Office of Research and Development

Biosolids Webinar
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Update on PFAS Analytical Methods

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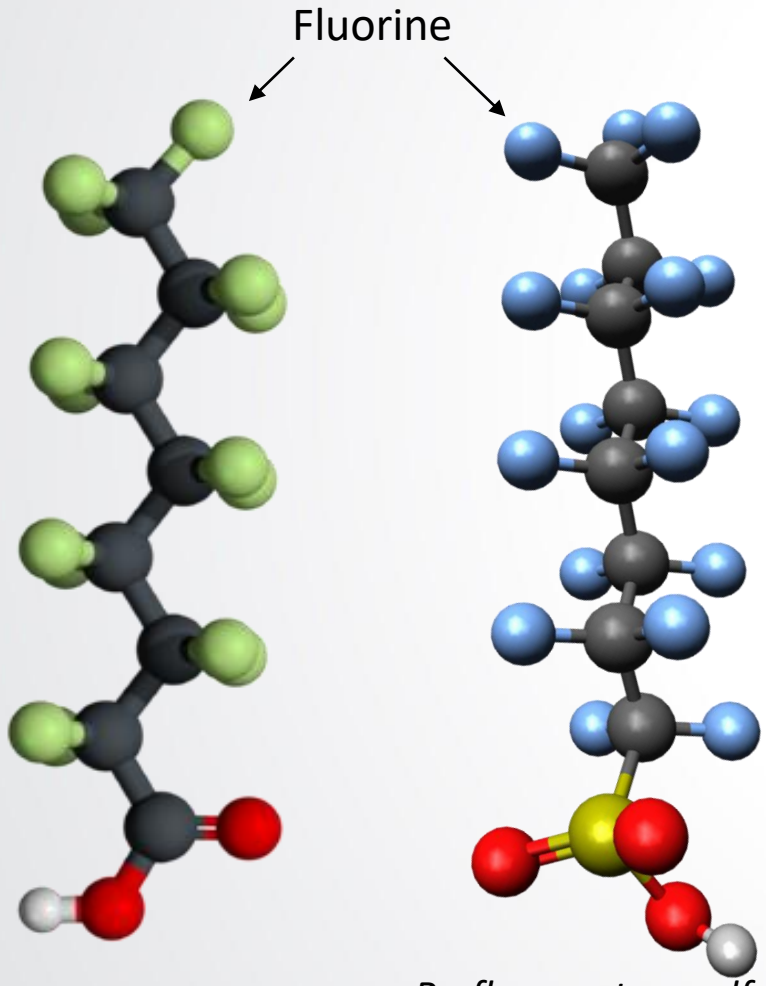
Per- & Polyfluoroalkyl Substances (PFAS)



Perfluorooctanoic acid (PFOA)

Perfluorooctanesulfonic acid (PFOS)

- **A class of man-made chemicals**
  - **Chains** of carbon (C) atoms surrounded by fluorine (F) atoms, with different terminal ends
  - **Complicated chemistry** – thousands of different variations exist in commerce
  - **Widely used** in industrial processes and in consumer products
  - **Some** PFAS are known to be **PBT**:
    - **Persistent** in the environment
    - **Bioaccumulative** in organisms
    - **Toxic** at relatively low (ppt) levels

Methods-Sampling

Guidance to avoid cross contamination in sampling

- No teflon
- Avoid contact with clothes, materials containing PFAS (e.g. some food wrappers)
- See: PFAS Analytical Methods Website https://www.epa.gov/water-research/pfas-analytical-methods-development-and-sampling-research
  - 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 issues epa.gov/fedfac/technical-fact-sheet-perfluorooctane-sulfonate-pfos-and-perfluorooctanoic-acid-pfoa-0

Targeted vs Non-Targeted

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

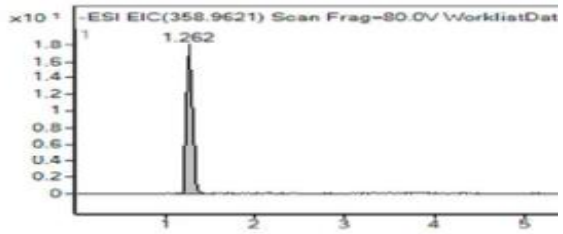
Non-targeted methods involve the use of High Resolution Mass Spectrometry (HRMS) capable of identifying all analytes in a sample, known and unknown

