

# Analytical methods for PFAS in environmental media

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*8 January 2023*

- Types of analytical and standard methods
- Drinking Water Sample Methods
  - Method 537
  - Method 533
- Non-Drinking Water Sample Methods
  - SW-846 Method 8327 – Direct Injection
  - CWA/SW846 Method—Isotope Dilution
- PFAS Analysis in Marine Waters
- PFAS Analysis in Fish Tissue
- Total Organofluorine Analysis using Combustion Ion Chromatography (TOF)
- Total Oxidizable Precursors (TOP)
- Summary of EPA PFAS Methods as of January 2023

## Guidance to avoid cross contamination in sampling

- No teflon, fluoropolymers, etc.
- Avoid contact with clothes, materials containing PFAS (e.g. some food wrappers)
- See [PFAS Analytical Methods Development and Sampling website](#)
  - Sampling guidance from states (e.g. MI)
  - Interstate Technology and Regulatory Council Fact Sheet: *Site Characterization Considerations, Sampling Precautions, and Laboratory Analytical Methods for PFAS*
- [PFAS Quality Assurance Plan and Data Review](#) technical brief

**Targeted methods** are methods which are applicable to a specific defined set of known analytes

- Analytical standards exist for quantitation
- Method only 'sees' analytes on the targeted list – will not measure others
- 'One and done' – once the analysis is complete, can't look for other analytes

**Non-targeted methods** involve the use of High Resolution Mass Spectrometry (HRMS) capable of identifying all analytes in a sample, known and unknown

- Can quantitate those for which laboratory standards exist, otherwise may semi-quantitate based on known, structurally similar analytes
- Can screen for lists of known suspects, can discover new/unknown analytes
- Can store the HRMS data and go back later to look for analytes which were unidentified at the time of analysis, but which later become known



# Types of Analytical Methods

## Three broad classes of methods:

- EPA Standard Methods
  - Methods which have been through a multi-lab validation following a particular rulemaking or guidance effort and are available to support Agency regulatory or guidance activities
- Research Methods
  - Methods which have been developed by an EPA ORD laboratory for research purposes; QA'ed and peer reviewed via publication, but not multi-lab validated, not considered EPA Standard Methods
- Developmental Methods
  - Methods which are currently undergoing research, development and testing; might become Standard Methods or Research Methods



# 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.



# PFAS Test Methods - Overview

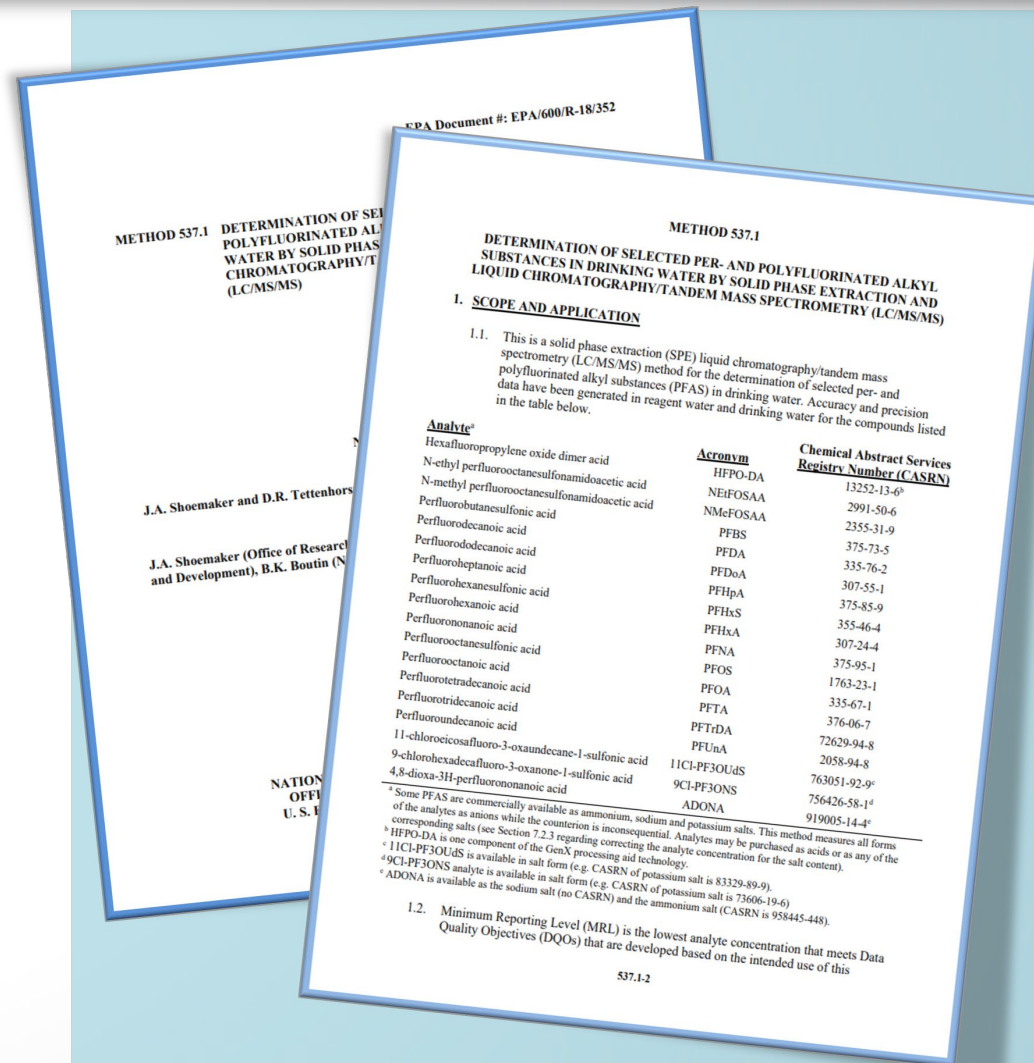
**Table E-2. Analytical Methods**

Media	Agency	Method	Validation: Intra-Lab	Validation: Inter-Lab	Individual PFAS	Total PFAS	Organofluorine Compounds (including, but not limited to PFAS)	Publicly Available	Agency Use Only
Drinking Water	EPA	Method 537 Version 1.1	X	X	X	-	-	X	-
Drinking Water	EPA	Method 533	X	X	X	-	-	X	-
Groundwater, Surface Water, Wastewater Effluent	EPA	SW-846 Method 8327	X	X	X	-	-	X	-
Aqueous (non-Drinking Water), Solids, and Tissue	EPA/DoD	CWA Method 1633	X	Ongoing	X	-	-	X	-
Aqueous - Wastewater	EPA	CWA Method 1621	x	Ongoing	X	-	x	X	-
Air	EPA	OTM-45	X	-	X	-	-	X	-
Blood Serum	CDC	Method 6304.9	X	-	X	-	-	X	-
Food	FDA	C-010.01	X	-	X	-	-	X	-
Cattle, Swine, Poultry, Siluriformes muscle and bovine plasma	USDA-FSIS	CLG-PFAS 2.03	X	-	X	-	-	X	-
Groundwater, Surface Water, Wastewater Effluent	USGS	LC 9660 (Direct Aqueous Injection)	X	-	X	-	-	-	X
AFFF and AR-AFFF	DoD	DoD AFFF01	x	x	-	-	-	x	-



