



# Targeted and Non-targeted Analytical Methods for PFAS Detection and Quantification In Environmental Matrices

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Workshop on Recent advances in PFAS Detection and Measurement Techniques  
Clarkson University, Capital Region Campus Schenectady, NY

*The views expressed in this presentation are those of the author(s) and do not necessarily represent  
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## Goals

- Validated analytical methods provide the means to collect data for research and regulatory applications
- Demonstrated and validated method performance in specific sample matrix types to collect data of known quality that is reproducible and defensible
- EPA standard methods are recognized nationally and internationally as the “gold standard” for analytical methodology by industrial, academic and other governmental agencies
- EPA research methods are tailored to specific research needs, often using standard methods as a starting point



# PFAS analytical methods

- Drinking Water Methods
  - Method 537
  - Method 533
- Non-Drinking Water Methods
  - SW-846 Method 8327 – Direct Injection
  - Draft CWA 1633—Isotope Dilution
- PFAS Analysis in Marine Waters
- PFAS Analysis in Fish Tissue
- Total Organofluorine Analysis using Combustion Ion Chromatography (TOF)
- Total Oxidizable Precursors (TOP)



# Types of Standard Methods

## Three broad categories of EPA Standard Methods:

- [Safe Drinking Water Act Methods](#)
- [Clean Water Act Methods](#)
- [SW846 Methods](#)

These are generally targeted methods for solids and water.

- Safe Drinking Water Act (SDWA) method developed by ORD for finished drinking water
- Revision 2 published March 2020
- 18 PFAS, including perfluorocarboxylic and sulfonic acids (chain length  $\leq C14$ ), perfluoroethers (HFPO-DA), and sulfonamides
- Solid Phase Extraction, LC/MS/MS data acquisition, quantification by internal standard calibration technique
- Limit of Detection (LOD) capable of regulatory application to PFOA/PFOS advisory level



- Agilent MassHunter Quantitative Analysis for QQQ - Precursor\_2021-2025\_16562921.batch.in

File Edit View Methods Tools Help

New Batch Open Batch Save Batch Add Samples Delete Samples Analyze Batch Calibration Clear Results Generate Report Open Report Folder Queue Viewer Library Search Results Edit Report Metadata Query Copy Paste Find

Batch Table

Sample: 3052\_L02 Sample Type: <All> Compound: 1031.FTHL 43.670 MIN

Compound Method				EIOAc_19555				3052_L1				3052_L2				3052_L5				3052_L10				3052_L20			
Name	Transition	RT	Final Conc.	Accuracy	Ave	RT	Final Conc.	Accuracy	Ave	RT	Final Conc.	Accuracy	Ave	RT	Final Conc.	Accuracy	Ave	RT	Final Conc.	Accuracy	Ave	RT	Final Conc.	Accuracy	Ave		
101.FTHL	610.0 > 531.0	13.676	864.7312		426	13.676	0.5265	105.9	96.4	2141	13.676	0.5640	96.4	2141	13.676	0.5640	96.4	2141	13.676	0.5640	96.4	2141	13.676	0.5640	96.4	2141	
102.FTHL	610.0 > 266.564	18.05	12.148	0.574	1149	18.05	0.9909	96.9	1167	13.13	1.9598	98.0	2160	12.148	4.9200	96.6	1961	12.153	93.84	10145	12.153	18.0595	96.2	2149			
102.FTHL	565.0 > 86.9	14.031	1475.3654		459	14.031	2.7802	1112	1262	14.037	5.0801	101.6	2710	14.037	10.0520	100.2	3910	14.037	24.5000	97.4	9823	14.037	47.6241	95.2	17039	14.037	93.0887
102.FTHL	565.0 > 527.0	13.371	2194.3521		278	13.365	3.1071	1243	2089	13.373	6.0426	120.9	3316	13.371	9.5392	96.4	4580	13.365	22.2936	98.2	9365	13.365	41.2509	92.5	17113	13.365	85.5596
111.FTHL	601.0 > 280.0	16.054	528.3290		278	16.049	0.5571	105.5	449	16.048	0.1034	103.0	817	16.043	1.9192	95.9	1341	16.043	5.0007	100.2	3141	16.043	9.3180	93.2	5970	16.049	19.2776
112.FTHL	265.0 > 270.0	15.588	82.0050		374	15.583	0.5175	103.5	2886	15.586	0.9887	99.0	6580	15.583	2.0510	102.5	11119	15.547	5.1720	103.5	26873	15.543	9.5452	95.5	15725	15.548	10.9916
4.2.FTHL	301.0 > 281.0	7.735	99.3656		458	7.724	0.5066	104.1	2901	7.724	1.0245	102.5	3024	7.724	1.9666	98.3	10740	7.718	5.0341	101.9	26736	7.724	9.5732	95.7	52463	7.724	19.4592
6.1.FTHL	351.0 > 331.0	8.572	153.0862		484	8.572	0.5338	106.5	2158	8.566	1.0277	102.8	4114	8.566	1.9249	96.2	7304	8.566	4.9198	98.4	17733	8.566	9.8448	94.8	36917	8.566	19.3300
6.1.FTHL	410.0 > 73.0	4.701	32.3430		130	4.695	0.5276	105.5	2491	4.694	1.034	103.4	3212	4.695	1.9516	97.6	9605	4.695	5.0302	100.4	24266	4.695	9.4152	96.2	48335	4.695	19.3810
6.1.FTHL	423.0 > 87.0	7.962	48.0569		79	7.957	0.5041	110.4	8917	7.967	0.9941	98.4	1885	7.967	1.9883	94.3	3560	7.961	5.2000	104.3	9627	7.967	9.5742	95.7	18560	7.967	19.4000
6.2.FTHL	365.0 > 327.0	8.160	139.0966		215	8.165	0.5327	106.5	104.0	8.166	0.9651	96.5	1889	8.160	2.0543	102.7	3811	8.160	4.9663	99.3	8786	8.165	9.7109	97.1	17080	8.160	19.4307
7.1.FTHL	301.0 > 281.0	9.558	203.8817		655	9.																					





