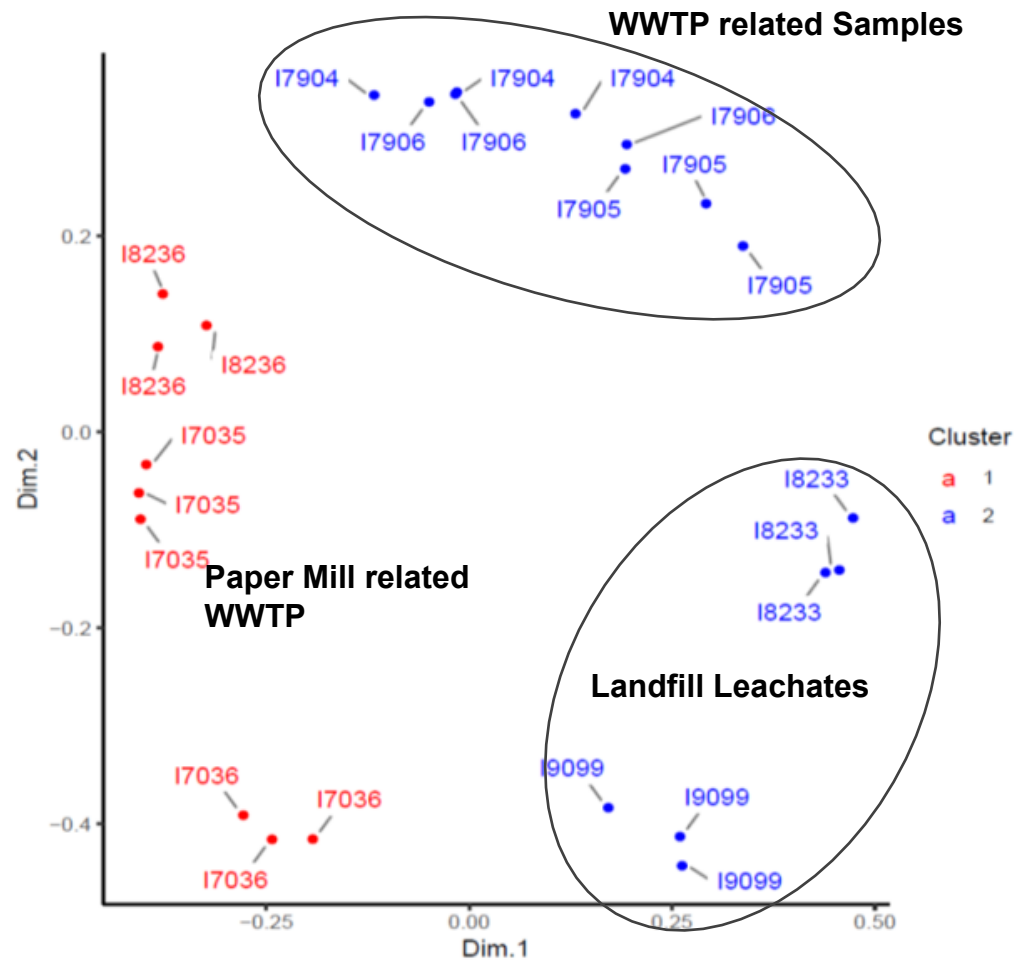
# Analyte Importance for an Example Data Set

10 of 30 Most Important Analytes are non-target!

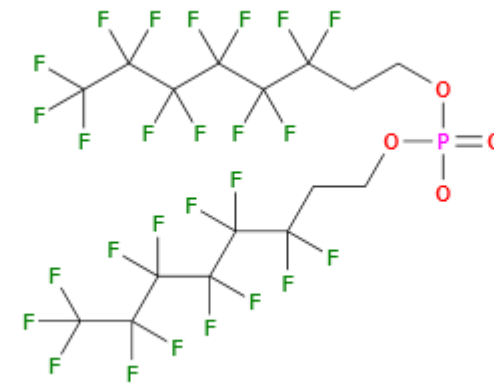
- 6:2 FTAB (+)
- N-SPAmP FASAPS C5 (-)
- FHxSAA (-)
- N-SPAmP FASAPS C4 (-)
- N-SPAmP FASA C4 (-)
- 8:2 FTAB (+)
- H-PFOS (-)
- N-SPAmP FASA C6 (-)
- N-SPAmP FASAPS C6 (-)
- 8:2 FASO2PA MePS (-)



