United States Environmental Protection Agency

Analytical methods for PFAS in environmental media

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Outline

- 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

Methods – Sampling

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 vs Non-Targeted Analysis

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.

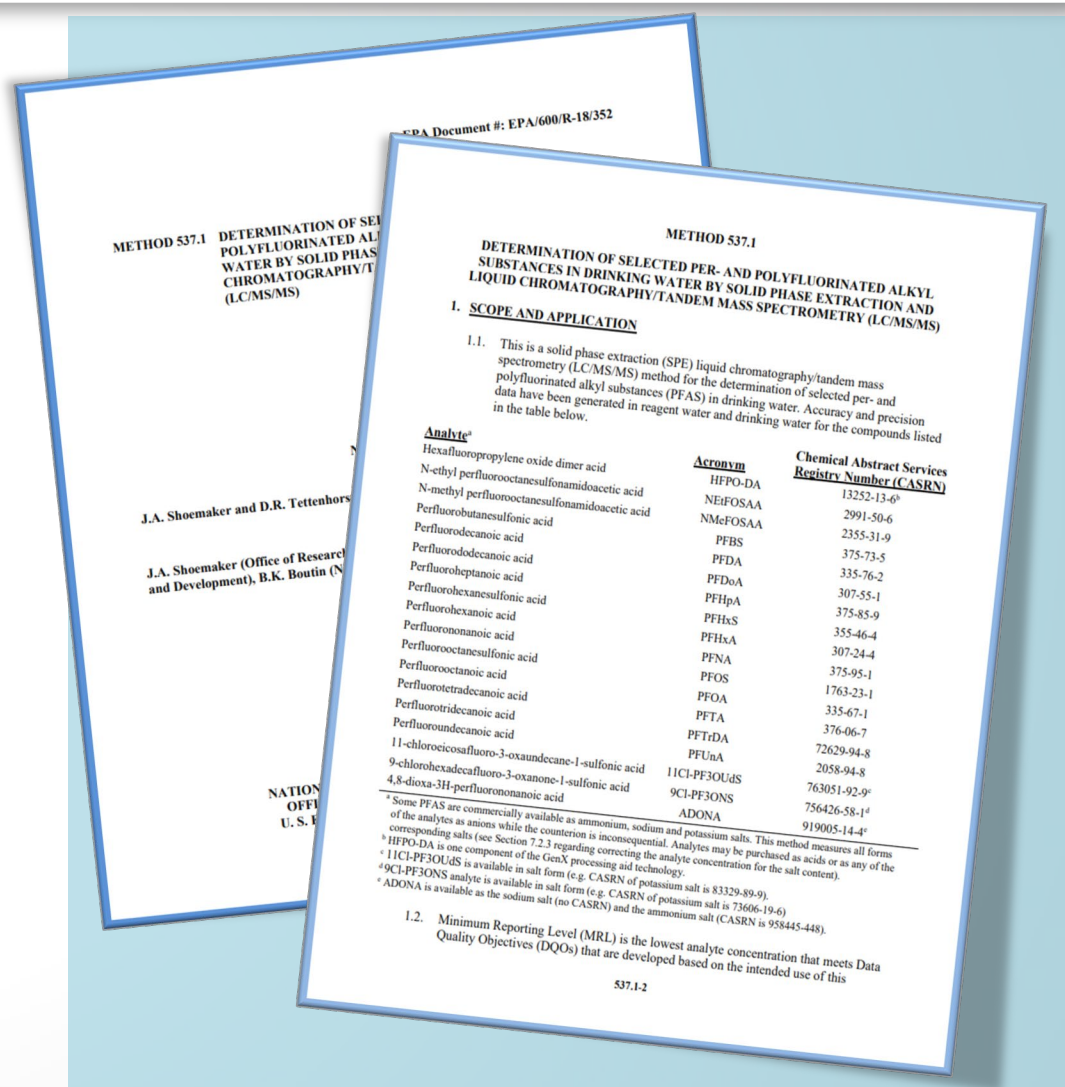
PFAS Test Methods - Overview

Table E-2. Analytical Methods

Media	Agency	Method	Validation		Individual PFAS	Total PFAS	Organofluorine Compounds (including, but not limited to PFAS)	Publicly Available	Agency Use Only
			Intra-Lab	Inter-Lab					
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	
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	
Ground Water, 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-dioxanonate (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



METHOD 537.1

DETERMINATION OF SELECTED PER- AND POLYFLUORINATED ALKYL SUBSTANCES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

1. SCOPE AND APPLICATION

1.1. This is a solid phase extraction (SPE) liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the determination of selected per- and polyfluorinated alkyl substances (PFAS) in drinking water. Accuracy and precision data have been generated in reagent water and drinking water for the compounds listed in the table below.