# Non-Drinking Water Sample Methods: CWA 1633—*Isotope Dilution*

## Flexibility in Application

- Columns
- Elution schemes

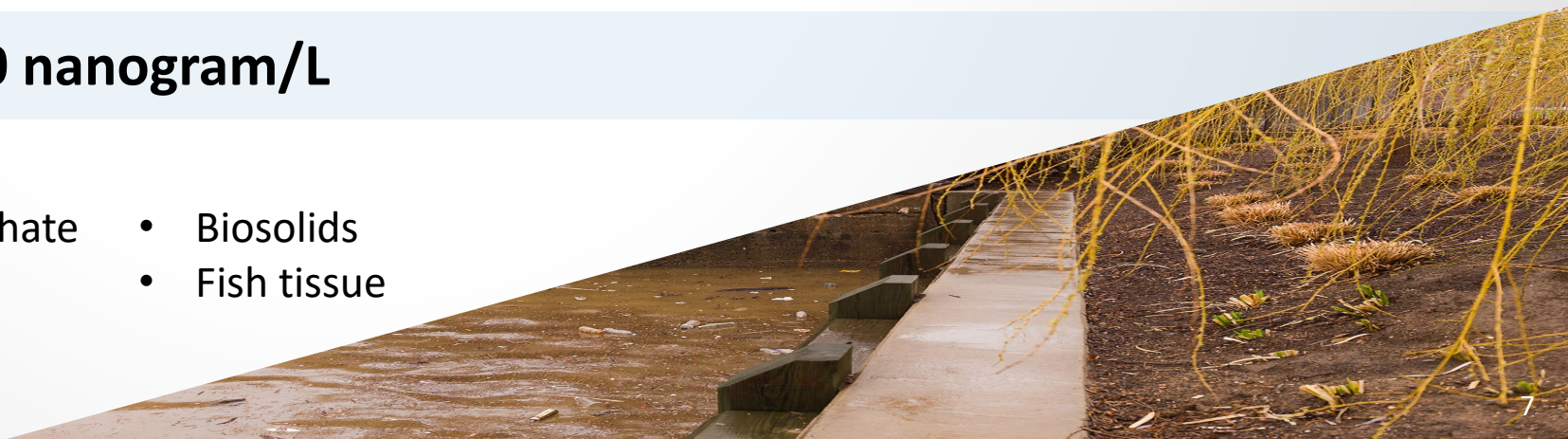
## Single laboratory validated and released as draft CW1633 in Sept 2021

- Collaborative effort among DoD, EPA Office of Water, EPA Office of Land and Emergency Management, and EPA ORD
- Multi-laboratory validation will start in 2021
- Method being developed in accordance SWA-846 protocols for method development

## Target Quantitation Limits: 1-10 nanogram/L

### Matrices include:

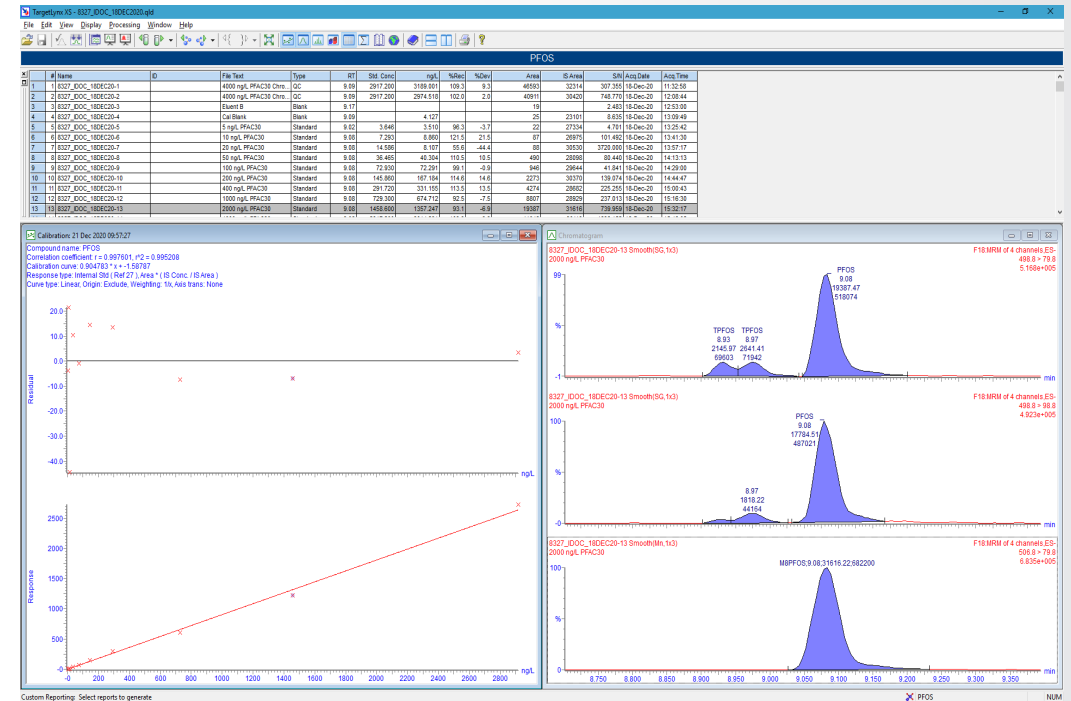
- Wastewater (influent and effluent)
- Groundwater
- Surface water
- Landfill leachate
- Soil
- Sediment
- Biosolids
- Fish tissue