# Drinking Water Method 537: Revision I

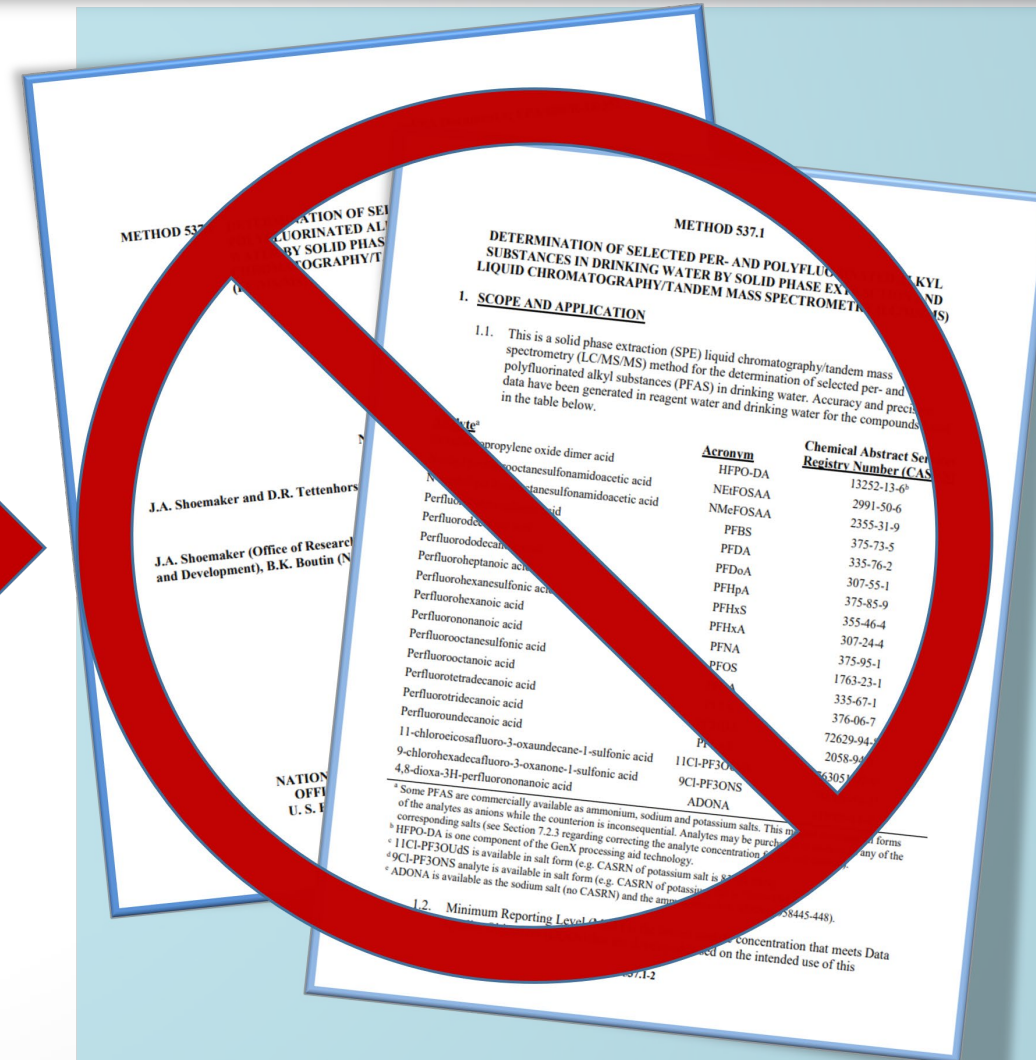
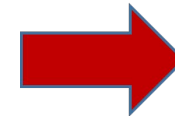
- Update: External lab validation for additional analytes by 537
  - Perfluoro-2-propoxypropanoic acid (GenX chemical HFPO-DA, CAS 13252-13-6)
  - Potassium 9-chlorohexadecafluoro-3-oxanone-1-sulfonate (9Cl-PF3ONS, CAS 73606-19-6)
  - Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (11Cl-PF3OUdS, CAS 83329-89-9)
  - Sodium dodecafluoro-3H-4,8-dioxanone (ADONA, CAS 958445-44-8)
- Incorporated clarifications issued in [EPA Technical Advisory-Laboratory Analysis of Drinking Water Samples for Perfluorooctanoic Acid \(PFOA\) Using EPA Method 537 Rev. 1.1](#)
- [Final published method](#) (November 2018)
- LC/MS/MS with internal standards. Single lab lowest concentration minimum reporting levels (LCMRLs) range from 0.53-6.3 ng/L





# Drinking Water Method 537: "Modified"

- Method 537 is often modified by analytical laboratories for use on non-drinking water samples
- If modifications are made that are not explicitly listed in 537 or 537 Revision 1, the method is **not** considered 537 by EPA
- The most common modification is inclusion of isotope dilution





## Drinking Water Method 533

### Solid phase extraction/isotope dilution method targeting PFAS <C12

- Method 537 generally performs poorly for C4 compounds (e.g. PFBA, PFBS).
- Solid phase extraction, LC/MS/MS, Isotope dilution
- Will allow EPA to consider additional PFAS for monitoring under the fifth Unregulated Contaminant Monitoring Rule
- Released December 2019
  - [Analytical Methods Developed by EPA for Analysis of Unregulated Contaminants](#)





# Drinking Water Method 533 (continued)

Method 533	Both Methods	Method 537.1
1H, 1H, 2H, 2H-perfluorodecane sulfonic acid (8:2 FTS)	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA)
1H, 1H, 2H, 2H- perfluorohexane sulfonic acid (4:2 FTS)	9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS)	N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA)
1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS)	4,8-dioxa-3H-perfluorononanoic acid (ADONA) <sup>3</sup>	Perfluorotetradecanoic acid (PFTA)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	Hexafluoropropylene oxide dimer acid (HFPO-DA)	Perfluorotridecanoic acid (PFTTrDA)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	Perfluorodecanoic acid (PFDA)	-
Perfluoro-3-methoxypropanoic acid (PFMPA)	Perfluorododecanoic acid (PFDoA)	-
Perfluoro-4-methoxybutanoic acid (PFMBA)	Perfluorohexanoic acid (PFHxA)	-
Perfluorobutanoic acid (PFBA)	Perfluoroundecanoic acid (PFUnA)	-
Perfluoroheptanesulfonic acid (PFHpS)	<b>Perfluorobutanesulfonic acid (PFBS)</b>	-
Perfluoropentanesulfonic acid (PFPeS)	<b>Perfluoroheptanoic acid (PFHpA)</b>	-
Perfluoropentanoic acid (PFPeA)	<b>Perfluorohexanesulfonic acid (PFHxS)</b>	-
-	<b>Perfluorononanoic acid (PFNA)</b>	-
-	<b>Perfluorooctanoic acid (PFOA)</b>	-
-	<b>Perfluorooctanesulfonic acid (PFOS)</b>	- <b>Bold indicates analytes listed on UCMR 3</b>