Analyte[a]	Acronym	Chemical Abstract Services Registry Number (CASRN)
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6[b]
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluorononanoic acid	PFNA	375-95-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorotetradecanoic acid	PFTA	376-06-7
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluoroundecanoic acid	PFUnA	2058-94-8
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9[c]
9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	9Cl-PF3ONS	756426-58-1[d]
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4[e]

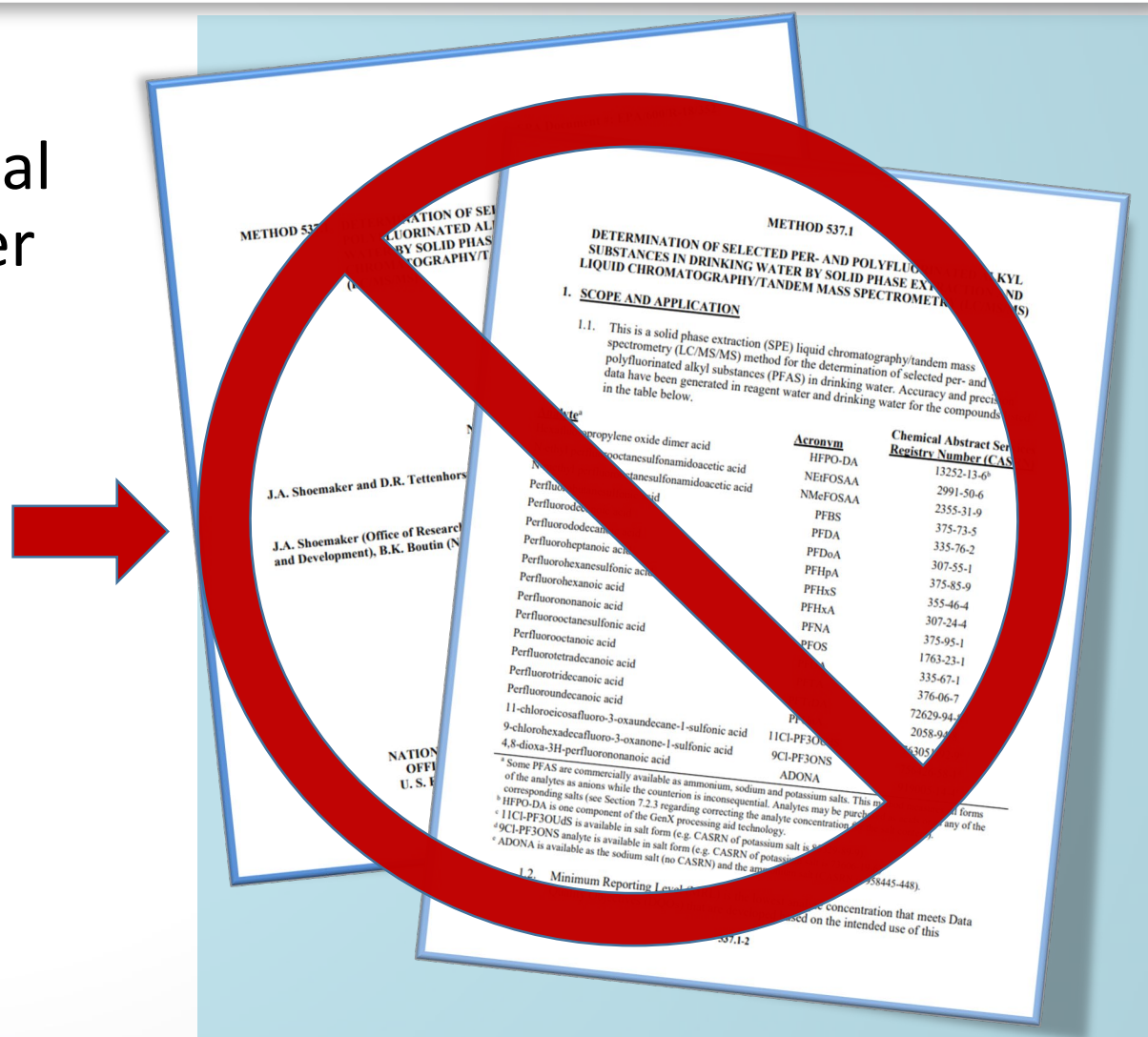
[a] Some PFAS are commercially available as ammonium, sodium and potassium salts. This method measures all forms of the analytes as anions while the counterion is inconsequential. Analytes may be purchased as acids or as any of the corresponding salts (see Section 7.2.3 regarding correcting the analyte concentration for the salt content).
[b] HFPO-DA is one component of the GenX processing aid technology.
[c] 11Cl-PF3OUdS is available in salt form (e.g. CASRN of potassium salt is 83329-89-9).
[d] 9Cl-PF3ONS analyte is available in salt form (e.g. CASRN of potassium salt is 73606-19-6)
[e] ADONA is available as the sodium salt (no CASRN) and the ammonium salt (CASRN is 958445-448).

