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Per- and Polyfluoroalkyl Substances – Non-Targeted Analysis Interlaboratory Study Final Report

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Abstract

Non-targeted analysis (NTA) is a technique for the identification of unknown and known chemicals in complex materials, such as novel per- and polyfluoroalkyl substances (PFAS) in environmental materials and commercial formulations. The PFAS NTA Interlaboratory Study (PFAS-NTAILS) was designed and administered to understand the measurement comparability of laboratories using NTA techniques for the identification of PFAS. Participating laboratories analyzed three samples containing varying amounts of known and unknown PFAS and other chemicals and reported the identified PFAS in each of the samples. This report discusses the design of the PFAS-NTAILS study, the description of the participants and their methods, and the initial results of the study. Generally, majority of the participating laboratories reported the known PFAS in each test sample, although there was a wide variability between the total number of PFAS reported in each sample among the different laboratories.

Keywords

Analytical chemistry; interlaboratory study; non-targeted analysis; mass spectrometry; per- and polyfluoroalkyl substances (PFAS)

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1. Introduction

1.1. Background

Non-targeted analysis (NTA) is a category of techniques that aim to detect and identify chemicals in complex mixtures with little to no prior knowledge regarding the composition of the mixture. NTA has been used for environmental mixtures (such as water or soil samples) to identify previously unknown environmental contaminants. While NTA can be performed using many different types of analytical instruments, for the scope of this interlaboratory study, NTA will be limited to the use of liquid chromatography with high resolution mass spectrometry (LC-HRMS). In lieu of analytical standards, NTA techniques that use LC-HRMS depend on the availability of reference mass spectra to provide probable compound identification.

Per- and polyfluoroalkyl substances (PFAS) are a class of anthropogenic chemicals, a subset of these chemicals are considered persistent, bioaccumulative, and/or toxic, and are contaminants of concern for human health. PFAS have been identified in a wide range of biological and environmental samples, including drinking water. Depending on the definition of PFAS [1], there can be over 10,000 individual structures that fit within the PFAS class. The use of NTA techniques aid in the identification of novel PFAS, as there are a limited number of commercially available analytical standards for PFAS and thousands of potential structures that could exist in a sample.

While researchers are routinely using NTA for the identification of PFAS in complex materials, there is a limited understanding of the comparability of NTA results between laboratories. The EPA Non-Targeted Analysis Collaborative Trial (ENTACT) was an interlaboratory comparison of NTA performance, although the scope was not limited to PFAS. The initial results showed a wide performance range with participants both under-reporting and over-reporting the expected number of chemicals in a spiked solution [2]. The results of ENTACT suggest the need to understand interlaboratory performance of NTA methods with more constrained study parameters, such as a smaller number of potential compounds to identify (i.e., a limited number of PFAS).

The NIST PFAS-NTA Interlaboratory Study (PFAS-NTAILS) was designed to provide a comparison of laboratory results using LC-HRMS for identifying PFAS. To enable laboratories to self-evaluate their performance, NIST provided samples, access to a database of PFAS reference mass spectra, and data analysis tools.

1.2. Database Infrastructure for Mass Spectrometry (DIMSpec) – PFAS Database

Conventionally, libraries of mass spectra are produced through the analysis of analytical standards for individual compounds, enabling the production of high quality, authenticated reference mass spectra for compound identification. Due to the lack of available analytical standards, the construction of a traditional library for PFAS would be extremely limited in comparison to the total number of PFAS that exist. Researchers have been generating quality mass spectra for decades using targeted and suspect screening approaches for PFAS in complex

mixtures, such as environmental samples and technical commercial mixtures, and these PFAS identities have been externally reviewed via the peer review process for journals. Mass spectra from such studies could be extremely useful to researchers performing NTA for PFAS identification, but often are not publicly available and/or not provided in a vendor-independent format that allows for broad interoperability.