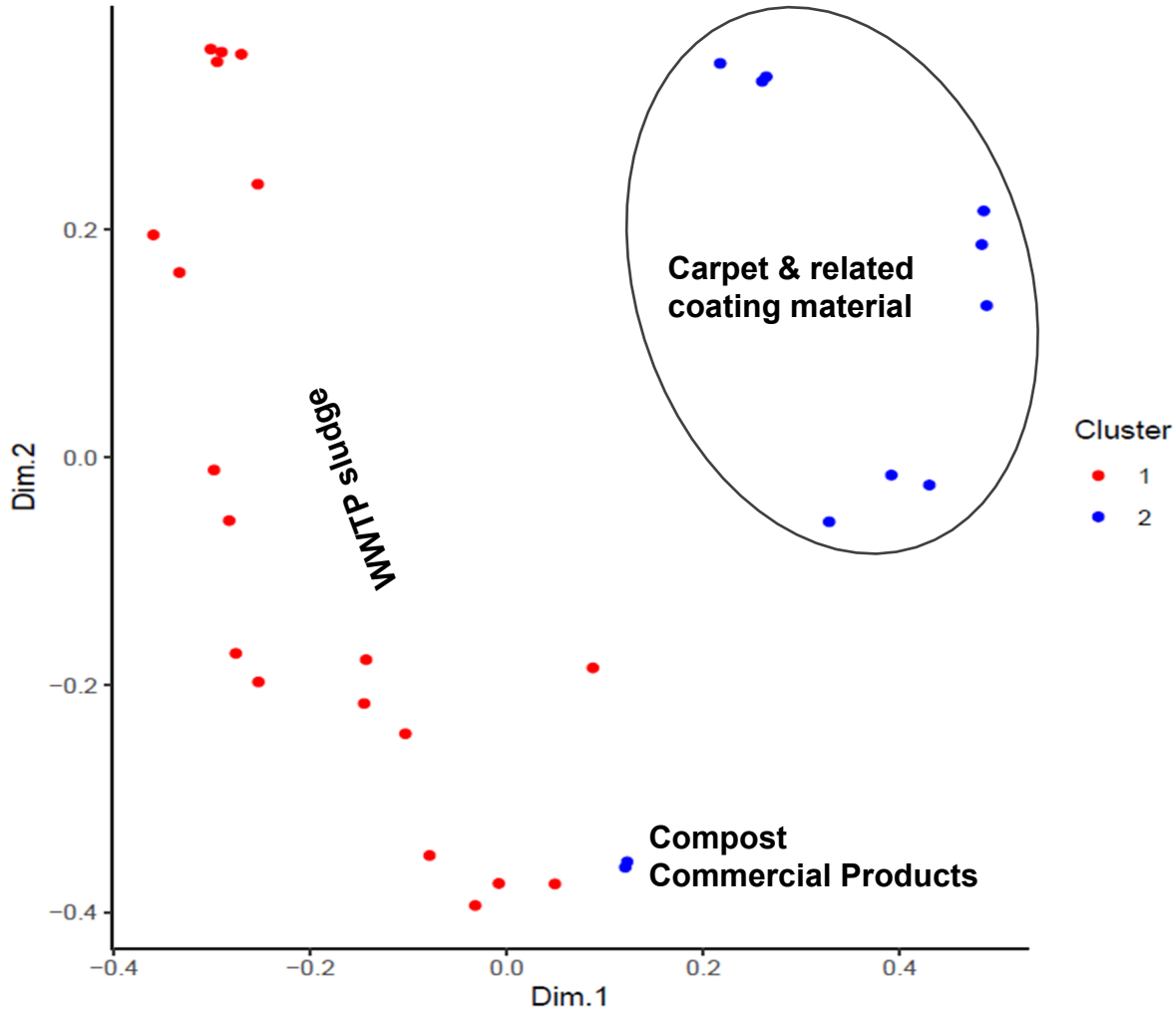
# PFAS Signature<sup>®</sup> Identifies Commercial and Waste Related Signatures