- 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

Non-Targeted Analysis

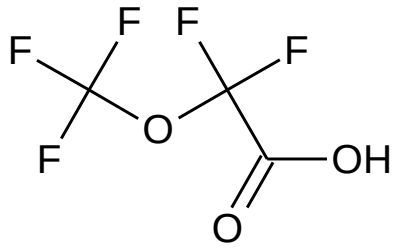
Explore Unknown compounds using High resolution mass spectrometry. Identify a peak in a chromatogram and to ultimately predict the identity of this unknown

- **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]-: 178.977308 Da

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

- **Compare peak to commercial to confirm identification if possible**

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; QAed 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

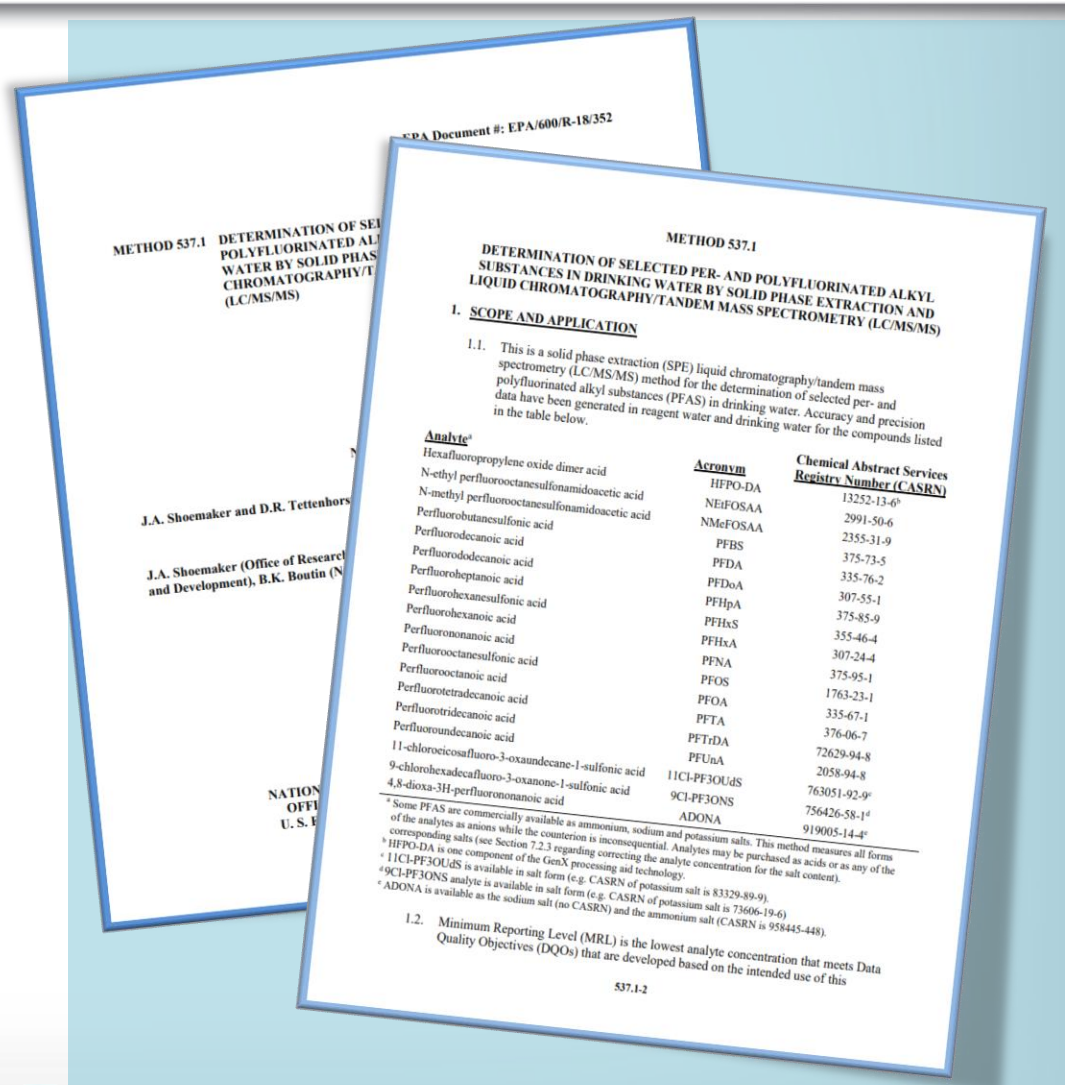
Three broad categories of EPA Standard Methods related to water:

- Safe Drinking Water Act Methods
  - https://www.epa.gov/dwanalyticalmethods
- Clean Water Act Methods
  - https://www.epa.gov/cwa-methods
- SW846 Methods
  - https://www.epa.gov/hw-sw846/guidance-methods-development-and-methods-validation-resource-conservation-and-recovery-act

These are generally targeted methods for solids and water

Drinking Water Method 537: Revision 1

- 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 epa.gov/sites/production/files/2016-09/documents/pfoa-technical-advisory.pdf
- Final published method (November, 2018) epa.gov/water-research/epa-drinking-water-research-methods
- 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]

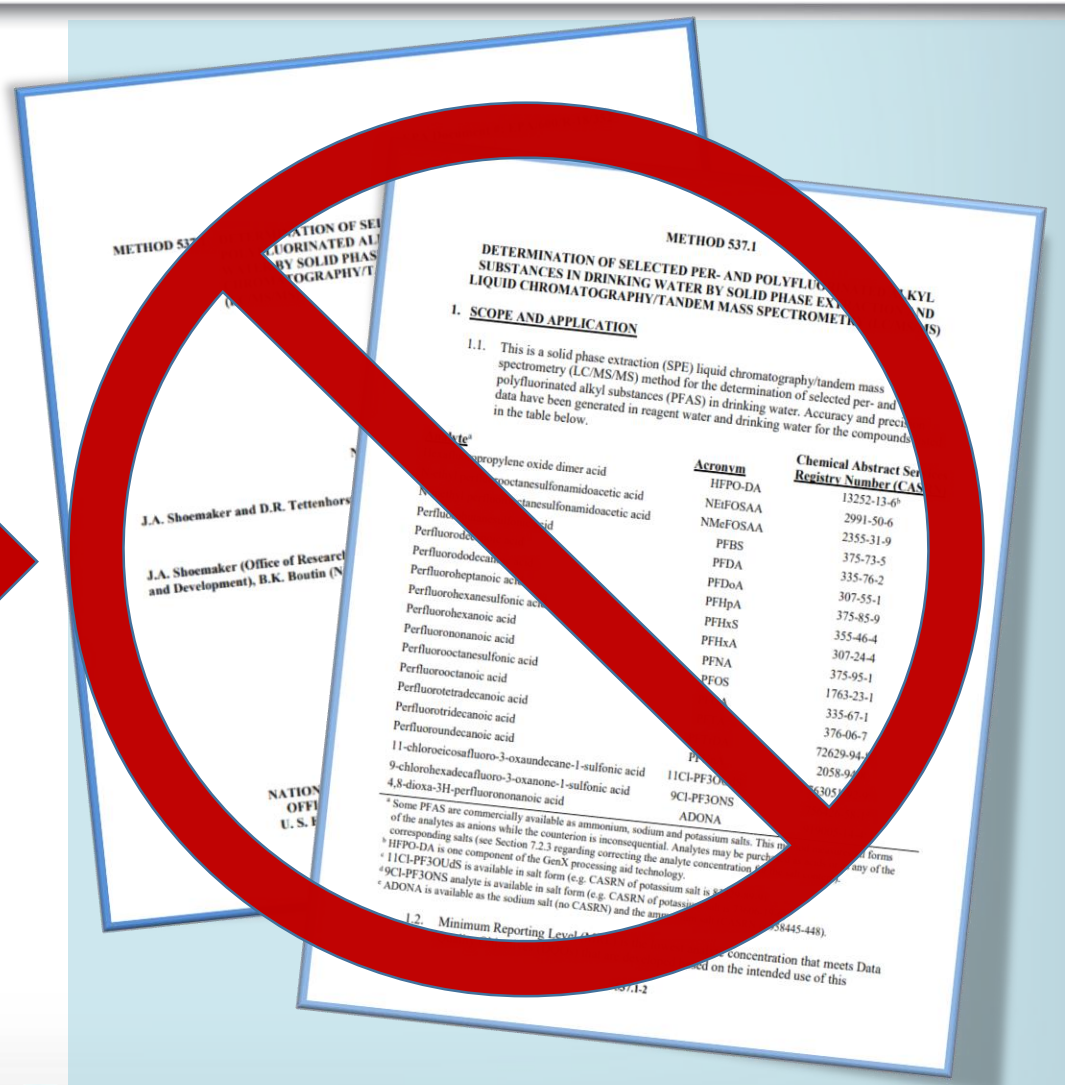
[a] Some PFAS are commercially available as ammonium, sodium and potassium salts. This method measures all forms of the analytes as anions while the counterion is inconsequential. Analytes may be purchased as acids or as any of the corresponding salts (see Section 7.2.3 regarding correcting the analyte 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 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 support the fifth Unregulated Contaminant Monitoring Rule
- Released December 2019
  - https://www.epa.gov/dwanalyticalmethods/analytical-methods-developed-epa-analysis-unregulated-contaminants