1.2. Minimum Reporting Level (MRL) is the lowest analyte concentration that meets Data Quality Objectives (DQOs) that are developed based on the intended use of this

537.1-2

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

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)[3]	Perfluorotetradecanoic acid (PFTA)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	Hexafluoropropylene oxide dimer acid (HFPO-DA)	Perfluorotridecanoic acid (PFTrDA)
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)	**Perfluorobutanesulfonic acid (PFBS)**	
Perfluoropentanesulfonic acid (PFPeS)	**Perfluoroheptanoic acid (PFHpA)**	
Perfluoropentanoic acid (PFPeA)	**Perfluorohexanesulfonic acid (PFHxS)**	
	Perfluorononanoic acid (PFNA)	
	Perfluorooctanoic acid (PFOA)	
	Perfluorooctanesulfonic acid (PFOS)	Bold indicates analytes listed on UCMR 3

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

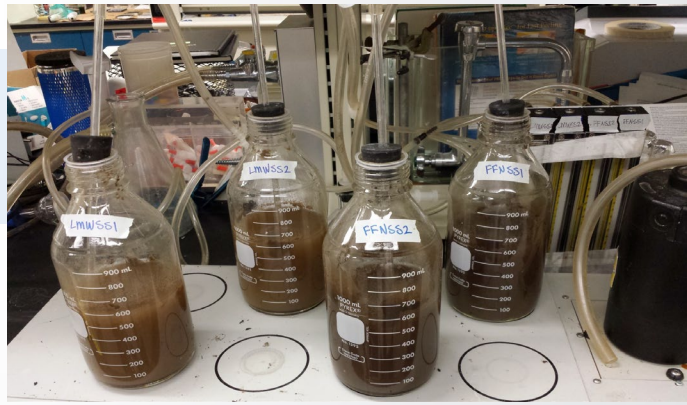
Non-drinking water aqueous matrices:

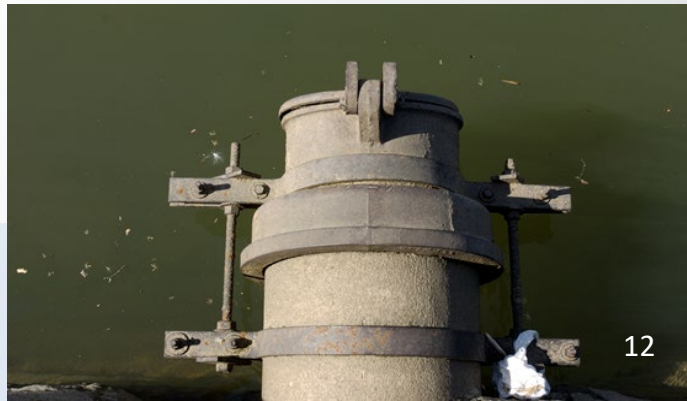
- Groundwater
- Surface water
- Wastewater

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)

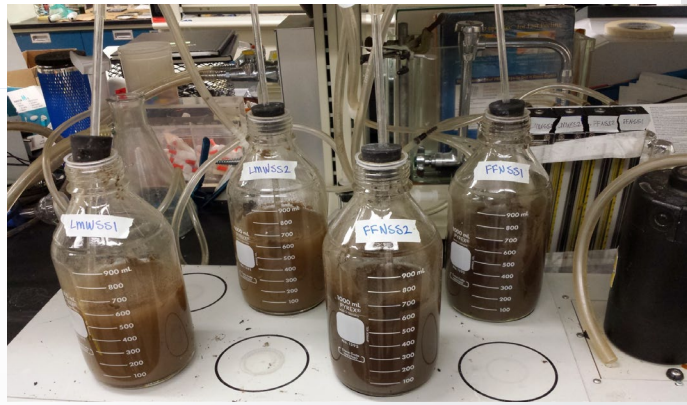
- Commercially available standards (“native” and isotopically labeled)

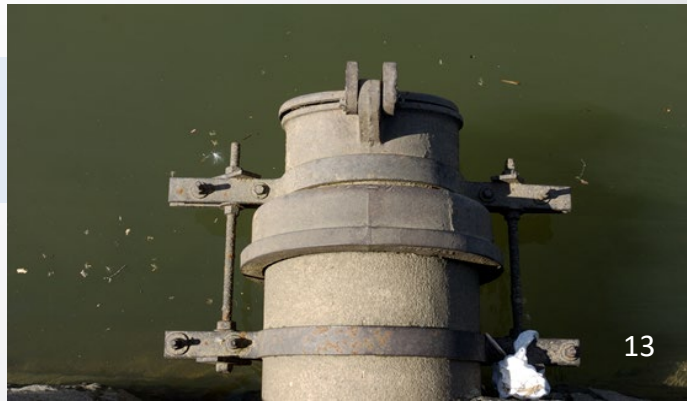
Direct injection-EPA Region 5/Chicago Regional Lab SOP

- 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

- **<u>Status</u>: 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 µm filter
  - Add 0.1% acetic acid by volume
  - Analyze by LC/MS/MS

Advantages	Disadvantages
• Small sample size (5 mL) • Rapid sample preparation • Few process steps	• Small dilution factor (2x) • Need modern LC/MS instrument to achieve low ng/L sensitivity • Not consistent with current practice in many testing laboratories

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

Quantitation Limits: 1-10 nanogram/L for 40 PFAS - includes all 537.1, 533, & SW-846 8327

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 consistent with 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.

