Agenda

- Background
- What type of Data is necessary for Forensics?
- Analytical Methods
- Fingerprinting and PFAS
- Non-Target Analysis
- PFAS Forensics
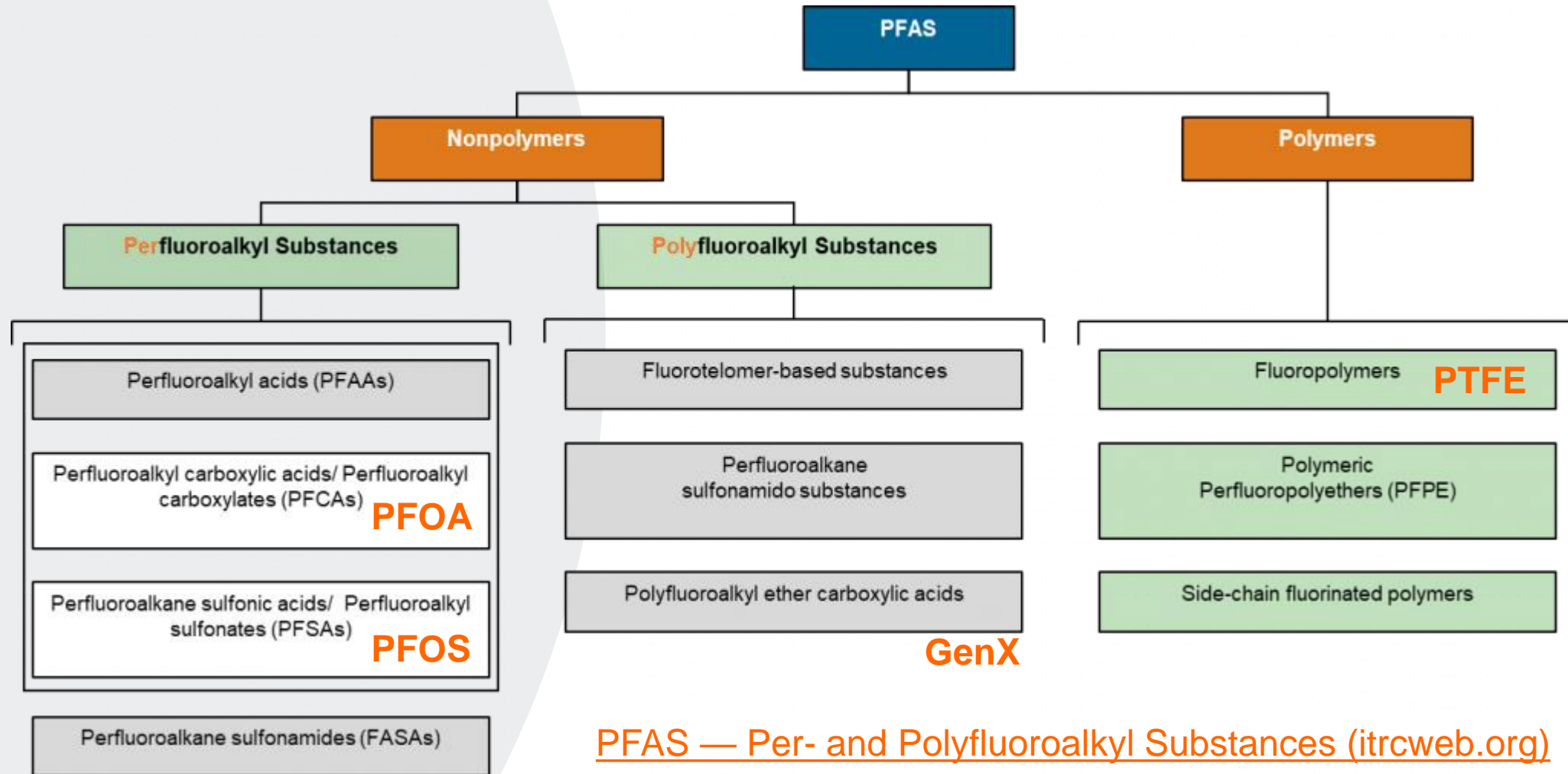- Summary

Background

Background: PFAS Compounds

- Standards Availability
- Current methods for measuring PFAS are limited in scope
- ~ 4000 to 6000 PFAS compounds
- TRI reporting requirements – 189 compounds
- How do we acquire the best possible data for Forensics?

Background: PFAS Terminology and Family Tree

PFAS — Per- and Polyfluoroalkyl Substances (itrcweb.org)

Background:
Traditional Forensics Approach

- Sophisticated Data Acquisition
- Analytical Methods:
  - LC/MS/MS
  - AOF
  - TOP Assay
  - LC qTOF HRMS
  - GC qTOF HRMS
  - Non - Targeted Analysis (NTA)
  - Complex Libraries
  - Mass Spectral Interpretation

Background:
Pioneering PFAS Analysis

- Isotope dilution analysis of ~ 100 targets with some of the lowest reporting limits in the industry
- Total Oxidizable Precursor Assay (TOP)
- Branched Isomer Characterization for Forensics
- Short Chain PFAS analysis
- AOF analysis
- AFFF and Non PFAS AFFF
- Custom Method Development and Validation
- Leadership on analytical issues, including stability, filtration and subsampling

Background:
Pioneering PFAS Analysis

- SGS AXYS performed the single-lab validation of the isotope-dilution PFAS method in water, solids, and tissue for the US EPA/US DoD (1633)

- Fish tissue, wastewater treatment mass balance, ambient monitoring, drinking water, ambient air, site investigation, remediation, human biomonitoring, product testing, passive sampling

Background: AFFF Products Method for US-DoD

Method Features

- 10 ppb PFOS and PFOA
- 31 other targets
- Isotope dilution, DoD QSM 5.3 compliant method

Status

- Validated for AFFF, AR-AFFF and tested on fluorine-free foams for DoD

What Type of Data is Necessary for Forensics?