Researchers at NIST sought to create a database infrastructure that enabled NTA researchers to access and use these empirical mass spectra. This work culminated in the development of the Database Infrastructure for Mass Spectrometry (DIMSpec), which is thoroughly described in Ragland and Place [3]. This tool, along with training documents and videos, was provided to all participants of the PFAS-NTAILS for optional use. Of the 27 participating laboratories that submitted data, seven labs reported using the Mass Spectral Match (MSMatch) data analysis tool, part of the DIMSpec toolkit used for matching experimental spectra with reference spectra. Further analysis of the reporting differences between laboratories that did and did not use DIMSpec and MSMatch will be performed at a later date. In addition, all participants were asked to report the identities of individual PFAS using the NIST PFAS ID number (referred to as NIST ID in this report), which is an identifier that connects to the NIST Suspect List of Possible Per- and Polyfluoroalkyl Substances [4] and disambiguates chemical identity [5].

1.3. Description of Samples

Three samples were created to represent an increasing amount of material complexity and were comprised of a variable number of PFAS. All samples were primarily methanol solutions. Generally, a 1-mL aliquot of the respective solution was transferred to a 2 L clear glass ampoule, the headspace of the ampoule was purged with argon gas, and then the ampoule was flame sealed. All solutions were stored at 4 °C until shipment. Samples were shipped to participants around March 6, 2024.

- **Sample A** was a solution consisting of a methanolic dilution of multiple analytical standards of PFAS (also in methanol). The nominal concentration was 0.1 µg/g for all PFAS components listed in Table 1.

Table 1. PFAS included in Sample A using analytical standards.

PFAS Preferred Name	Acronym	NIST ID
5:3 fluorotelomer betaine	5:3 FTB	NISTPFAS003794
Chloro-perfluorooctane sulfonate	8Cl-PFOS	NISTPFAS003238
Perfluorohexane sulfonamido amine	N-AP-FHxSA	NISTPFAS000878
Perfluorobutanoic acid	PFBA	NISTPFAS002649
Perfluoropentanoic acid	PFPeA	NISTPFAS002646
Perfluorohexanoic acid	PFHxA	NISTPFAS002643
Perfluoroheptanoic acid	PFHpA	NISTPFAS002640
Perfluorooctanoic acid	PFOA	NISTPFAS002637
Perfluorononanoic acid	PFNA	NISTPFAS002635
Perfluorodecanoic acid	PFDA	NISTPFAS002632
Perfluorobutanesulfonic acid	PFBS	NISTPFAS003045

PFAS Preferred Name	Acronym	NIST ID
Perfluorohexanesulfonic acid	PFHxS	NISTPFAS003043
Perfluorooctanesulfonic acid	PFOS	NISTPFAS003041

- **Sample B** was a solution consisting of a methanolic dilution of two aqueous film-forming foam (AFFF) commercial solutions. One of the AFFF solutions was an electrochemical fluorination (ECF) based product, and the second solution was a fluorotelomerization based product. The nominal dilution of the individual AFFF products in methanol was 1000 µg AFFF product per 1 g of solution.

- **Sample C** was a methanolic extract of an AFFF-impacted soil amended with an analytical standard of a single PFAS in methanol. For preparation of this sample, approximately 400 g of AFFF-impacted soil was extracted by ultrasonication in 600 mL of methanol. The solvent was filtered and concentrated 20-fold. An aliquot of an analytical standard containing N-AP-FHxSA (NISTPFAS000878) was added with a nominal concentration of 0.1 µg/g in the sample.

1.4. Description of Reporting Format

Participants were individually provided with a Microsoft Excel workbook template to report their NTA methods and the PFAS they identified in each of the samples. An example of the reporting format is provided in the supporting information (access to this information is provided in the Appendix). To list the identities, the workbook included a look-up function that searched compound common names, aliases, acronyms, structural descriptions (InChI Key), and other database identifiers (such as EPA's DTXSID), where available.