Drinking Water Method 533

Method 533[C]	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-Drinking Water Sample Methods: *SW-846 Method 8327—Direct Injection*

Non-drinking water aqueous matrices:

- Groundwater
- Surface water
- Wastewater

Find a balance among 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)

https://www.epa.gov/hw-sw846/validated-test-method-8327-and-polyfluoroalkyl-substances-pfas-using-external-standard

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 American Society for Testing and Materials (ASTM) Method D7979
- Multi-laboratory validation study completed in 2018
- OLEM addressing public comments (closed August 26, 2019)
- Finalize in Fall (?) 2020

Target Quantitation Limits: 10 nanogram/L

Associated preparation method 3512 for aqueous matrices

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

More complex method relative to direct injection, however will

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

40 PFAS analytes-includes all analytes listed in 537.1, 533, and SW846 8327

Non-drinking water samples

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

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

Build in flexibility

- Columns
- Elution schemes

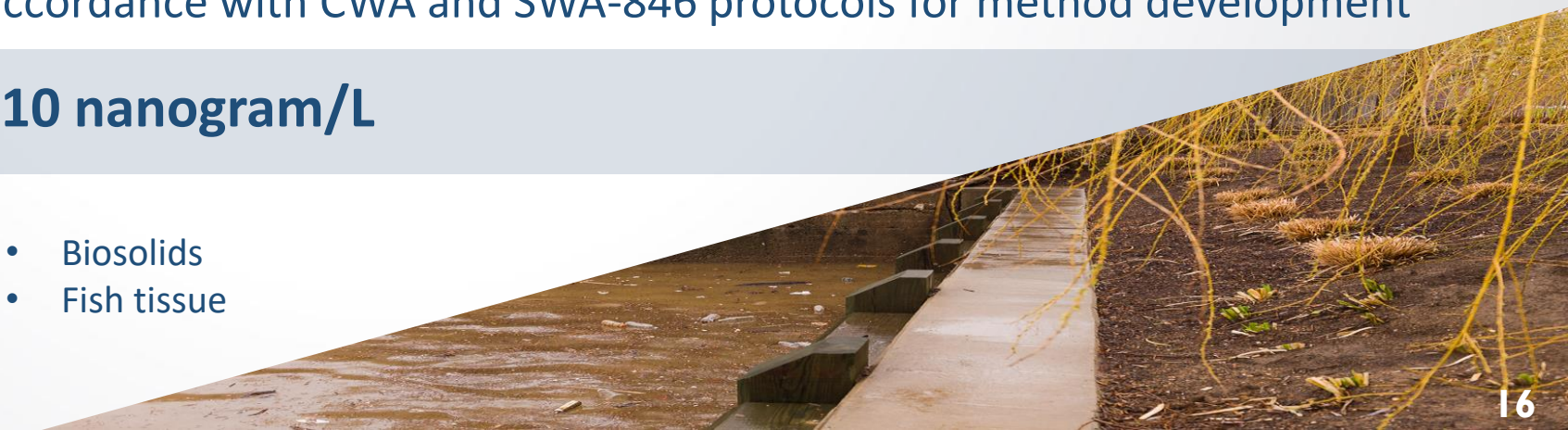
Single laboratory validation in progress