What Type of Data is Necessary for Forensics?

- Historical site use data
- Fire fighting activities on site
- Types of foams used in fire-fighting activities over time
- Any type of polymers used on site
- Due to ubiquitous nature of PFAS any information regarding products used onsite
- Site Maps

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What Type of Data
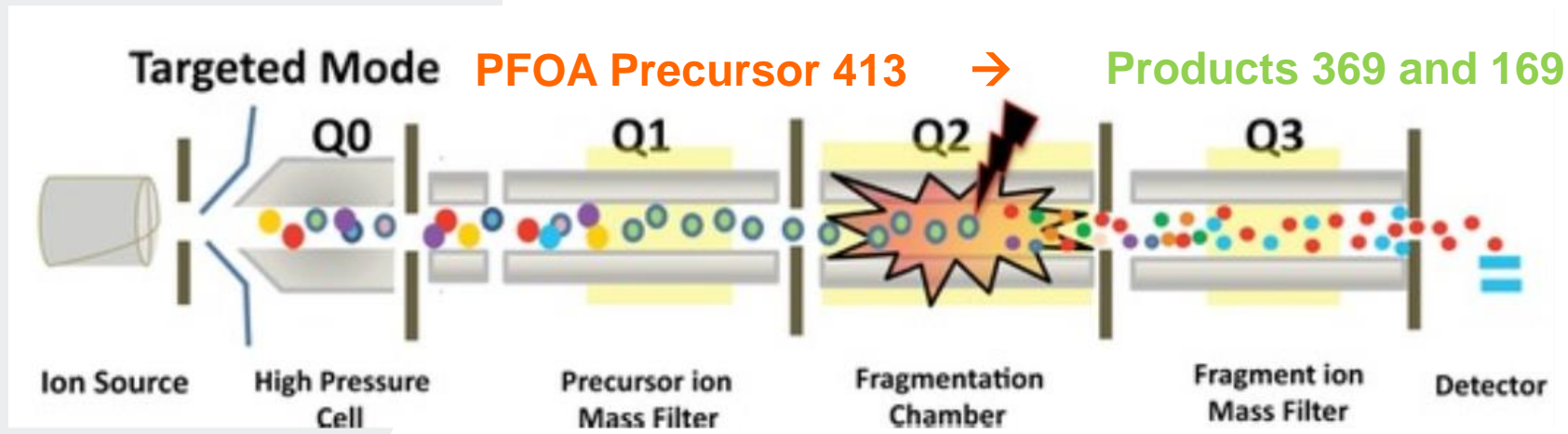# is Necessary for Forensics?

Analytical Data

- LC/MS/MS

- AOF

- TOP Assay

- LC qTOF HRMS

- GC qTOF HRMS

- Non - Targeted Analysis (NTA) Software

- Understanding Complex Work-Flows

- Expertise in processing data

- Libraries – example EPA DSS TOX database (~875,000 unique substances) (MS ready structures)

- Complex Software and Mass Spectral Interpretation

Analytical Methods
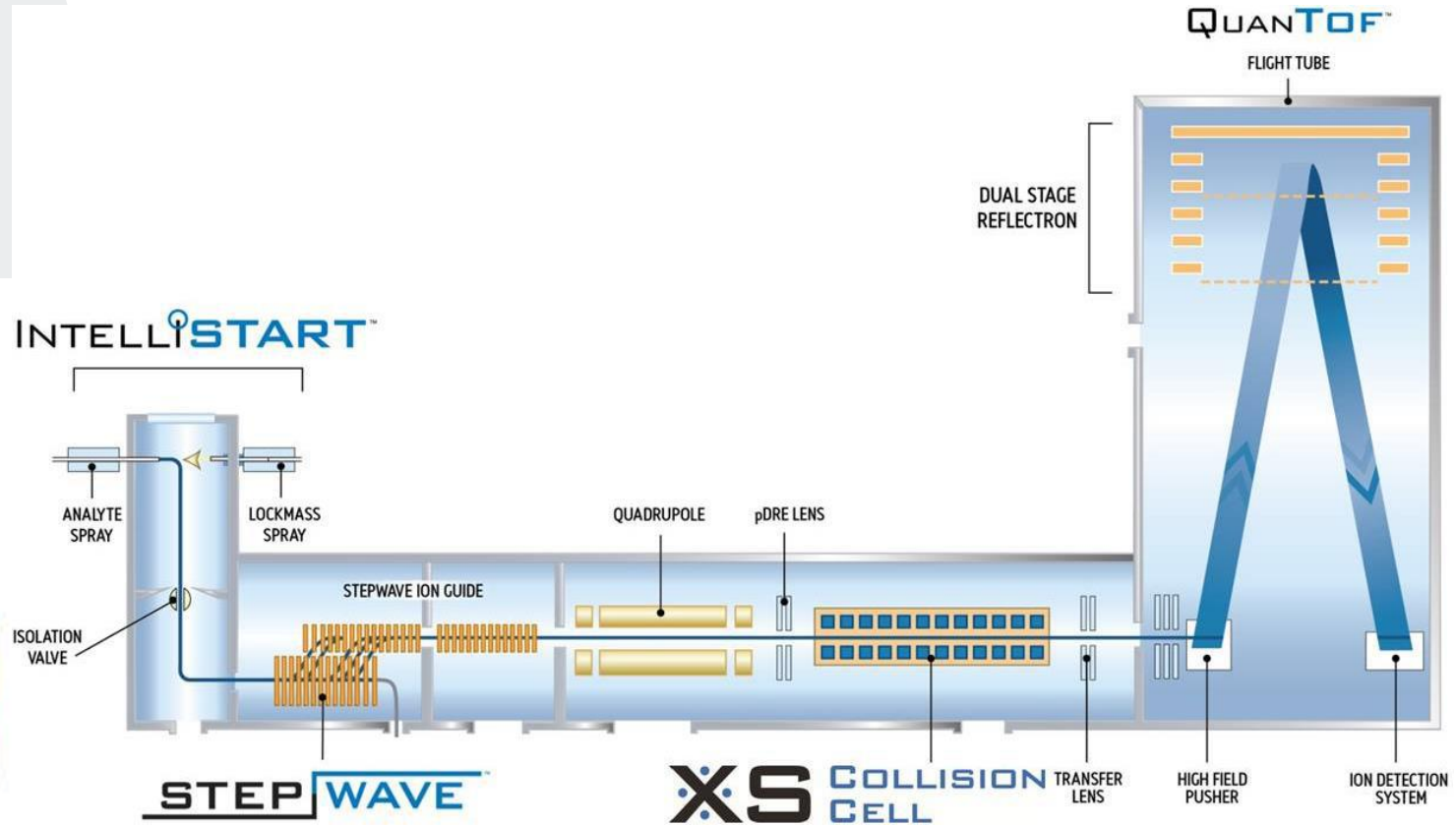
Analytical Methods: LC/MS/MS