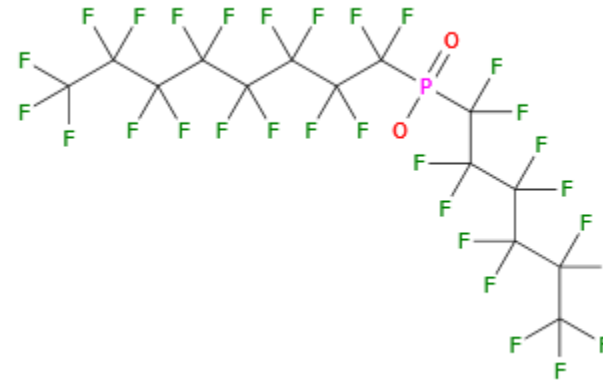
- Methyl/Ethyl FASAAs, FASAs, n:3 FTCAs found in waste sector
- n:2 DiPAPs found in commercial products



# PFAS Signature® Identifies Commercial and Waste Related Signatures



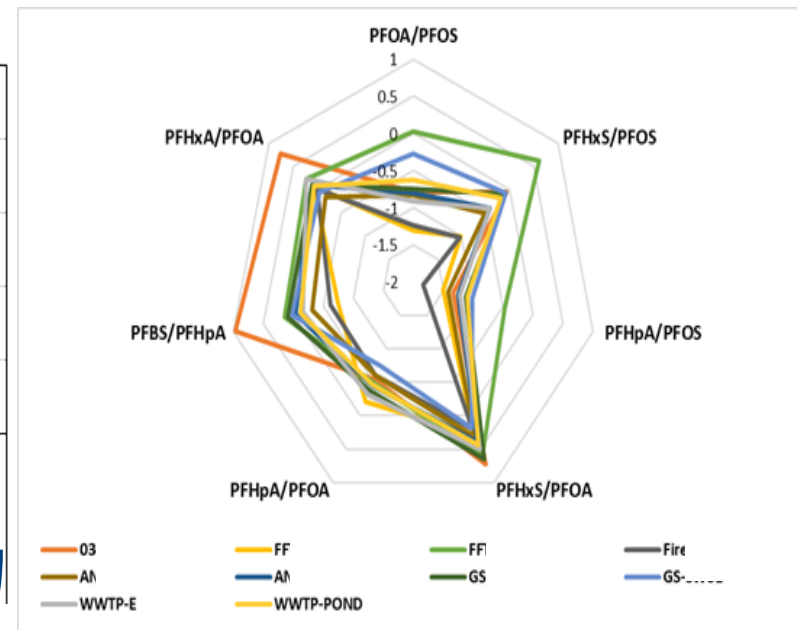
- Methyl/Ethyl FASAAs, FASAAs, n:3 FTCAs found in waste sector
- n:2 diPAPs, diSAmPAP, Methyl/Ethyl FASAAs found in commercial products