- Collaborative effort among DoD, EPA Office of Water, EPA Office of Land and Emergency Management, and EPA ORD
- Assuming single lab validation success (December 2020?), multi-laboratory validation will follow over 2021
- Method being developed in accordance with CWA and 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



PFAS Analysis Marine Waters

No EPA Approved method for PFAS in Marine Waters – ORD has a research method…

- LC-MS/MS Isotope dilution method

Method Details

– Covers 24 PFAS, Commercially available standards (“native” and isotopically labeled)

- Similar to those in EPA 537.1, 533, and SW 846 8327
- Target quantitation limits <1ng/L, with extracted samples
- SPE sample concentration – matrix elimination (up to 500 mL, Weak-Anion Exchange (WAX))
- Accounts for matrix effects (e.g., high ionic strength and DOM)

– Adapted to estuarine sediments and TOP assay

Contact: David Katz & Mark Cantwell EPA/ORD/CEMM (katz.david@epa.gov; cantwell.mark@epa.gov)

PFAS Analysis in Fish Tissue

No EPA Approved method for PFAS in fish tissue – EPA uses commercial laboratories' 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
- Injected into LC-MS/MS for analysis

Longer chain PFAS C8+ most consistently present

Contact: Sara Hisel-Mccoy, US EPA/OW/OST (hisel-mccoy.sara@eap.gov)

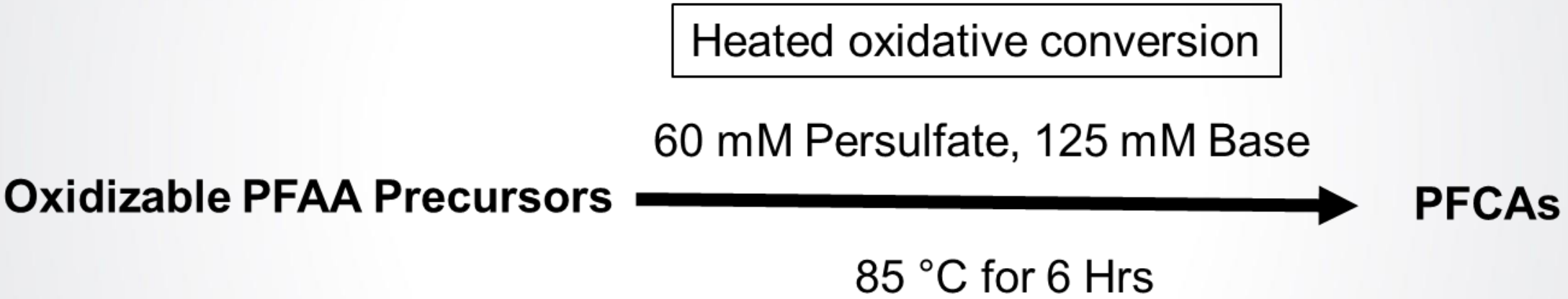
Total Organofluorine Analysis using Combustion Ion Chromatography (TOF)

- No specialized or costly instrumentation required
- Applied for aqueous matrices and blood samples
- Removing the background inorganic F^- from the sample is important to make sure that the reported F^- is organic
- Can be developed on a wide commercial scale
- High priority for EPA. ORD working with OW-OST to develop a draft method in 2020

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

Total Organic Precursors (TOP)

- Developed by Houtz et al. No multi-laboratory validated standard methods.
- Available from some contract 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)

Summary: EPA PFAS Methods, July 2020

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 and undergoing final review for non-potable water (24 PFAS)
- Method CWA-1600 undergoing single and multi lab validation for non-potable water and 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
- **High resolution mass spectrometry –** Continued development and application of HRMS methods for discovery of novel PFAS, suspect screening, and non-targeted analysis

For More Information

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