- **LC/MS/MS: First separate analytes in solution by liquid chromatography**
- **Triple Quadrupole or Tandem Mass Spec**
  - Q1/MS1 separates the precursor ion(s) from everything else in the sample and allows them into the collision cell
  - Q2 is the collision cell, ions are fragmented forming product ions
  - Q3/MS2 allows only selected product ions to pass through to the detector

Targeted Mode   **PFOA Precursor 413** →   **Products 369 and 169**

Ion Source | High Pressure Cell (Q0) | Precursor ion Mass Filter (Q1) | Fragmentation Chamber (Q2) | Fragment ion Mass Filter (Q3) | Detector

Analytical Methods: LC/QTOF/HRMS

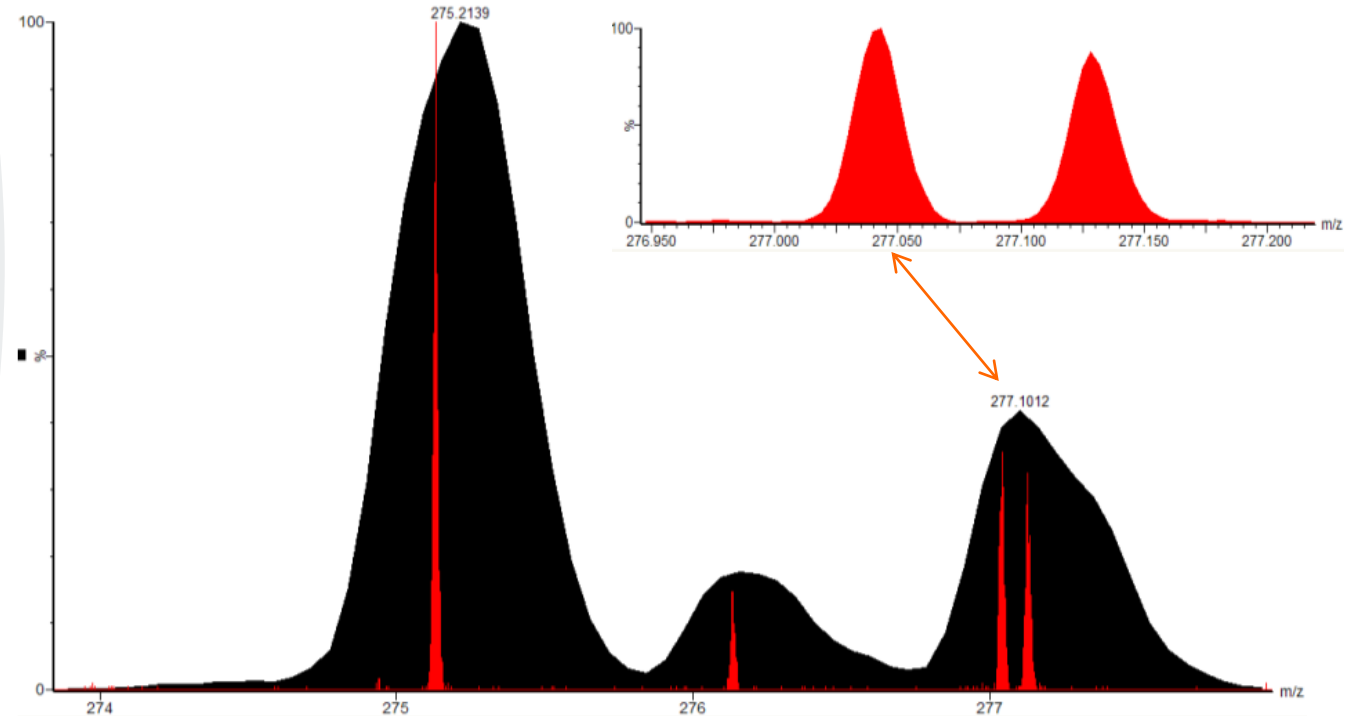- Accurate Mass Screening
- Peak Resolution
- Sensitivity

Courtesy: Waters

Analytical Methods: LC/QTOF/HRMS

- Accurate Mass Screening
- Power of High Resolution

Analytical Methods: GC/QTOF/HRMS

- Accurate Mass Screening

- Utilized for Air Samples[1]