PFAS Analysis in Fish Tissue

Currently, CW1633 method includes PFAS in fish/bivalve 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

Contact: Sara Hisel-McCoy, EPA OW/OST (Hisel-Mccoy.Sara@epa.gov)

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" PFAS measurements

- 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.

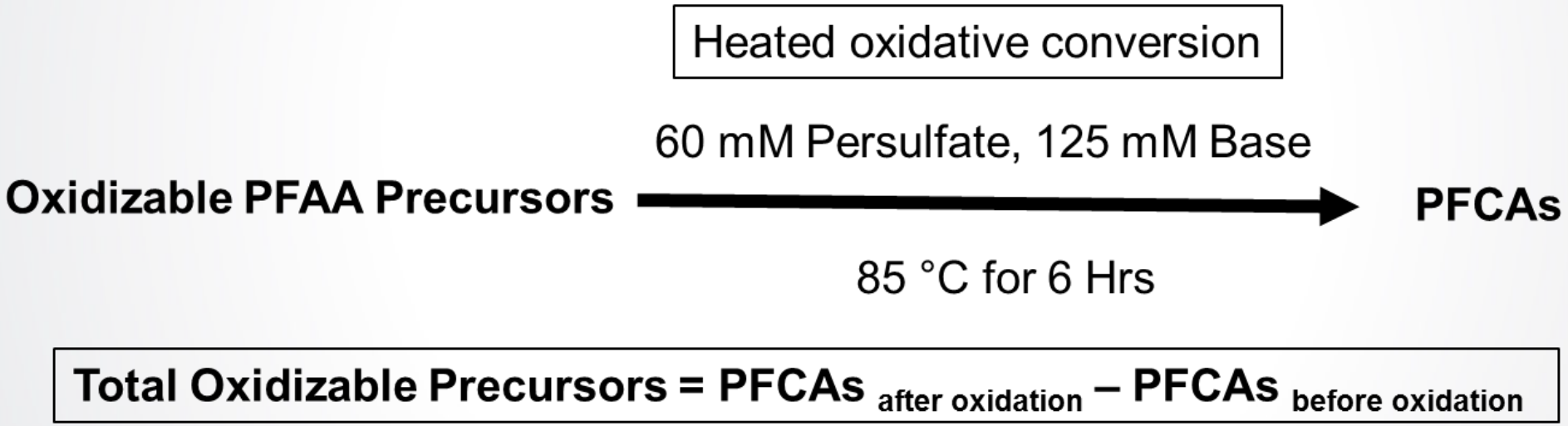
Total Organofluorine (TOF)/AOF Analysis using Combustion Ion Chromatography

- 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

Contact: Marc Mills, EPA ORD/CESER (Mills.Marc@epa.gov)