# Non-potable Aqueous Sample Methods: *SW-846 Method 8327—Direct Injection*

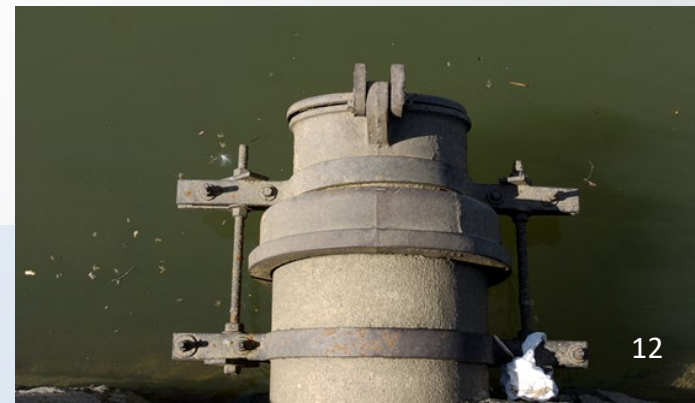
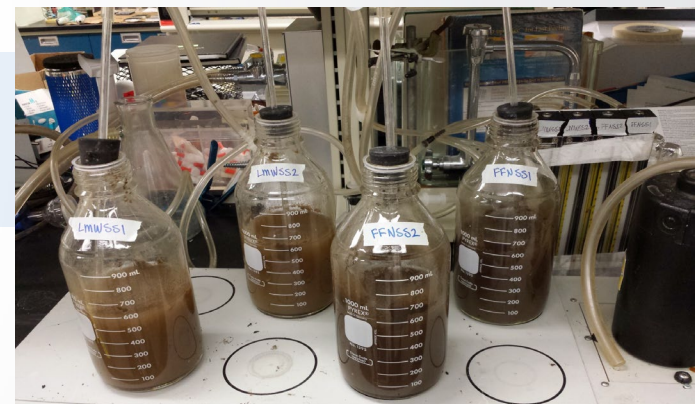
## Non-drinking water aqueous matrices:

- Groundwater
- Surface water
- Wastewater

## Finds a balance between sensitivity, ease of implementation, and monitoring requirements

- Simplicity
- Robustness
- Maximizing throughput for production lab use
- Minimizing sample transfers, extractions, filter steps, chemical additions (e.g., pH adjustments)

[Validated Test Method 8327: PFAS Using External Standard Calibration and MRM LC-MS/MS](#)







# Non-Drinking Water Sample Methods: *SW-846 Method 8327—Direct Injection*

## 24 PFAS (including all target analytes in EPA Method 537)

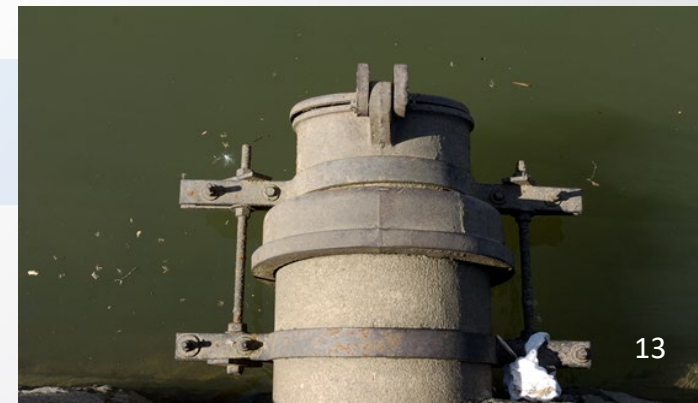
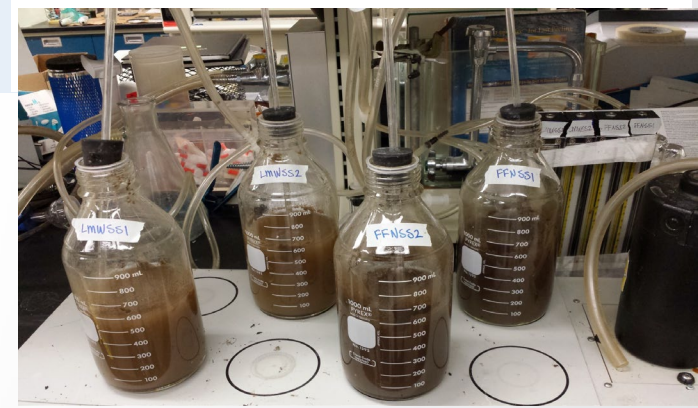
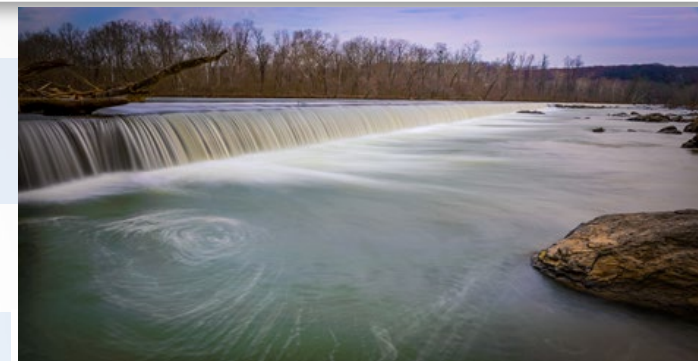
- Commercially available standards (“native” and isotopically labeled)

## Direct injection-EPA Region 5/Chicago Regional Lab SOP

- Similar to draft ASTM Method D7979
- Multi-laboratory validation study completed
- OLEM addressing public comments
- Validated method available for use

**Target Quantitation Limits: 10 nanogram/L**

**Associated preparation method 3512 for aqueous matrices**





## Targeted methods: SW-846 Methods 3512 and 8327

- **Status**: Final methods were published in July 2021
    - Tested in wastewater, groundwater surface water
    - Validated at Lower Limits of Quantitation (LLOQs) as low as 10 ng/L
  - **Process**:
    - Dilute sample 1:1 with methanol
    - Vortex for 2 min
    - Filter through 0.2  $\mu\text{m}$  filter
    - Add 0.1% acetic acid by volume
    - Analyze by LC/MS/MS
- | Advantages  | Disadvantages   |
|---|---|
| <ul style="list-style-type: none"><li>• Small sample size (5 mL)</li><li>• Rapid sample preparation</li><li>• Few Process steps</li></ul> | <ul style="list-style-type: none"><li>• Small dilution factor (2x)</li><li>• Need modern LC/MS instrument to achieve low ng/L sensitivity</li><li>• Not consistent with current practice in many testing laboratories</li></ul> |