- Resolving power

*Ref[1]: Seth Newton, Jonathan Casey, Office of Research and Development,
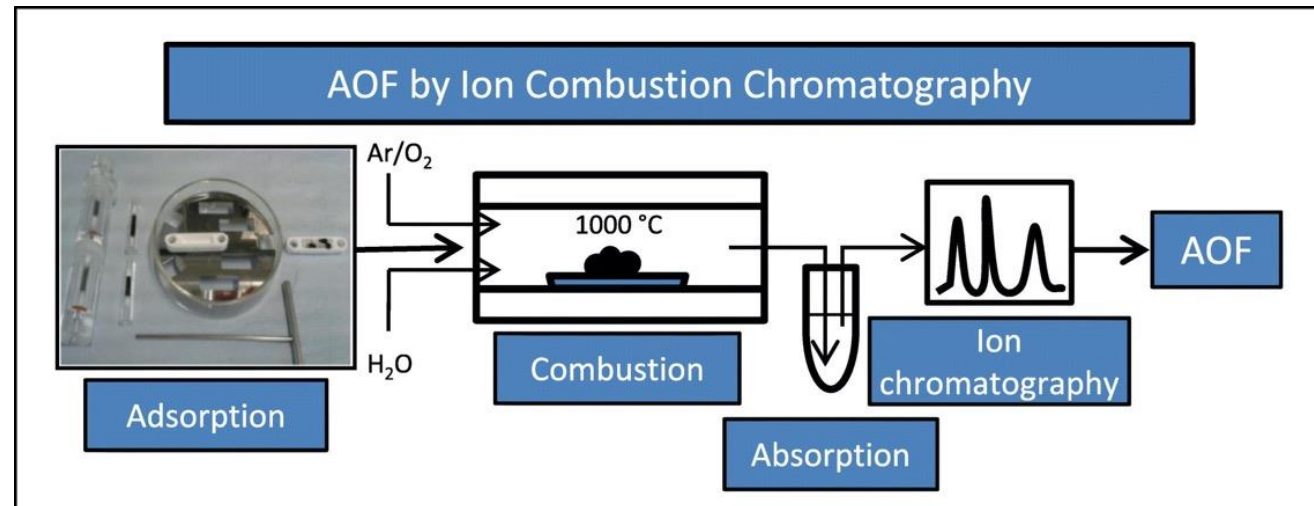US Environmental Protection Agency*

Courtesy: Agilent

Analytical Methods: Adsorbable Organic Fluorine

- Good potential for quickly understanding total fluorine, EPA Method 1621 in progress
- Challenges
  - Fluorine background
  - Reporting limits 100-1000 times higher than LC-MS/MS
  - No chain length information

Technique for estimating organic fluorine in a sample by combustion ion chromatography.
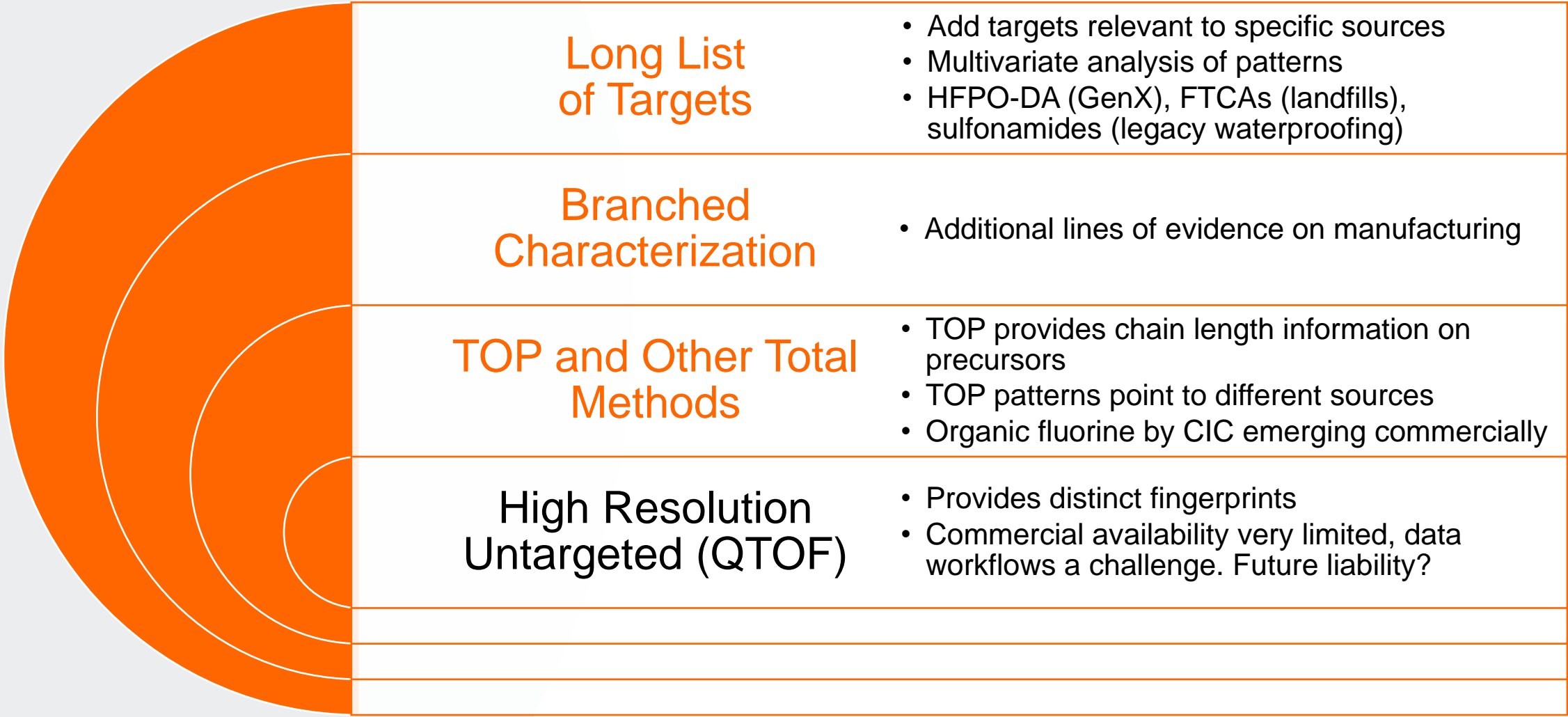