# Site Applications

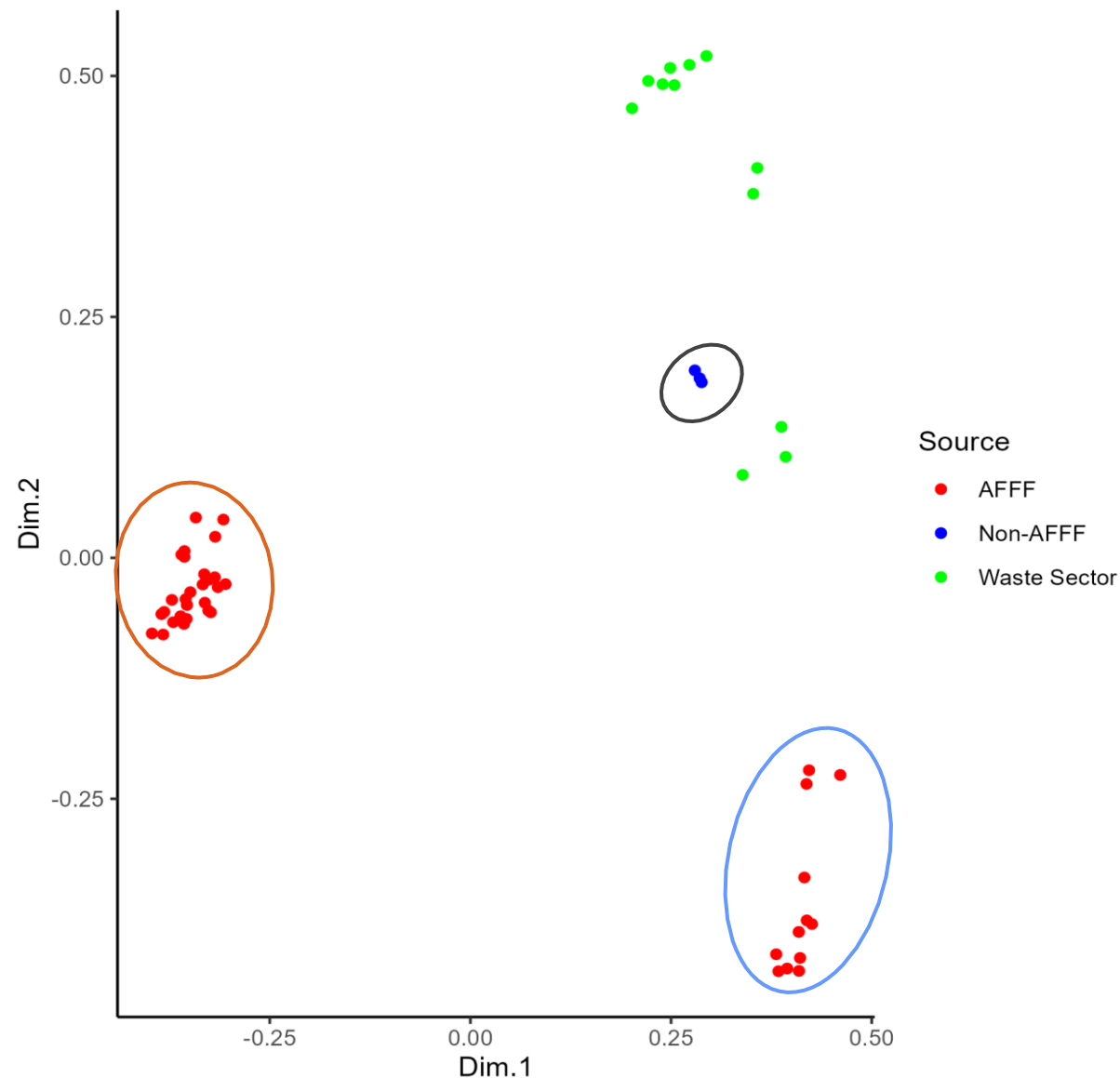


# Targeted Analysis - Data Interpretation Using Radial Plots



- Radial plots using ratios of commonly found PFAS
- Based on the targeted analysis data it is uncertain to inform whether there is an AFFF/Non AFFF source of contamination

# PFAS Signature<sup>®</sup> Analysis – AFFF-impacted Site



AFFF related with both ECF and FT chemistry near source locations

High levels of PFAAs and other transformation products with few detections of ECF precursors – **Downstream** from the source locations, detections resulting from environmental biotransformation and fractionation

No detections of AFFF related analytes

6:2, 8:2, 10:2 FTABs found only in current FTA and Waste sector samples  
PFHxSaAm, found only in WWTP influent  
6-2 & 8:2 FASO2PA-MePS found in both WWTP influent and effluent (2 orders of magnitude less)