# SW846 Method 8327

- Methods 3512 (extraction) and 8327 (Analysis) published July 2021
- Method developed by R5/OLEM for 24 PFAS in non-potable water
- Direct Injection, LC/MS/MS data acquisition, quantification by external standard calibration technique
- A simple and robust high throughput analytical method for screening level characterization of surface water, groundwater, wastewater
- Limit of Detection (LOD) higher than the SDWA methods







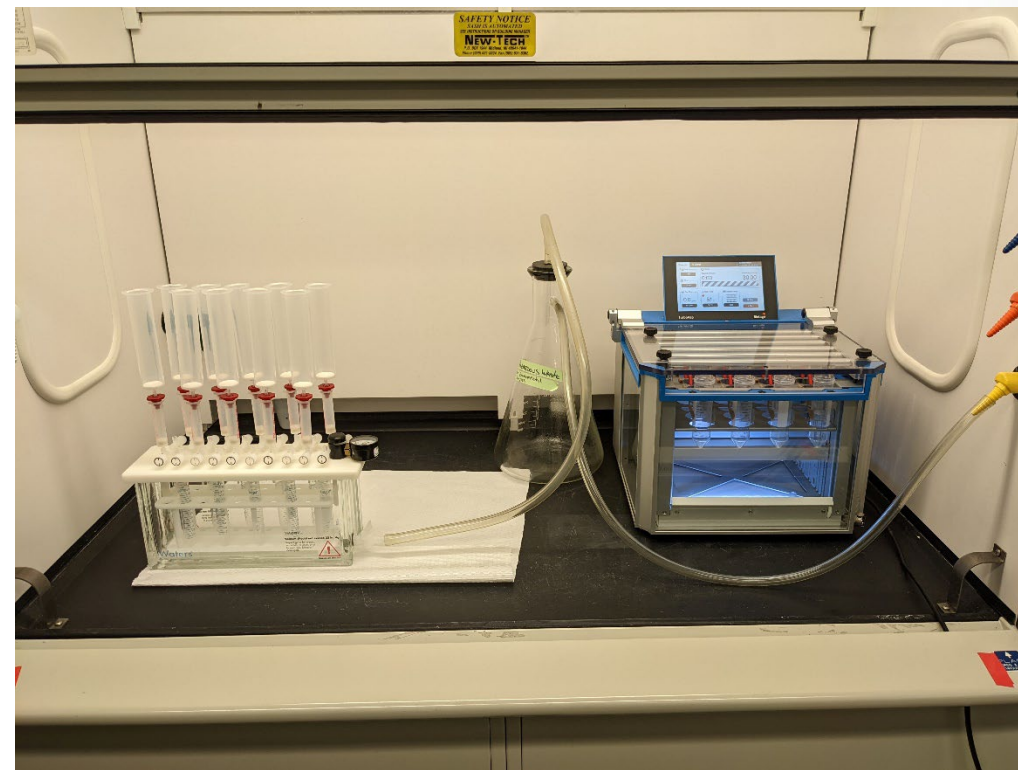
## CESER PFAS Analytical Research Support Capabilities

- CESER research analytical chemistry staff includes staff with extensive experience in the development of analytical chemistry methods for PFAS and other emerging contaminants
- SDWA EPA methods 537.1, 533 (supporting the EPA Office of Water, OW)
- SW846 methods 8327 (supporting EPA region 5 and OLEM), draft method 1633 (supporting OLEM), draft method 1621
- CESER Standard Operating Procedures (SOPs) for research applications
- Supporting internal and external collaborations for PFAS analytical
- Technical support to EPA regional partners, State and Municipal entities