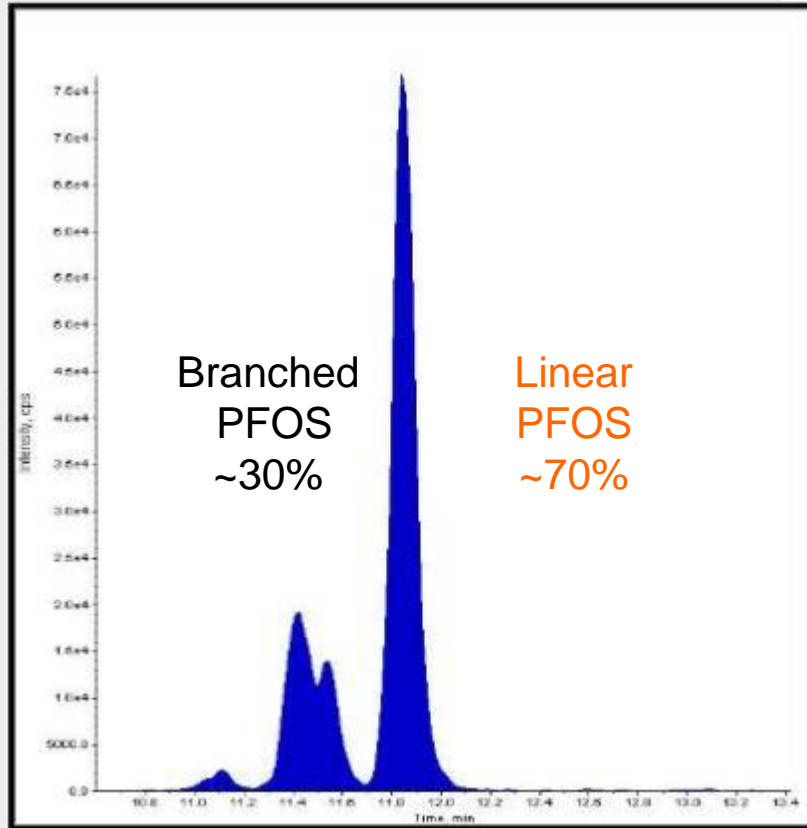*Science of The Total Environment* **673**, 384–391 (2019).

Fingerprinting and PFAS

Fingerprinting

Long List of Targets
- Add targets relevant to specific sources
- Multivariate analysis of patterns
- HFPO-DA (GenX), FTCAs (landfills), sulfonamides (legacy waterproofing)

Branched Characterization
- Additional lines of evidence on manufacturing

TOP and Other Total Methods
- TOP provides chain length information on precursors
- TOP patterns point to different sources
- Organic fluorine by CIC emerging commercially

High Resolution Untargeted (QTOF)
- Provides distinct fingerprints
- Commercial availability very limited, data workflows a challenge. Future liability?

Linear vs. Branched Isomers

Branched PFOS ~30%

Linear PFOS ~70%

*Riddell, N. et. al, Environ Sci. Technol. 2009 (43) 7902-7908.*

- Eleven known isomers of PFOS in Electrochemical Fluorination (ECF) process
- Transitions have different relative response factors for the linear and the branched isomers for most PFAS
- Quantitative biases possible depending on standard type and MRM transitions used for quantitation
- Distribution/half lives in tissue are different between linear and branched
- All best-practice methods, and US DoD QSM require measurement of all identified isomers
- Speciation adds line of evidence on fingerprinting

Linear vs. Branched Isomers

- Eleven known isomers of PFOS in Electrochemical Fluorination (ECF) process
- Distribution/half lives in tissue are different between linear and branched
- All current EPA methods, and US DoD QSM require measurement of all identified isomers
- Separating branched/linear isomers adds a forensics line of evidence on PFAS provenance
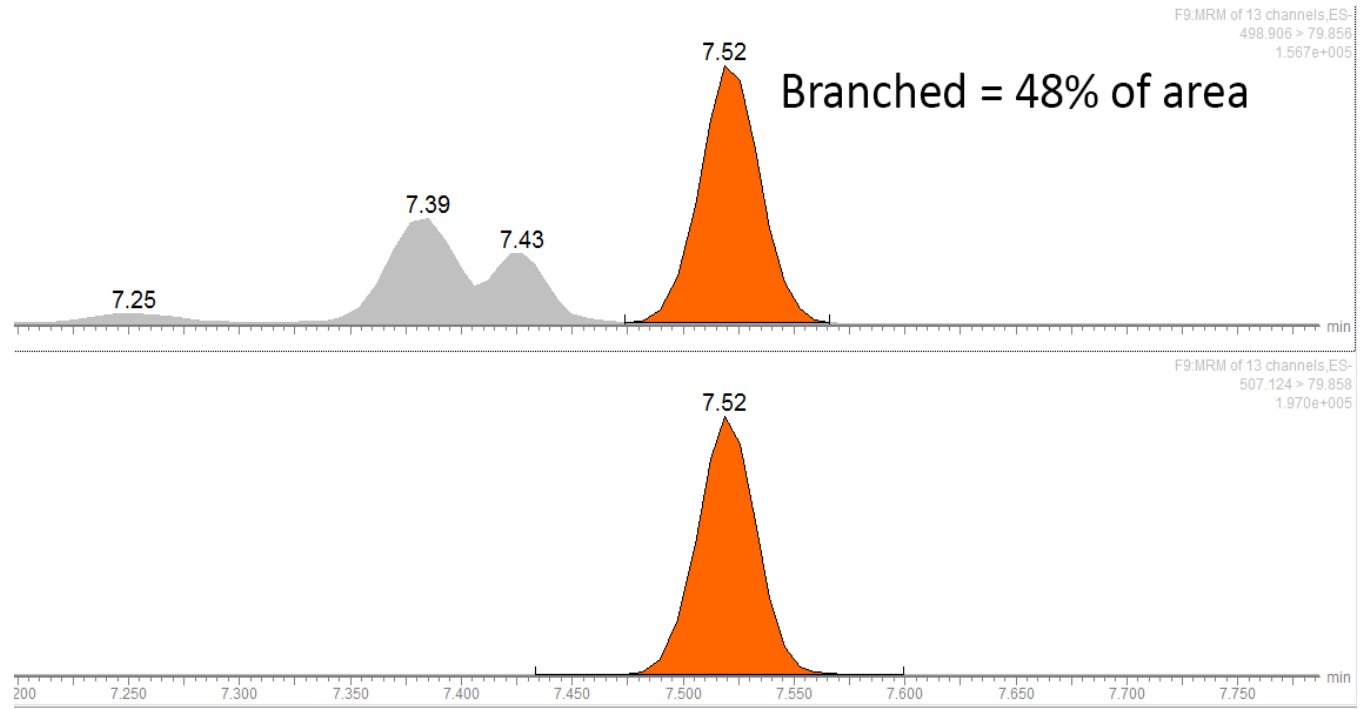
Chromatogram of PFOS in a surface water sample with significant branched isomer content. Linear isomer shaded in orange, branched in gray. Bottom chromatogram shows isotopically labeled linear standard for reference