# Example PFAS Signature® Application

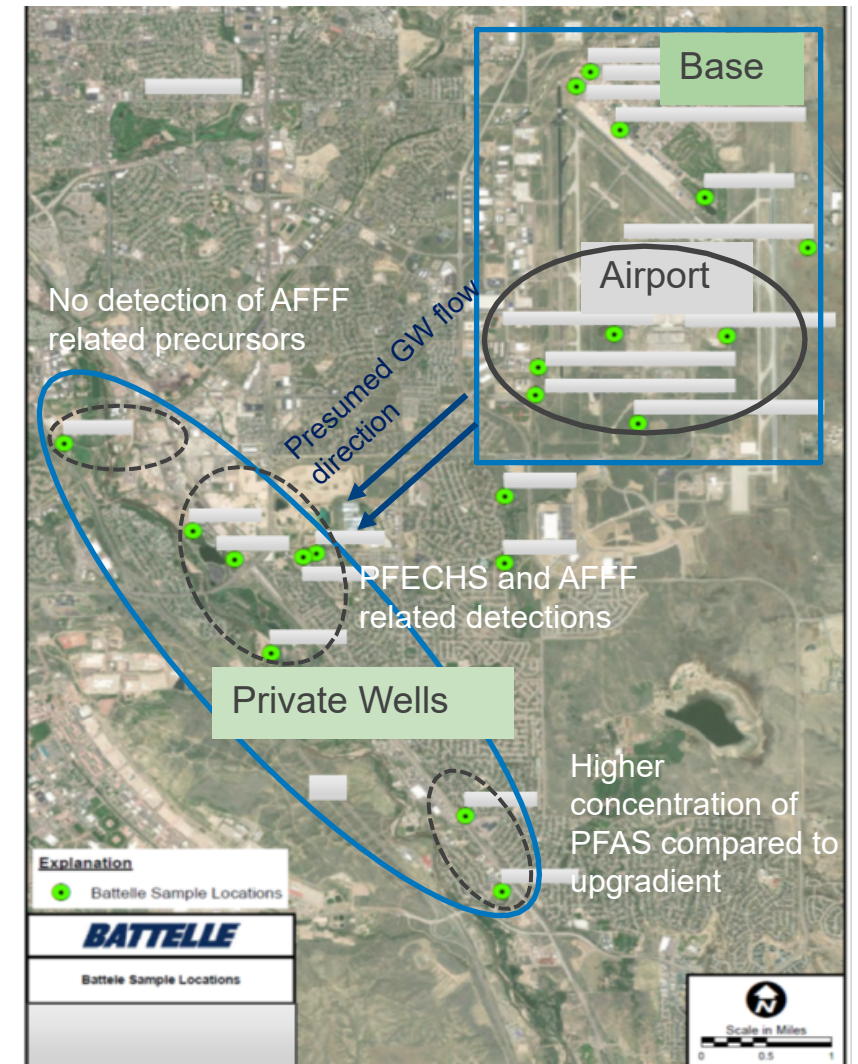
**Objective:** Compare PFAS detections on-base and off-site locations

## Limited Sample Collection:

- 20 samples collected from – on-base; nearby airport and private well water from surrounding areas

## Summary:

- Highest PFAS concentration on-base (up to 100 ppb);
- Downgradient migration of cyclic PFOS analog from airport and other AFFF related precursors;
- Downgradient private well samples had slightly higher PFAS concentrations compared to upgradient wells indicating fate and transport and other potential sources
- Sampling one year later suggested further downgradient migration



# PFAS Signature® Summary



**PFAS Signature® is a source discrimination/characterization tool using a combination of advanced analytical tools and data analytics**



**PFAS focused data filtering tools provides confidence on the PFAS related data.**



**Good reproducibility of the workflow demonstrated on triplicate data sets.**



**PFAS Signature® Tool has been demonstrated on environmental samples collected from a variety of different source scenarios.**

# Acknowledgements to Battelle Team

- PI/PM

- Kavitha Dasu

- Analytical Chemists

- Larry Mullins
- Cameron Orth

- Targeted Analysis

- Jonathan Thorn

- Data Scientists

- Dave Friedenber
- Brandon Hill

- Data Filtering

- Brannon Seay



**It can be done**

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