## Solid Phase Extraction by LC/MS/MS

- CESER SOPs for extraction/analysis of aqueous and solid sample matrices
- Potable and non-potable water, wastewater, landfill leachates, soil, sediment and biosolids.
- 30 PFAS, including perfluorocarboxylic and sulfonic acids (chain length  $\leq C14$ ), perfluoroethers (HFPO-DA), and sulfonamides
- Solid Phase Extraction, LC/MS/MS data acquisition, quantification by internal standard and isotope dilution calibration
- Limit of Detection (LOD) capable of regulatory application to PFOA/PFOS advisory level and lower



## Grab Sampling

- **Grab sampling is commonly used for PFAS**
- **Consideration needed of personal care products and clothing for potential contamination**
- **Sample bottle should be opened prior to the sampling and dipped inside the water and closed with the lid immediately after sample collection**
- **Pack the sample bottles with individual PFAS free zip-lock bags to separate samples and pack with regular ice**
- **Overnight shipment to analytical laboratory**



## ■ Advantages

- Low-cost and time-integrative sampling approach proven useful for broad range of contaminants
- Accumulates dissolved contaminants through diffusion
- QC samples include field blanks and laboratory blanks

## ■ Considerations

- Commonly used for neutral compounds
- PFAS are ionic - anions
- Challenges for sampling ionized compounds



- **Polar Organic Chemical Integrative Sampler (POCIS)**
  - Anions (e.g. PFOA, PFOS)
  - Used for water sampling
- **Polyethylene samplers**
  - Neutral species - Precursors
  - (e.g. 6:2, 8:2 and 10:2 FTOH, Me/Et-FOSA, Me/Et-FOSE)
  - Used for both water and air sampling
- **New passive sampler materials specific to PFAS?**



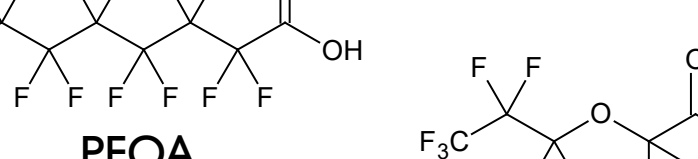


# Air Methods

## Other Test Method (OTM)-45

- OTM-45 is a method for measuring 50 PFAS in air emissions from stationary sources.
- OTM-45 is a draft method that is under evaluation and will be updated and revised as data from stakeholders becomes available.
- [Measurement of Selected Per- and Polyfluorinated Alkyl Substances from Stationary Sources](#)
- EPA is currently refining methods to characterize PFAS in emissions from stationary sources. Candidate methods for evaluation and validation expected in 2022.



- 
- PFOA**  
FC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(=O)O
- PFOS**  
FC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)S(=O)(=O)O
- HFPO-DA (GenX)**  
FC(F)(F)C(F)(F)OC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(=O)O



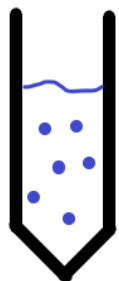
# Method Development Approach

- Simplify sample preparation as much as possible
  - \*\* Nothing but dilution with methanol and aliquoting (to reduce PFAS sorption loss)
- Concentrate samples using anion-exchange-based SPE to improve sensitivity, 5 mL analysis aliquots
  - \*\* Online SPE to automate this process/remove separate preparation stages
- Track analyte recovery using isotope dilution to account for losses in concentration/analysis stages
  - Particularly vital for competition/anion studies where method performance is challenged



# Isotope Dilution Mass Spectrometry

Sample solution,  
diluted with  
methanol



Analysis  
aliquot



Fortification with  
labeled PFAS (e.g.  
 $^{13}\text{C}_8$ -PFOA)



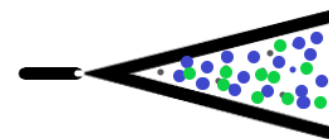
On-line Solid-Phase  
Extraction (Anion  
exchange resin)



Both PFOA and  $^{13}\text{C}_8$ -  
PFOA are retained  
equally by sorbent,  
and concentrated



Analysis (Electrospray  
Mass Spectrometry)



Quantified as ratio of  
PFOA/ $^{13}\text{C}_8$ -PFOA vs  
calibration

- Addition of isotopically labeled PFAS (in green) allows for tracking of SPE and mass spectrometric interferences to correct for method performance
- Without labeled materials, matrix, anions, and other interferences can compromise method accuracy and reproducibility.