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

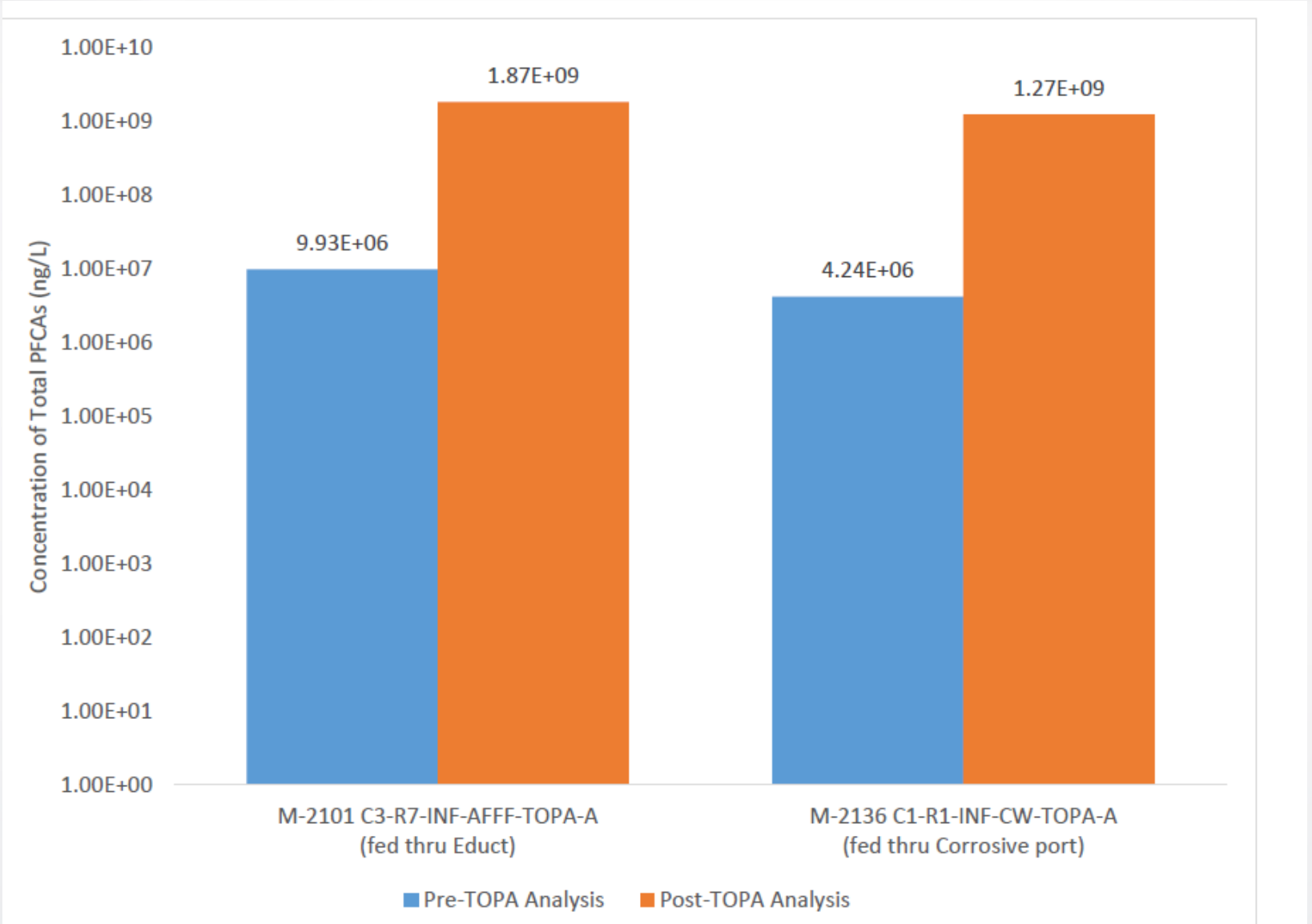


Total Oxidizable Precursors = PFCAs $_{\text{after oxidation}}$ – PFCAs $_{\text{before oxidation}}$

- 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

Contact: Marc Mills, EPA ORD/CESER (Mills.Marc@epa.gov)

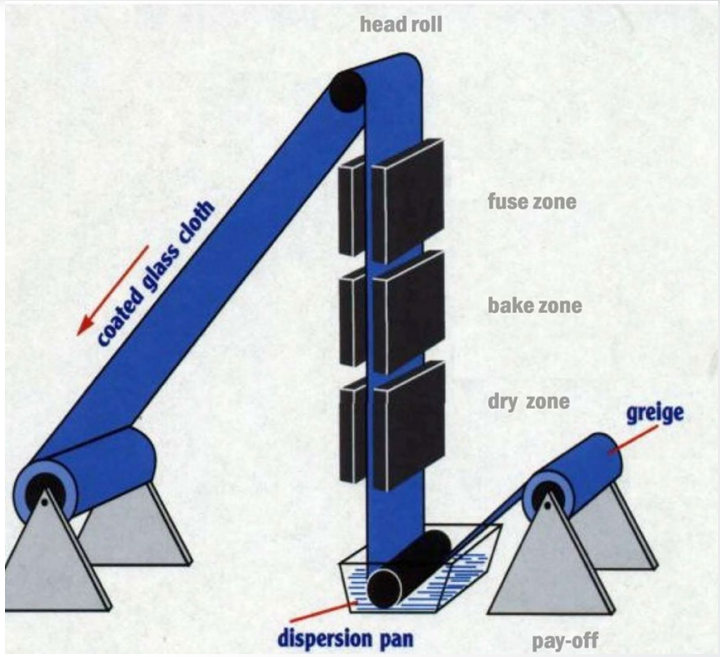
PFCAs in AFFF concentrate: pre- and post-Total Oxidizable Precursors Assay



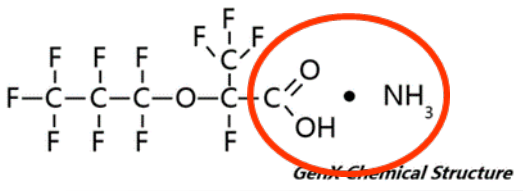
Jennifer Guelfo, Texas Tech

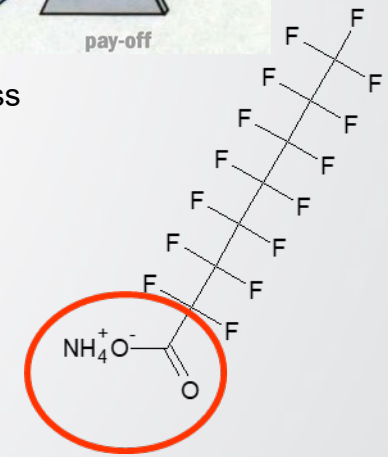
Emissions Measurement Considerations/Challenges

- 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)
- **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
- What about Products of Incomplete Combustion (PICs)?