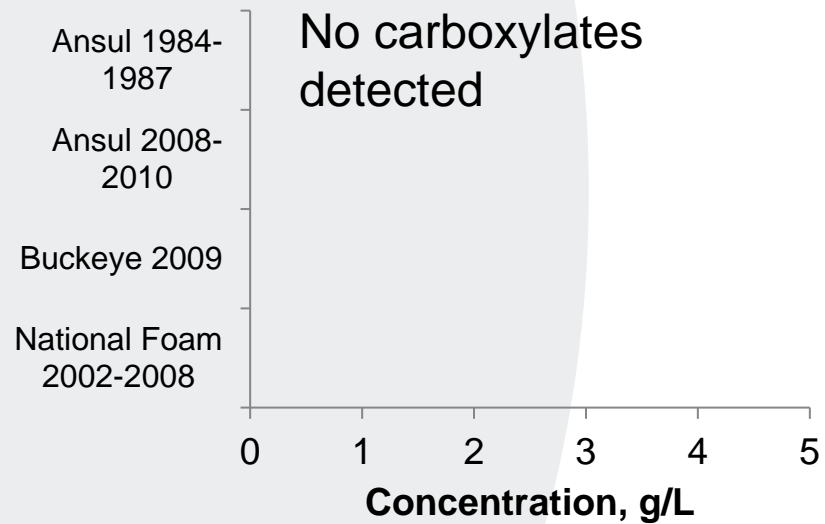# TOP Shows Significant Non-target PFAS at AFFF Sites
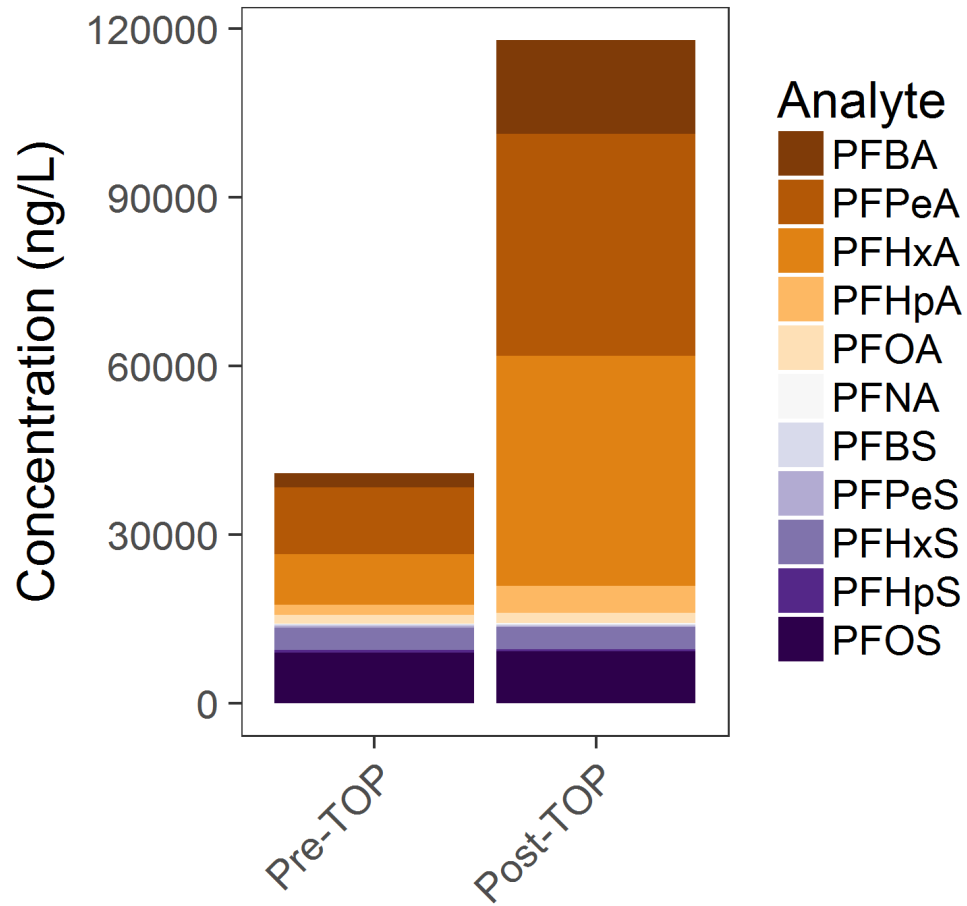
- 300% increase
- Increases primarily in C4, C5 and C6, indicating use of a 6:2 AFFF product
- Sulfonates constant
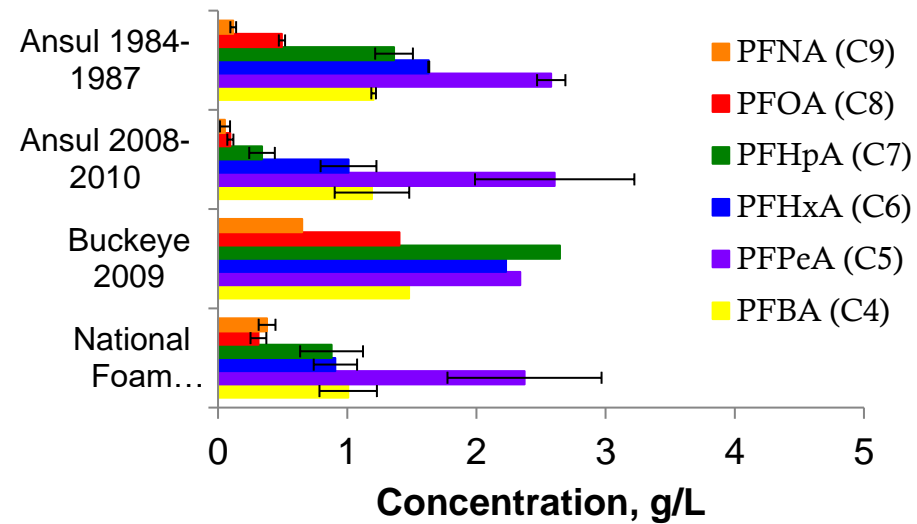- FTS disappears completely