# Non-Drinking Water Sample Methods: CWA-1633/SW-846 Method—*Isotope Dilution*

## Build in flexibility

- Columns
- Elution schemes

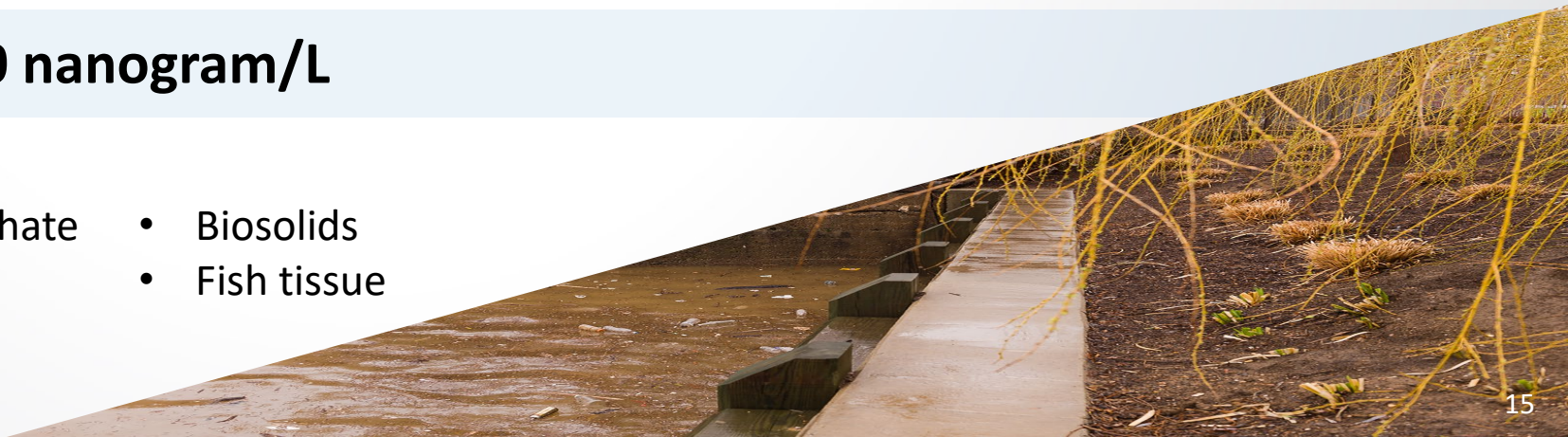
## Single laboratory validated and released as draft [CWA-1633](#) in Sept 2021

- Collaborative effort among DoD, EPA Office of Water, EPA Office of Land and Emergency Management, and EPA ORD
- Multi-laboratory validation started in 2021
- Method being developed in accordance SW-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







## Non-Drinking Water Sample Methods: CWA/SW846 Method—*Isotope Dilution*

**More complex method relative to direct injection; however, the method:**

- Is more robust for complex matrices (e.g., wastewater influents, biosolids). Account for matrix effects (e.g., sorption) through isotopically-labelled standard recoveries;
- Is capable of meeting DoD requirements; and
- Allows users to perform lower-level analyses based on screening results (e.g. 8327).

**For all matrices, isotopically labeled standards added prior to extraction**

- Aqueous samples are extracted using solid-phase extraction (SPE) cartridges and undergo cleanup using carbon before analysis.
- Solid samples are extracted into basic methanol and cleaned up with carbon and SPE cartridges before analysis.
- Tissue samples are extracted in potassium hydroxide and acetonitrile followed by basic methanol and cleaned up with carbon and SPE cartridges before analysis.







## Non-Drinking Water Sample Methods: CWA-1633/SW-846 Method—*Isotope Dilution*

### More complex method relative to direct injection; however, will:

- be more robust for complex matrices (e.g., wastewater influents, biosolids). Account for matrix effects (e.g., sorption) through isotopically-labelled standard recoveries;
- afford options to meet DoD requirements; and
- allow users to perform lower-level analyses based on screening results (e.g., 8327, TOF).

### 40 PFAS analytes - includes all analytes listed in 537.1, 533, and SW-846 8327

### Non-drinking water samples

- Surface water, groundwater, wastewater
- Landfill leachates
- Solids (soils, sediments, biosolids, tissues)





# PFAS Analysis in Fish Tissue

**Currently, CW1633 method includes PFAS in fish tissue – EPA also uses commercial proprietary methods.**

- LC-MS/MS with solid phase extraction and isotope dilution
- Similar to DW 533 and SWA 1600 but don't dare call it a Modified Method...

## Method Details

- Covers 13 carboxylic and sulfonic acids from C4 to C12, plus PFOSA; now 33 analytes
- Quantitation limits ranged from 0.25 to 1.25 ng/g (ppb) for the 13 (0.38-4.09 ng/g for 33)
- Spike stable isotopically-labeled PFAS analogs into 1-2 g fillet tissue sample
- Sample digested with caustic (KOH or NaOH) methanol solution to release PFAS from tissue
- Solids removed by centrifugation; aqueous solution processed by SPE extraction
- LC-MS/MS for analysis and quantitation



**Longer chain PFAS C8+ most consistently present**



# PFAS Compounds Identified in Fish

Name	Abbreviation	Name	Abbreviation
Perfluorobutyric acid	PFBA	Perfluoroundecanoic acid	PFUnA*
Perfluoropentanoic acid	PFPeA	Perfluorododecanoic acid	PFDoA*
Perfluorohexanoic acid	PFHxA	Perfluorobutanesulfonic acid	PFBS
Perfluoroheptanoic acid	PFHpA	Perfluorohexanesulfonic acid	PFHxS
Perfluorooctanoic acid	PFOA	Perfluorooctanesulfonic acid	PFOS*
Perfluorononanoic acid	PFNA*	Perfluorooctanesulfonamide	PFOSA*
Perfluorodecanoic acid	PFDA*	-	-

\*Indicates PFAS compounds consistently found in fish tissue in our studies.