Example Coating Process

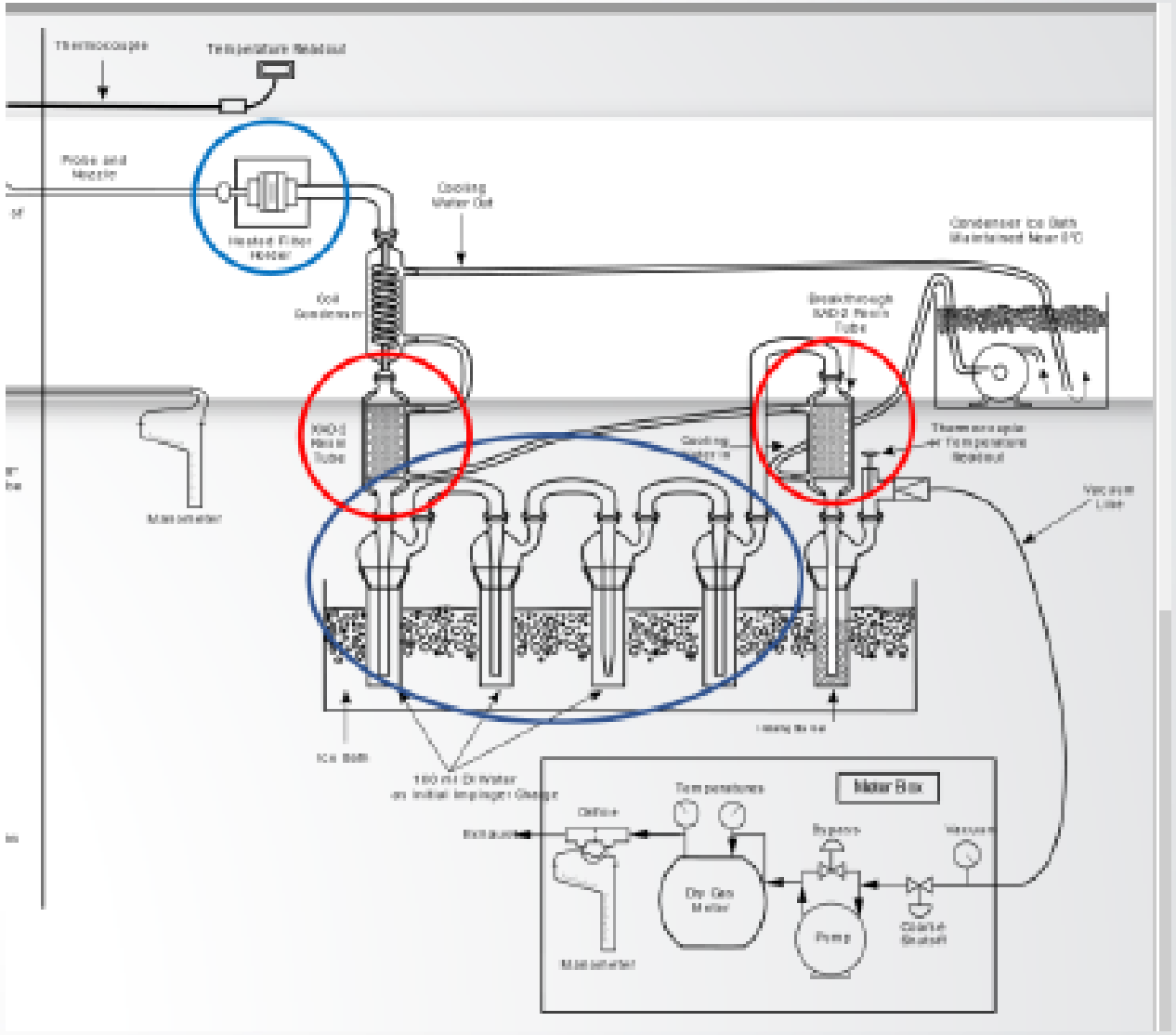




OTM-45 for emission sampling

- 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**

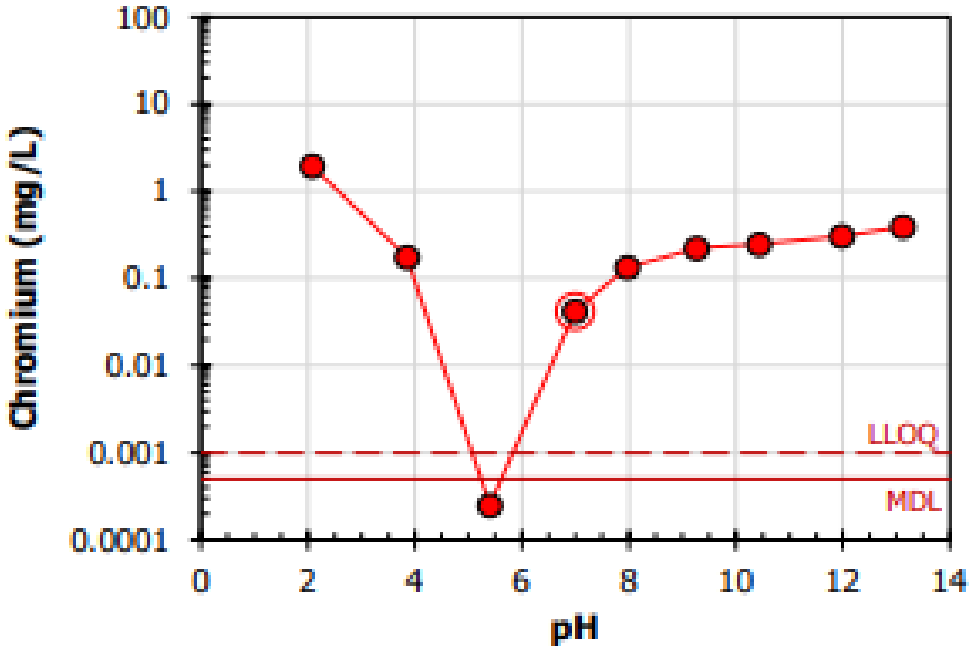


Leaching methods

SW-846 Methods Updates: LEAF

LEAF: Leaching Environmental Assessment Framework

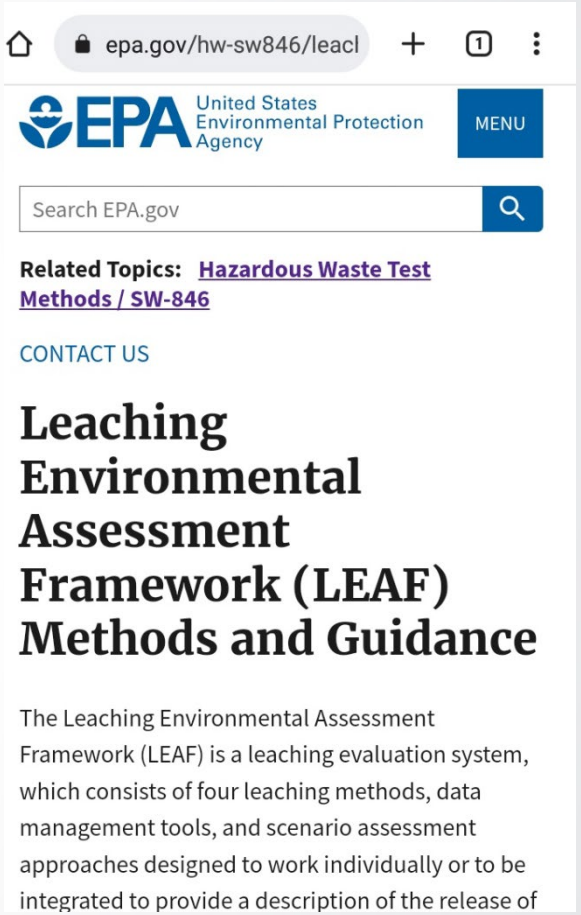
- Aqueous leaching methods, data management/visualization software, “How to” guide, case studies
- LEAF provides inputs for fate and transport modeling:
  - Identify key variable(s) affecting leaching behavior in source material, under range of exposure scenarios