Example: TOP Assay Conversion of AFFF

Before Oxidation

After Oxidation

No carboxylates detected

Legend:
- PFNA (C9) — orange
- PFOA (C8) — red
- PFHpA (C7) — green
- PFHxA (C6) — blue
- PFPeA (C5) — purple
- PFBA (C4) — yellow

Before Oxidation chart — categories (top to bottom): Ansul 1984-1987, Ansul 2008-2010, Buckeye 2009, National Foam 2002-2008. X-axis: Concentration, g/L (0 to 5)

After Oxidation chart — categories (top to bottom): Ansul 1984-1987, Ansul 2008-2010, Buckeye 2009, National Foam… X-axis: Concentration, g/L (0 to 5)

Ref: *Thomas Bruton, David Sedlak, Department of Civil and Environmental Engineering, University of California at Berkeley; Houtz et.al ES&T, 2013, 46:9342-9349.*

24

TOP Shows Large Presence of Non-target PFAS in Biosolids
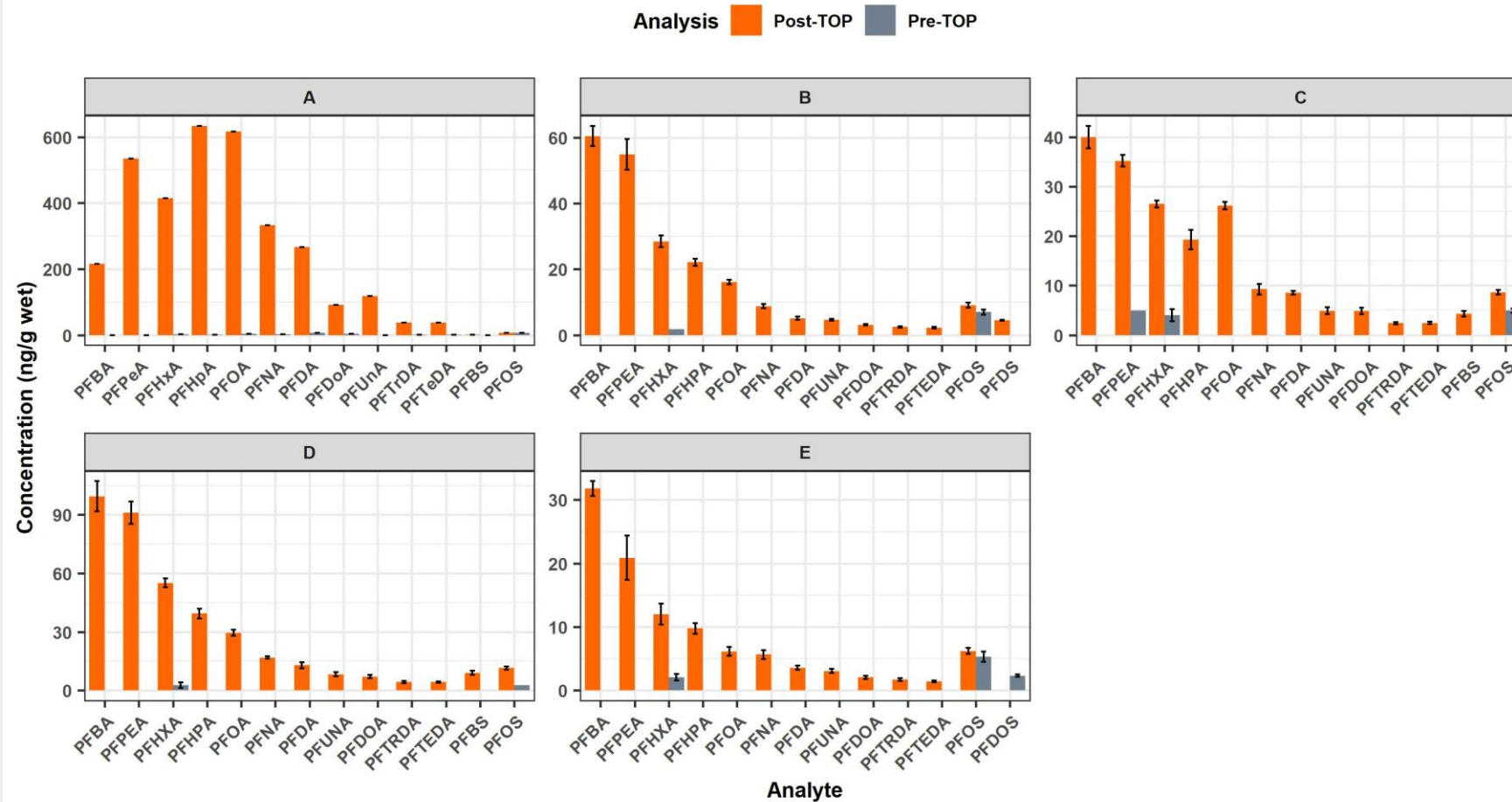
TOP Technical Issues

- Standardization of reaction: Is this a lab specific test?