- **Total Organic Fluorine (TOF) - Combustion – Ion Chromotography to measure total organic fluorine**
- **Particle induced gamma emission (PIGE) to measure total fluorine.**
- **Total Oxidizable Precursors (TOP) is a sample pre-treatment to characterize precursors.**



# Total Organofluorine (TOF) Analysis using Combustion Ion Chromatography



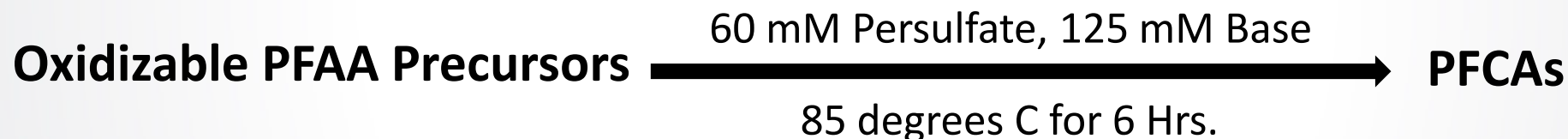
- Less specialized or costly instrumentation required
- Applied for aqueous matrices
- Removing the background inorganic  $F^-$  from the sample is important to make sure that the reported  $F^-$  is organic
- May be developed on a wide commercial scale
- High priority for EPA. ORD working with OW-OST and released a draft EPA Method 1621 in 2021



## Total Oxidizable Precursors (TOP)

- Developed by Houtz et al. No multi-laboratory validated standard method exist currently.
- Available from some commercial laboratories
- Does not identify individual precursor compounds

Heated oxidative conversion

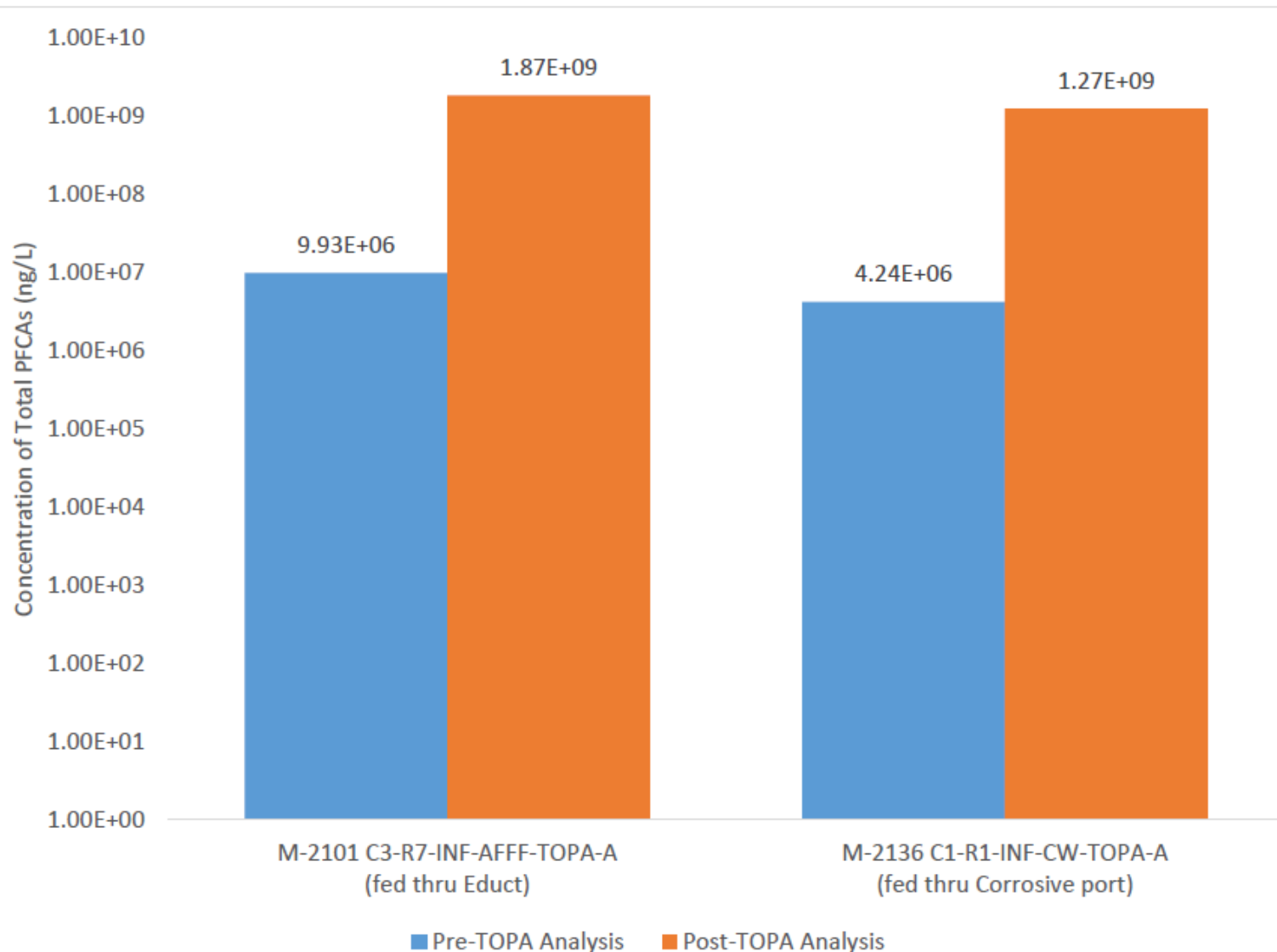


**Total Oxidizable Precursors = PFCAs (after oxidation) – PFCAs (before oxidation)**

- Applicable for aqueous and solid matrices
- Conservative estimate of the total concentration of PFAA precursors
- More expensive; sample needs to be analyzed twice for PFAS

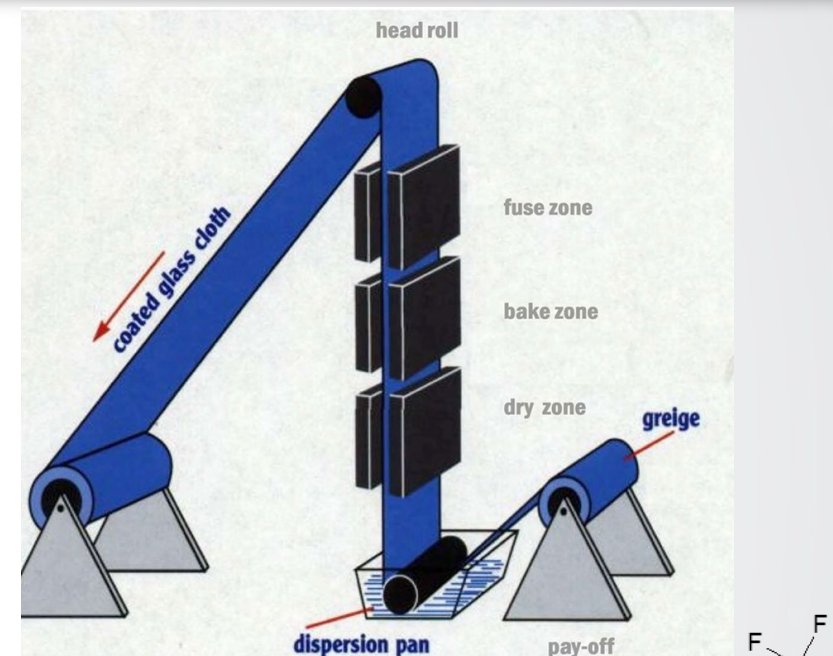


# PFCAs in AFFF concentrate: pre- and post-Total Oxidizable Precursors Assay

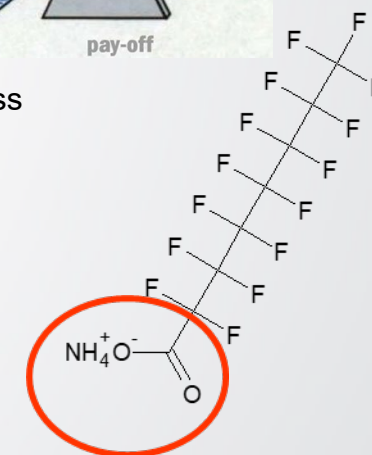
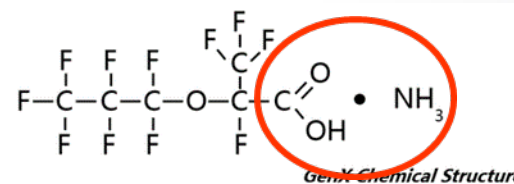