# Validating SPE Recoveries

Percentage Recovery of 200 ng/L PFAS mix spike, %RSD in parenthesis

Fortification	PFBA	PFBS	HFPO-DA	PFHxS	PFOA	PFOS	PFNA	PFDA
Control (unfortified)	98 (9)	110 (15)	100 (10)	102 (3)	108 (12)	104 (9)	106 (2)	107 (6)
10 µg/L PFBA Spike	NA	94 (2)	92 (5)	90 (6)	92 (3)	92 (3)	117 (12)	96 (2)
10 µg/L PFBS Spike	94 (8)	NA	100 (6)	99 (4)	101 (4)	103 ( )	109 (4)	105 (2)
10 µg/L HFPO-DA	106 (2)	105 (2)	NA	106 (1)	106 (7)	107 (4)	107 (5)	106 (10)
10 µg/L PFHxS	97 (7)	105 (2)	101 (5)	NA	104 (6)	104 (4)	105 (9)	108 (5)
10 µg/L PFOA	104 (1)	105 (1)	100 (11)	105 (5)	NA	104 (3)	102 (5)	104 (8)
10 µg/L PFOS	105 (0)	104 (2)	97 (2)	100 (9)	108 (3)	NA	(48) <sup>1</sup> (7)	108 (5)
10 µg/L PFNA	96 (2)	105 (3)	103 (7)	105 (5)	110 (5)	105 (12)	NA	108 (3)
10 µg/L PFBA, PFBS	NA	NA	98 (3)	103 (2)	102 (2)	102 (4)	111 (13)	104 (1)
10 µg/L PFOA, PFOS	107 (1)	105 (1)	102 (8)	108 (10)	NA	NA	104 (14)	106 (6)
100 µg/L PFBA	NA	93 (3)	88 (3)	93 (7)	95 (1)	92 (11)	96 (7)	97 (5)
100 µg/L PFNA	84 (9)	95 (5)	85 (3)	93 (5)	148 <sup>2</sup> (5)	(8%) <sup>1</sup>	(18%) <sup>1</sup>	97 (15)

1. Flagged as suspect due to low internal standard recovery. In all other cases internal standard recoveries were within the 50-150% tolerance for the concentrations reported.
2. Possible PFOA contamination in PFNA stock causing high recovery (max ~0.1%).





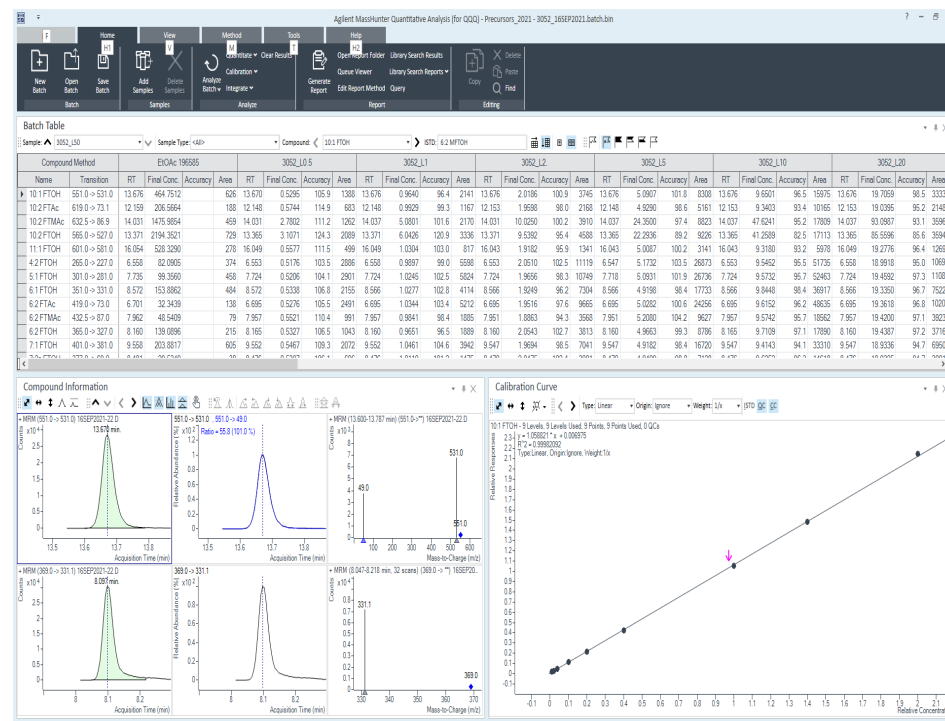
# PFAS Precursor Analysis by GC/MS/MS

## EPA ORD SOPs for Non-potable Waters and Solids

- Water - SPE, analysis by GC/MS/MS
- Solids – solvent extraction, analysis by GC/MS/MS, sample cleanup with SPE or GAC.

## Target Analytes:

- 12 Fluorotelomer Alcohols (FTOHs)
- 6 Fluorotelomer Monomers
- 3 Perfluorosulfonamido-ethanols (FOSE)
- Solid Phase Extraction, GC 3Q data acquisition, quantification by internal standard calibration.





## Summary: EPA PFAS Targeted Methods, June 2022

### EPA has validated Standard Methods complete or in development for PFAS in water

- Final SDWA Methods 533 and 537.1 for available for drinking water (29 PFAS)
- Method SW846-8327 validated for non-potable water (24 PFAS)
- Draft Method 1633 undergoing multi lab validation for non-potable water/solids (40 PFAS)

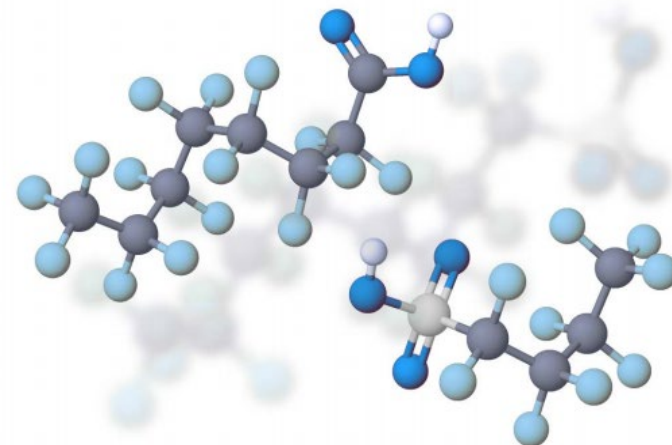
### EPA has or is developing additional methods for partner use