- Quality of underlying analytical procedures
  - Ensuring isotope dilution through the process
  - Reporting of precursors post-TOP: FTS, other precursor accuracy is not adequate without isotope dilution
  - Need for continued alignment with PFAS methods

- Monitoring reaction completeness
  - Many approaches used
  - The Queensland PFAS guidance (only instance of TOP in regulation) recommends precursors as a percent of total PFAS

- Ether PFAS?

See Chandramouli book chapter in Kempisty, D.M., Xing, Y., Racz, L., 2018. Perfluoroalkyl Substances in the Environment: Theory, Practice and Innovation
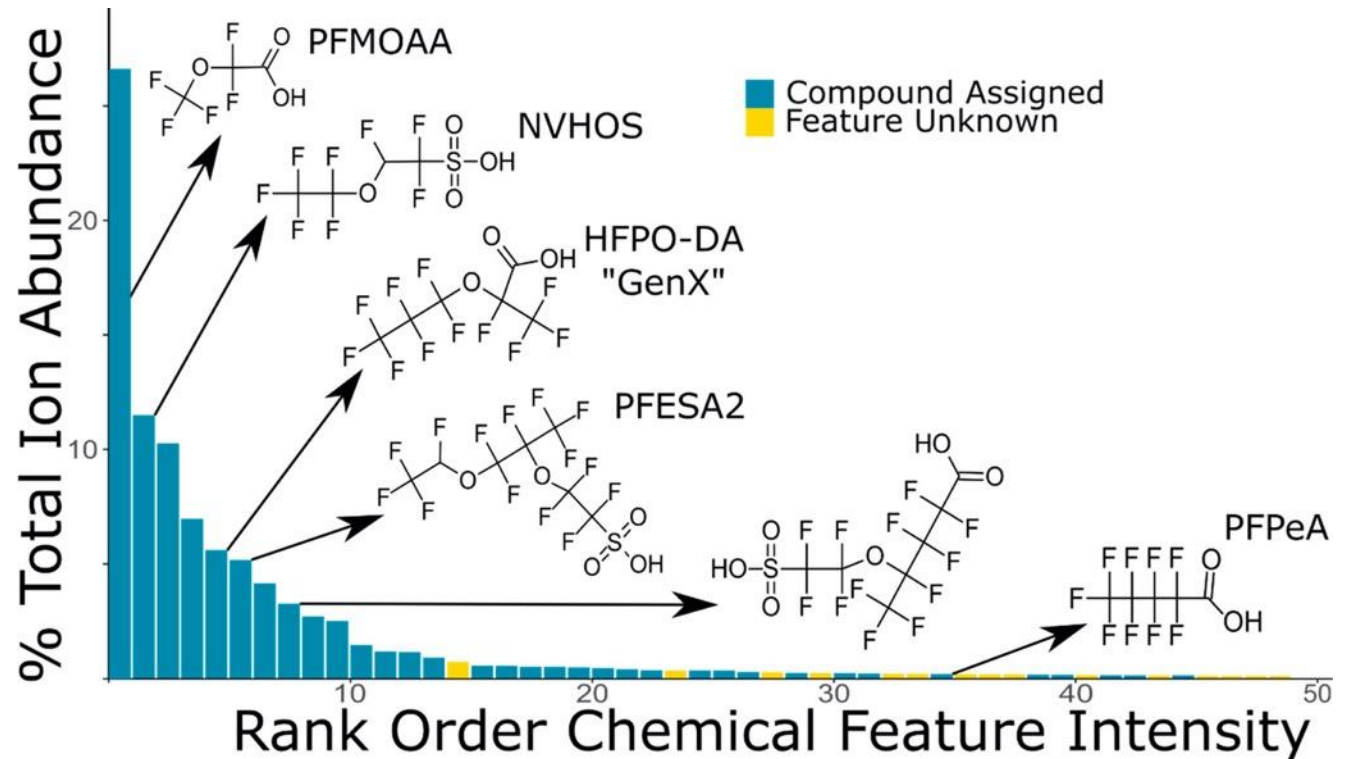
Non-Target Analysis

Non-Target Analysis; or 175.9591 Rather Than 175

- The wider availability of mass spectrometers that can scan samples at high-resolution unlocks another tool to understand and characterize unknown PFAS

- Lots of promise and widely available with academic and some government institutions

- Major questions on data workflow, quality and more

- Commercial availability for environmental analysis limited

Identification of Per- and Polyfluoroalkyl Substances in the Cape Fear River by High Resolution Mass Spectrometry and Nontargeted Screening

James McCord[†] and Mark Strynar[*,‡]

Non-Target Analysis

- Use of standard GC/qTOF/HRMS methods in addition to LC/qTOF/HRMS methods
- Data Dependent (DD) and Data Independent Acquisitions (DIA)
- Mass Spectral Deconvolution
- Selecting cleavage patterns to search
- Peak Picking
- Eliminating Targets from the Non-Targets
- Spectral interpretation
- Complex software – Public Domain and Instrument Vendors' NTA software
- Talent Needed – Available at Universities
- Availability of resources in Universities

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Non-Target Analysis

- Determination of Precursors and Degradation Products (DIA)
- Complex Spectral Interpretations
- Pattern Recognition in Totality
- Complex software
- Statistical Approaches

PFAS Forensics

PFAS Forensics

- Cluster analysis for scoring profile similarities
- Multivariate analysis for identifying source profiles
- Linear mixing models or dimension reducing analyses
  to find best fit for allocations

Summary

Summary

- Complex Analysis – Seek Expertise at the Start !!!
- Sample Collection and Laboratory Analytical protocols must be designed to achieve the desired outcome
- Develop method-specific technical (and robust QC) requirements
- Instrument vendors have NTA software imbedded
- For Forensics, detailed requirements must be written and agreed upon prior to sampling, analysis and data generation which include raw instrument files

Thank you!

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