- Industrial emission sources are diverse:
  - PFAS chemical manufacturers
  - PFAS used in commercial applications
  - Process can alter emission composition**
- PFAS incineration sources
  - PFAS wastes (e.g., AFFF)
  - Products of Incomplete Combustion (PICs)
- Accepted source and ambient air methods for PFAS do NOT exist
- Current emissions tests often target only a small number of PFAS compounds for analysis while significantly more may be present**
- Emissions measurements are needed for source characterization
- Emissions measurements are needed for control technology evaluation



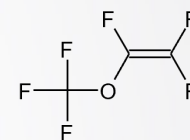
Example Coating Process





- **PFAS emission measurement methods are needed to inform regulatory decisions**

- Comprehensive emissions characterizations
- Technology evaluations

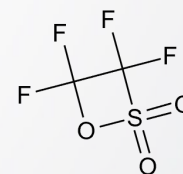


- **What kind of PFAS measurement methods are needed?**

- Ability to measure volatile/semivolatile/nonvolatile and polar/nonpolar PFAS compounds
- Ability to measure targeted PFAS compounds and identify nontargeted PFAS compounds

- **What PFAS to measure?**

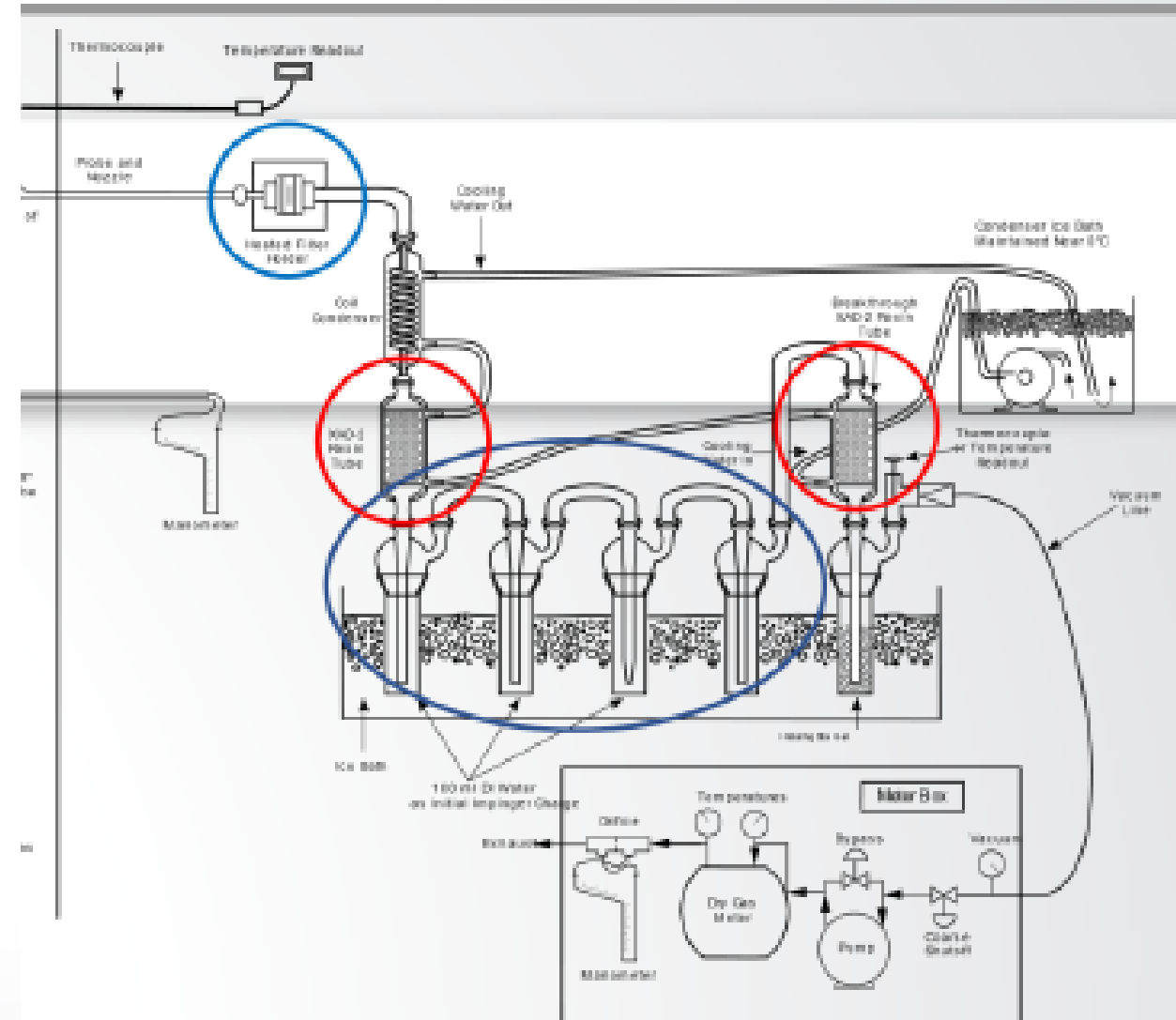
- Targeted compounds?
  - Legacy (537) compounds
  - What about PFAS wastes (e.g., AFFF) constituents?
- What about Products of Incomplete Combustion (PICs)?