- **Fish Tissue** – Isotope dilution method for 13 PFAS has been used in national surveys
- **Serum** – Isotope dilution method (targeted and non-targeted) used in biomonitoring
- **Ambient air and emissions** – Sampling and analysis methods undergoing development and testing
- **Total Organic Precursors (TOP)** – Identify total PFAS load which may degrade to most persistent PFAS
- **Total Organic Fluorine (TOF)** – Potential rapid screening tool to identify total PFAS presence/absence
- **Nontargeted analysis** – Continued development and application of HRMS methods for discovery of novel PFAS, suspect screening analysis, and identification of transformation and end products.

## Non-targeted analysis for Total PFAS

- Adsorbable Organic Fluorine (AOF) Screening Method with Detection by Combustion Ion Chromatography
- Suspect Screening and Identification using High Resolution Mass Spectrometry (HRMS)
- Research applications for identification of unknown organofluorine chemicals

### EPA's Per- and Polyfluoroalkyl Substances (PFAS) Action Plan



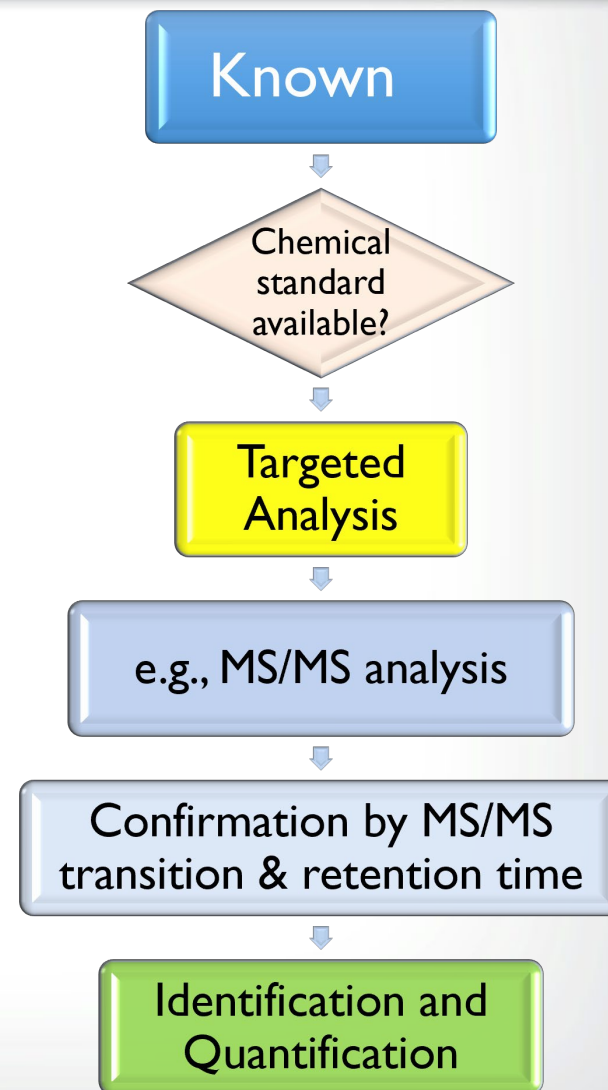


## Why “Total PFAS” Methods?

Currently, most common PFAS detection technique is mass spectrometry (MS) using targeted analysis

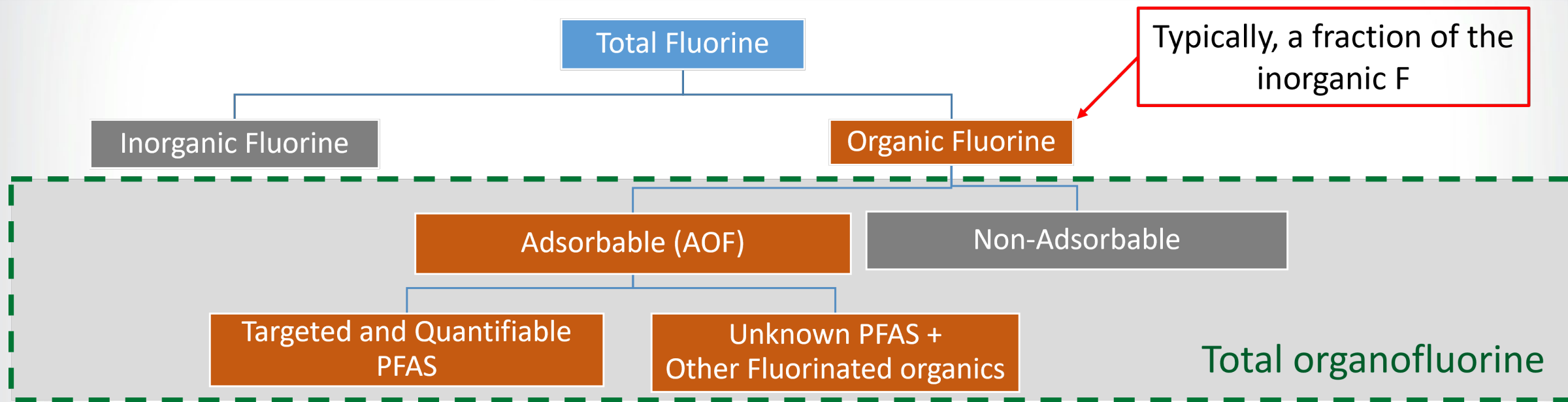
### ❖ Targeted Analysis:

- methods applicable to a specific defined set of known analytes
- analytical standards exist for quantitation
- methods only measure for analytes on the targeted list; once the analysis is complete, you can't look for other analytes.
- >4000 PFAS in existence





## Goals (continued)

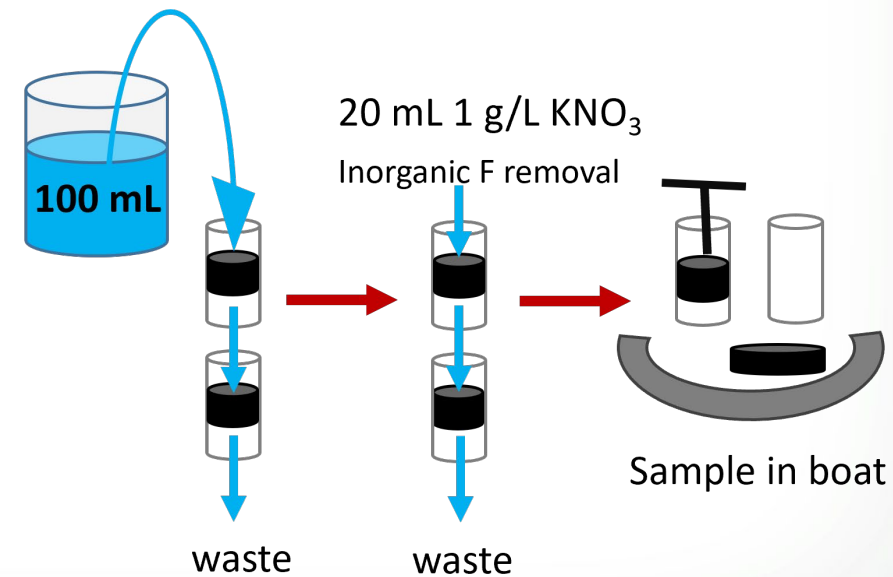
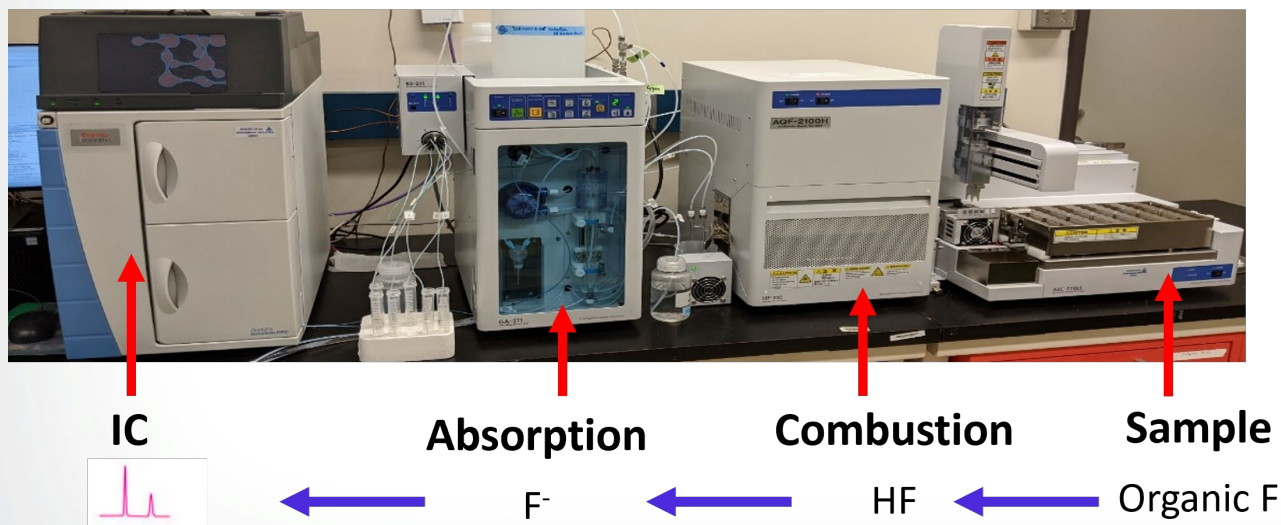


- Develop a screening method for wastewaters that measures adsorbable organic fluorine (AOF)
- Aid in screening for PFAS contamination or assessing removal of organofluorine contaminants in treatment streams
- AOF measurements must address removal of inorganic fluorine
- AOF measurements will include all adsorbable organic fluorine, e.g., fluorinated pharmaceuticals, agrochemicals