  - Estimate “source term” i.e., aqueous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



https://www.epa.gov/sites/default/files/2019-05/documents/final_leaching_environmental_assessment_framework_leaf_how-to_guide.pdf

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



https://www.epa.gov/hw-sw846/leaching-environmental-assessment-framework-leaf-methods-and-guidance

SW-846 Methods Updates: LEAF

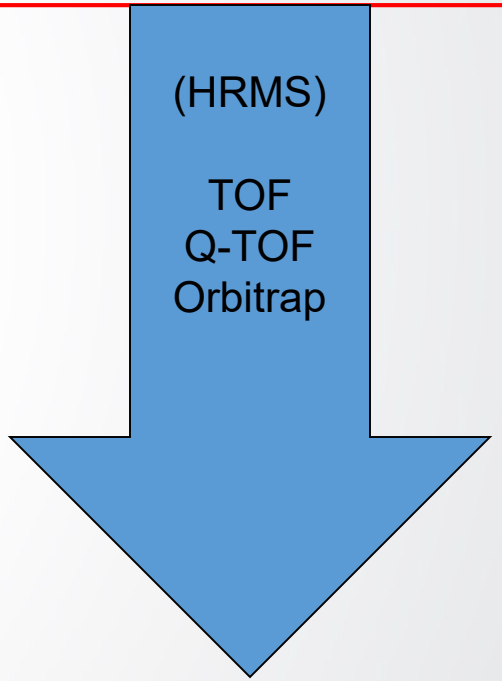
Current status of LEAF Methods for both organics and inorganics