# OTM-45 for emission sampling

- Application: stack sampling and testing
- Sampling train: probe/filter, XAD resin, impingers, breakthrough impinger (4 fractions)
- Target analytes: semivolatile PFAS (49 target analytes)
- Basic solvent extraction of sampling train components; WAX SPE for aqueous solution in impingers
- Analyze by LC/MS/MS
- **Points of Contact: Ray Merrill OAR/OAQPS, Jeff Ryan ORD**



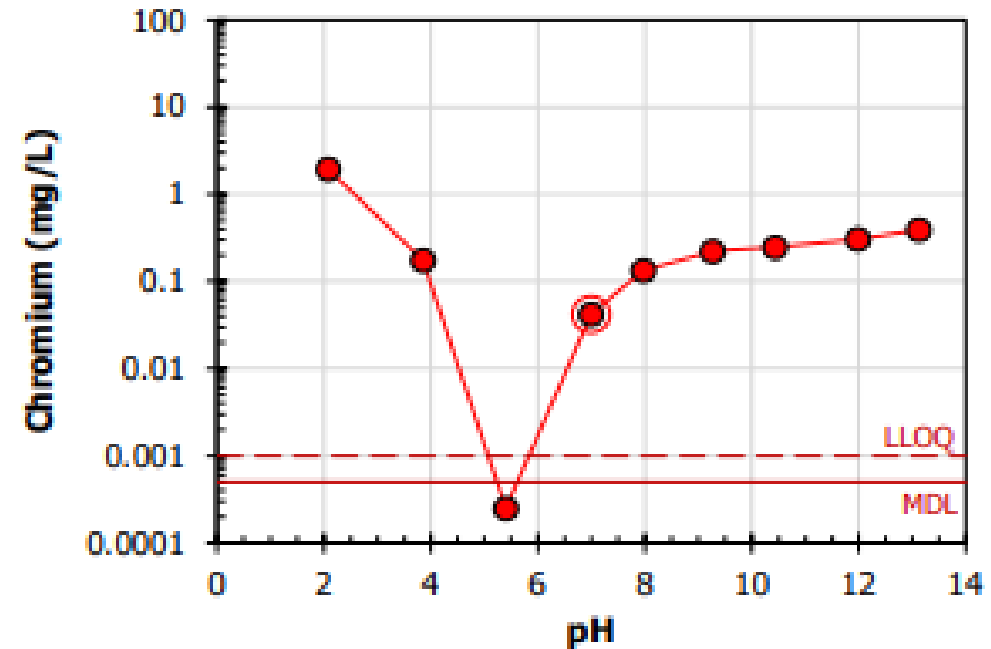


# Leaching methods

## SW-846 Methods Updates: LEAF

### LEAF: Leaching Environmental Assessment Framework

- Aqueous leaching methods, data management/visualization software, “How to” guide, case studies
- LEAF provides inputs for fate and transport modeling:
  - Identify key variable(s) affecting leaching behavior in source material, under range of exposure scenarios
  - Estimate “source term” i.e., aqueous concentration, release rate
  - Evaluate immobilization strategies in a laboratory environment prior to field deployment





# SW-846 Methods Updates: LEAF

- Initially developed and validated for inorganics
  - Final versions of Methods 1313-1316 and LEAF “How To” User’s Guide published in 2019
- Methods have been applied to organics
  - Clear need to standardize!
  - Need to evaluate materials compatibility, process changes to accommodate different classes of chemicals
- Interested stakeholders:
  - EPA Superfund and RCRA programs
  - Department of Energy, Department of Defense
  - National Academy of Sciences
  - Australian, European, Israeli governments

The screenshot shows the EPA website page for LEAF methods and guidance. The URL in the browser is [epa.gov/hw-sw846/leaf](https://www.epa.gov/hw-sw846/leaf). The page features the EPA logo and the text "United States Environmental Protection Agency". A search bar is present with the text "Search EPA.gov". Below the search bar, there are "Related Topics" listed as [Hazardous Waste Test Methods / SW-846](#) and a "CONTACT US" link. The main heading of the page is "Leaching Environmental Assessment Framework (LEAF) Methods and Guidance". The introductory text states: "The Leaching Environmental Assessment Framework (LEAF) is a leaching evaluation system, which consists of four leaching methods, data management tools, and scenario assessment approaches designed to work individually or to be integrated to provide a description of the release of". At the bottom of the screenshot, the full URL is provided: <https://www.epa.gov/hw-sw846/leaching-environmental-assessment-framework-leaf-methods-and-guidance>.

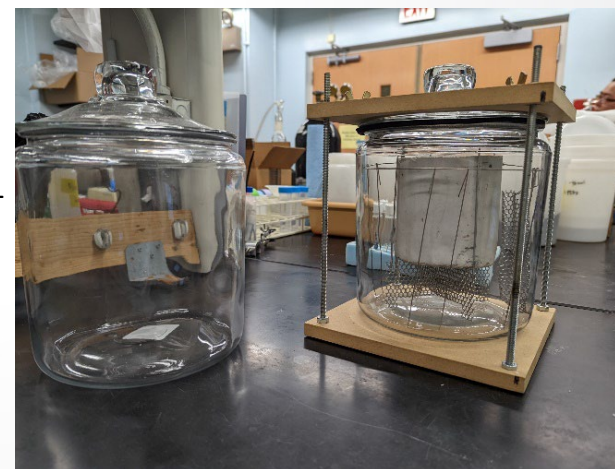
- **Equilibrium-based Tests (Method 1313, 1316)**
  - Batch tests on size-reduced material
  - *Contaminant concentration and release as function of:*
    - *Eluate pH* – Method 1313
    - *Liquid-solid ratio (L/S)* – Method 1316
- **Percolation Column Test (Method 1314)**
  - Up-flow column – saturated to minimize preferential flow
  - *Contaminant concentration and flux as a function of water percolated*
- **Mass Transport Rate Test (Method 1315)**
  - Tank-based leaching test, monolithic or compacted granular
  - *Rates of contaminant release*





## Current status of LEAF Methods for both organics and inorganics

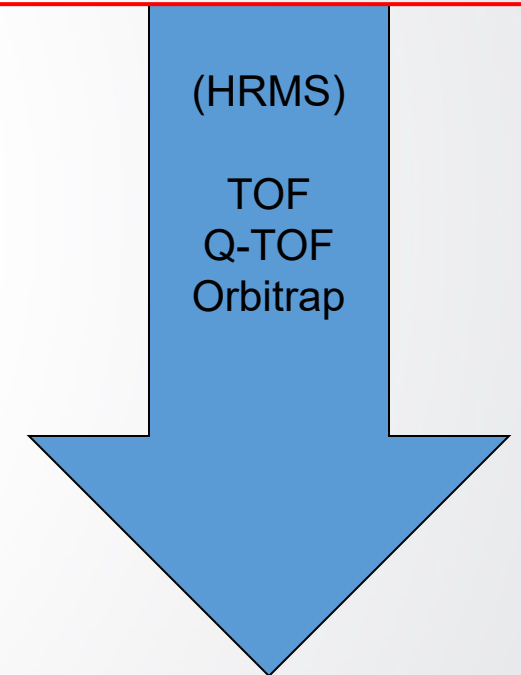
- Planning to begin multi-laboratory validation in 2023
- LEAF development and single laboratory demonstrations nearing completion for SVOCs, progressing for PFAS
  - Materials of construction
  - Volatile loss
  - Aqueous subsampling
- Planning multi-laboratory validation study with EPA ORD, Jacobs and Vanderbilt University
- PFAS LEAF method development funded by DoD through SERDP grant – joint effort by Texas Tech and Vanderbilt
- VOCs still needs development work