## How:

- Screening method adsorbs contaminants onto granular activated carbon, removal of inorganic fluoride with nitrate solution, followed by combustion of the carbon
- Organofluorine compounds are converted to fluoride in the combustion process and measured by ion chromatography



**Method Detection Limit:** 1.4 - 2.2 µg/L



## Current Status – AOF/CIC

### Status:

- Recovery data for 35 PFAS, 2 PFAS mixtures, 4 fluorinated pesticides and pharmaceuticals
- Spiked recovery data in 14 wastewaters and 2 surface waters
- Delivery of draft wastewater screening method to OW by October 2021
- Multi-lab validation to be conducted by OW



## CIC Method for Drinking Water

- Investigating development of a CIC method for drinking water
- Meaningful detection limit for DW is very challenging
- Increased sample volume using AOF following Draft Method 1621 resulted in loss of recovery
  - PFBA recovery in 500 mL tap water sample was 23.9%
- Extractable organofluorine (EOF) using solid phase extraction (SPE) with liquid injection into CIC may allow concentration factors as high as 2000X
  - Background fluoride contamination (reagents, materials) will significantly impact detection limit



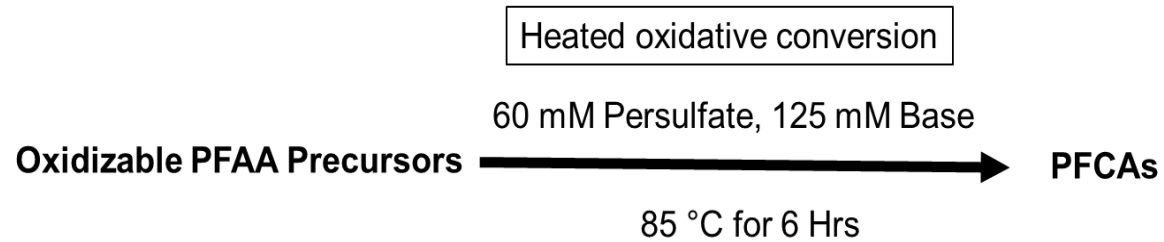
# Total Organic Fluoride for PFAS

## Potential Future Work:

- To evaluate options that would lead to lower detection levels for the AOF/CIC method
- To keep an eye on other approaches that may come closer to capturing all PFAS within the method while not capturing inorganic fluoride or fluoride associated with other organic molecules (e.g., pesticides)
- Evaluate AOF/CIC technique for other matrices



# Total Oxidizable Precursor Assay (TOP Assay)



$$\text{Total Oxidizable Precursors} = \text{PFCAs}_{\text{after oxidation}} - \text{PFCAs}_{\text{before oxidation}}$$

- Applicable for both aqueous and solid matrices
- Conservative estimate of the total concentration of PFAA precursors

- Does not identify individual precursor compounds
- More expensive technique - Sample needs to be analyzed 2 times for PFAAs – before and after oxidation
- Available in commercial labs and under evaluation at EPA, however, no standard methods currently exist.





## Target vs Non-Target Analysis

**The most common PFAS detection technique is mass spectrometry**

Target	Non-Target
Methods applicable to a specific defined set of known analytes	Methods use high resolution mass spectrometry (HRMS) capable of identifying all known & unknown analytes in a sample
Analytical standards must exist for quantitation	Can screen for lists of known suspects & can discover new or unknown analytes providing a tentative ID.
Methods only measure for analytes on the targeted list; once the analysis is complete, you can't look for other analytes.	HRMS data can be stored and analyzed later for newly identified analytes. If standard is available, quantitation can be performed.



Investigate the effect of known and unknown chemical formulations on water quality using non-suspect screening tools

- **Identify unknown chemicals by suspect screening analysis (SSA)/non-targeted analysis (NTA) using high resolution mass spectrometry (Orbitrap LC-MS).**

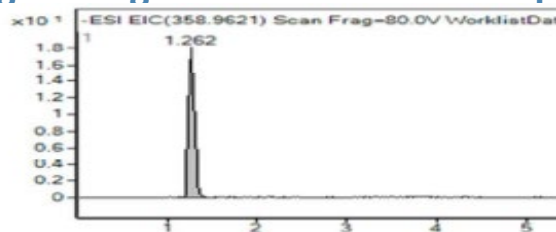




# Non-Targeted Analysis

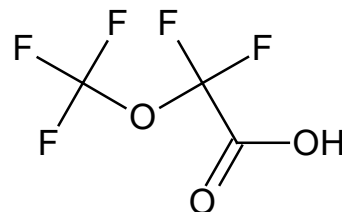
Explore Unknown compounds using High resolution mass spectrometry. Identify a peak in a chromatogram and to ultimately predict the identity of this unknown

↓ Mass spectrometer assigns a high resolution mass for peaks observed in the chromatogram



↓ Software calculates the exact number and type of atoms needed to achieve the measured mass.

↓ Fragmentation experiments allow determination of most likely structure:



Molecular Formula:  $C_3HF_5O_3$   
Monoisotopic Mass: 179.984585 Da  
[M-H]<sup>-</sup>: 178.977308 Da

↓ Using mass, formula, and structure, identity can be assigned by searching against databases of known compounds

↓ Compare peak to commercial material to confirm identification if possible



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