- 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

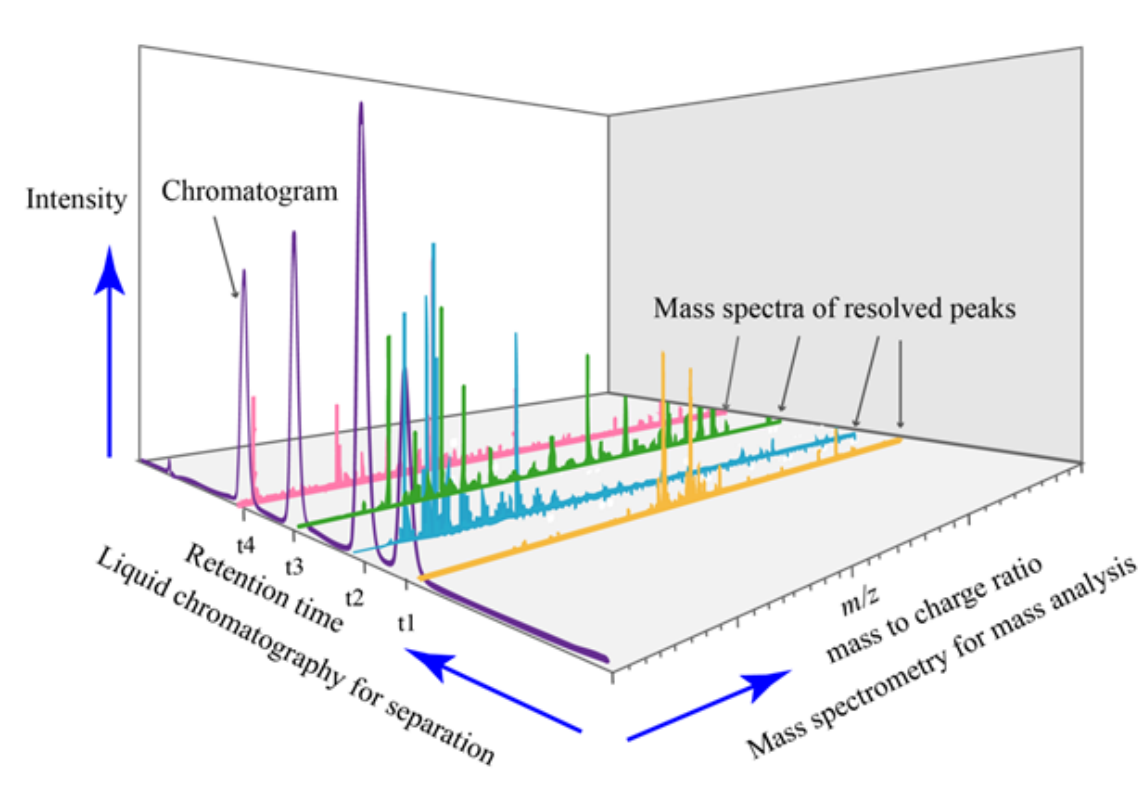
What is Non-Targeted Analysis?

- 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 ae proportional to the mass of PFAS in the sample
- Relative abundance only

Slide courtesy Mark Strynar

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		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.

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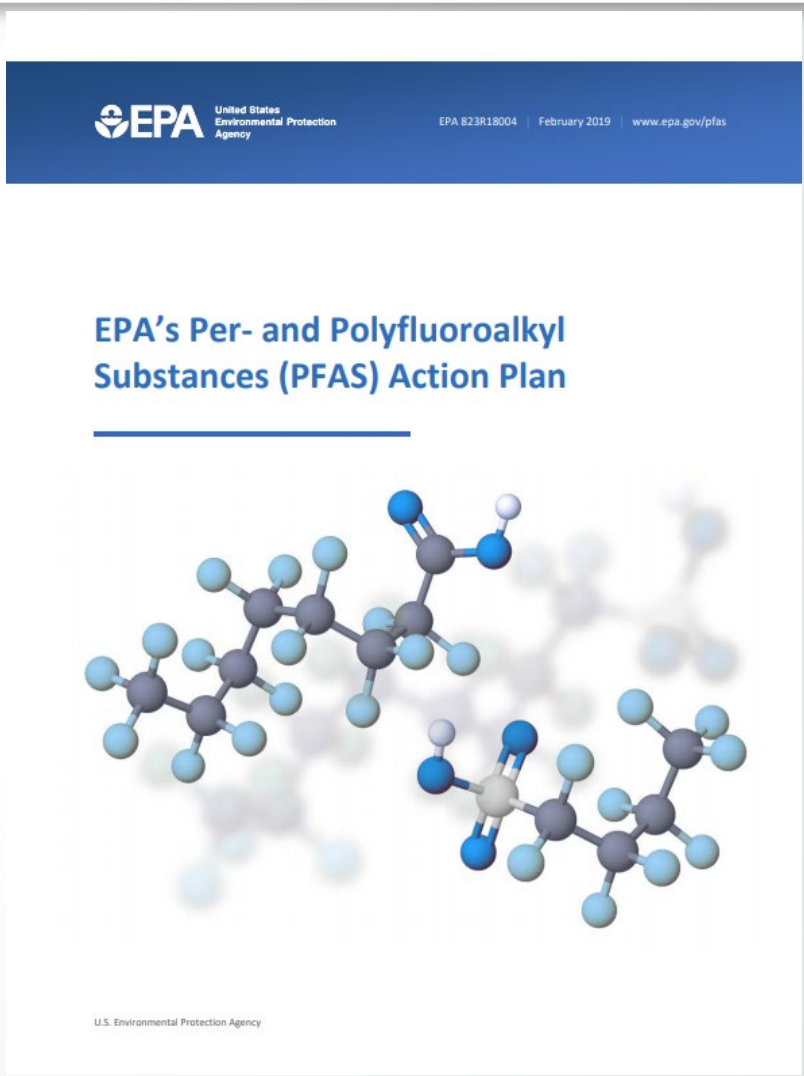

Questions?