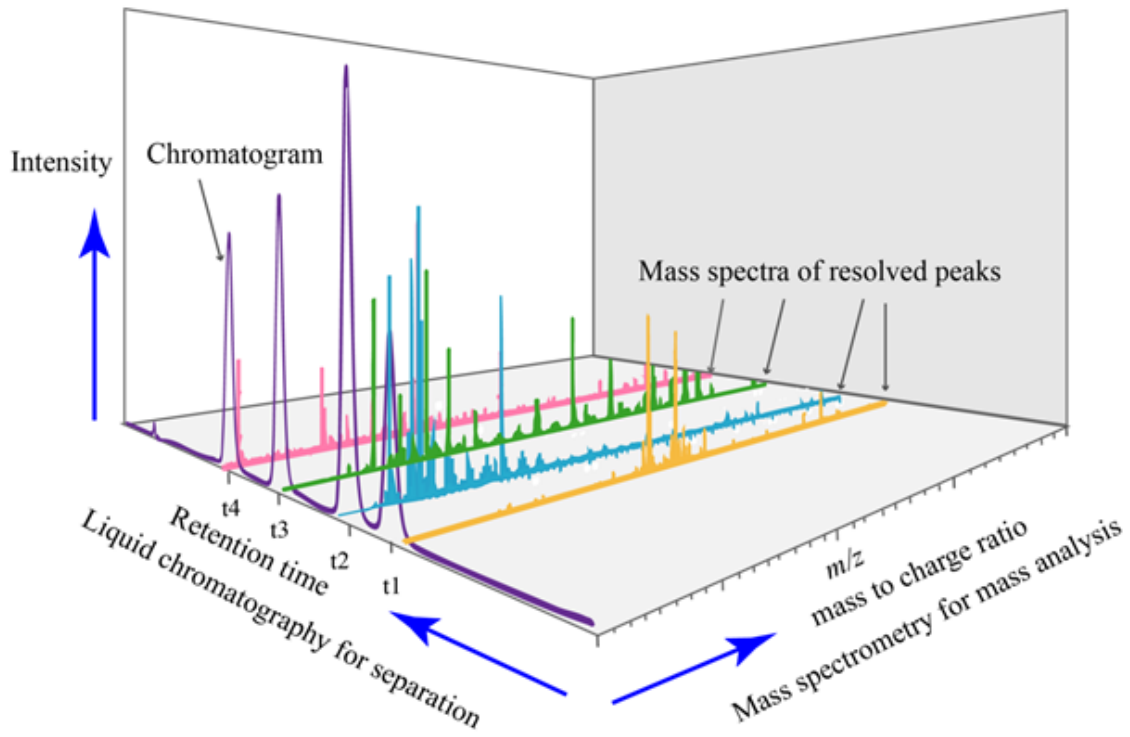


# What is Non-Targeted Analysis?

- Targeted Analysis
    - How much PFOA is in my sample?
- 
- Suspect Screening
    - Which chemicals in this database are in my sample?
  - Non-Targeted Screening
    - What are the chemicals in my sample?



# Non-Targeted Analysis Reporting



## 1) Compound Identification (tentative)

- A combination of mass spectral data along with patterns of fragmentation compared to on-line and in-house mass-spectral libraries
- Augmented by manual examination by analyst
- Identify monoisotopic mass, formula, and possibly chemical name

## 2) Indication of how much PFAS is present in the sample (Peak Area Counts)

- Peak area counts are proportional to the mass of PFAS in the sample
- Relative abundance only





## Published/Available EPA PFAS analytical methods:

- **Targeted analytical methods:**
  - LC/MS/MS based
  - Quantitative analysis
  - Defined list of target analytes for which certified reference materials are available
  - By program:
    - OAQPS – OTM-45
      - Stack sampling and testing
    - OGWDW – 533, 537.1
      - Drinking water
    - ORCR – SW-846 methods 3512, 8327
      - Non-potable waters
    - OST – 1633 (draft)
      - Wastewater, other non-potable waters, solids
- **Adsorbable organic fluorine, Method 1621 (draft):**
  - OST, aqueous (wastewater) matrices
  - Screening, semiquantitative - Could include chemicals that are not PFAS



# Summary: EPA PFAS Methods, January 2023

## 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)
- Method in CWA-1633 completed single lab and undergoing multi lab validation with DoD for non-potable water/solids (40 PFAS). SW-846 determination to follow.

## 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.



## Published/Available PFAS analytical methods, by matrix:

Matrix	Reference Method	Sample Preparation Technology	Extract Cleanup	Validated Limit of Quantitation
Drinking water	EPA 537.1	SPE (styrene-divinyl benzene)	None	as low as 0.5 ng/L (LCMRL)
Drinking water	EPA 533	SPE (weak anion exchange)	None	as low as 1.4 ng/L (LCMRL)
Non-potable Water	EPA 1633	SPE (weak anion exchange)	graphitized carbon	as low as 1.6 ng/L
Non-potable Water	EPA 3512/8327 ASTM D7979-20 ASTM D8421-21	Solvent Dilution (1:1)	None	as low as 10 ng/L
Solids	EPA 1633	Basic methanol (triplicate)	graphitized carbon, SPE	as low as 200 ng/kg
Solids	ASTM D7968-17a	1:1 basic methanol-water	none	as low as 25 ng/kg
Fish tissue	EPA 1633	Basic organic solvent extraction (triplicate)	graphitized carbon, SPE	as low as 500 ng/kg
Air	OTM-45	Basic methanol extraction	-	-

## Complex analytical problem:

- We still know very little about the physical-chemical behavior of the many, diverse chemicals classified as PFAS
- Very low concentrations are relevant.
- Background concentrations are an issue everywhere.
- Requires highly trained personnel and expensive equipment.
- Chemical formulation used in commercial and consumer products are evolving as new chemistries are developed.



## Contact

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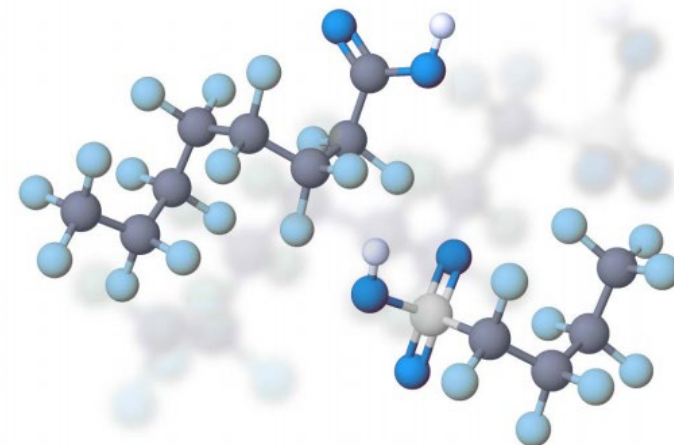
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### EPA's Per- and Polyfluoroalkyl Substances (PFAS) Action Plan



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**Questions?**