1.4.1. Reporting of Identification Confidence

Participants were asked to provide an identification confidence level for each PFAS they reported. Confidence levels were from Charbonnet et al. [6] and selected from a drop-down list containing those levels. Identification confidences levels and their meanings include:

Level 1a - Confirmed by reference standard
Level 1b - Indistinguishable from reference standard
Level 2a - Probable by library spectrum match
Level 2b - Probable by diagnostic fragmentation evidence
Level 2c - Probable by diagnostic homologue evidence
Level 3a - Positional isomer candidates
Level 3b - Fragmentation-based candidate
Level 3c - Circumstantial candidate based on fragmentation
Level 3d - Circumstantial candidate based on homologues
Level 4   - Unequivocal molecular formula
Level 5a - PFAS suspect screening exact mass match
Level 5b - Non-target PFAS exact mass of interest

2. Results

2.1. Description of Participating Laboratories

Overall, samples were shipped to 34 participating laboratories, 27 of which submitted results. Laboratories that submitted results before June 30, 2024, were emailed a preliminary report to review their overall results (no individual PFAS were identified in the preliminary report) and were provided an opportunity to submit any updates or corrections to their results. Of the laboratories that submitted data, 11 laboratories were from academic institutions, 3 laboratories were analytical instrument vendors, 6 laboratories were commercial or contract analytical laboratories, and 7 were from government agencies (US Federal, State, and non-US). Results in the following sections were analyzed as-provided by participants.
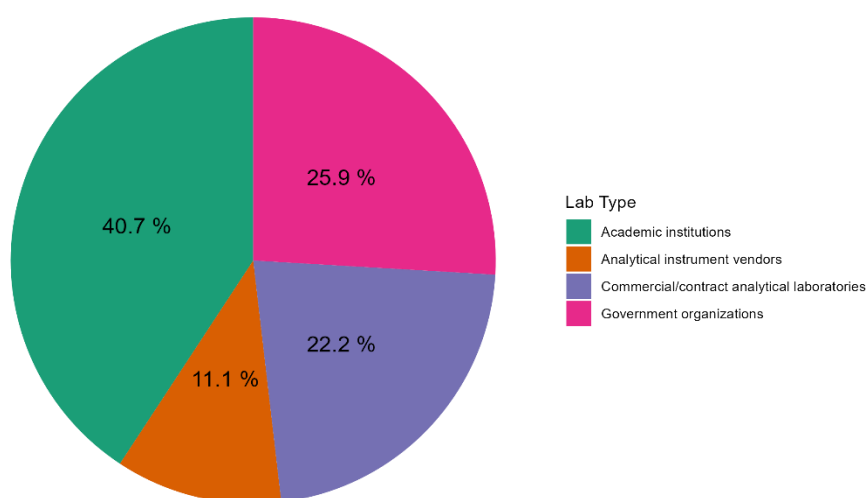
Fig. 1. Composition of the participating laboratories that submitted results, values within each sector are the percentage of the total.

2.2. Description of Laboratory Methods

Generally, laboratories used octadecyl (C18) stationary phases for separation, while the mass analyzers (the terminal mass analyzer) used by laboratories were split between Orbitrap and time-of-flight technologies. Less than half (44 %) of the laboratories used positive and negative ionization for the analysis of the samples, rather than negative ionization alone. Table 2 lists method information reported by the participating laboratories.

Table 2. Tabulated description of the methods used by the participating laboratories.

Parameter	Setting	Number of laboratories (% of total labs)
Column Stationary Phase	Octadecyl (C18)	25 (92.6 %)
	Pentafluorophenyl (PFP)	1 (3.7 %)
	Silica	1 (3.7 %)
Ionization Polarity	Negative Only	15 (55.6 %)
	Positive Only	0 (0 %)
	Negative and Positive	12 (44.4 %)
Mass Analyzer	Orbitrap	10 (37 %)
	Time-of-Flight	17 (63 %)

Additional method metadata was collected during this study, but the effect of these additional parameters on the individual laboratory results will require further analysis for a future report.

2.3. Sample Analysis Results

For brevity, only the top twenty identified PFAS in each sample are included in these sections. See the supporting information described in the Appendix for the full identification lists. Frequency of identification of individual PFAS among the laboratories is presented as a Reporting Rate for each PFAS in each respective sample, which is defined as:

$$Reporting\ Rate\ (\%) = 100\ \% \times \frac{[number\ of\ times\ PFAS\ identity\ was\ reported]}{[total\ number\ of\ participating\ laboratories]}$$

2.3.1. Sample A

Sample A was a solution of multiple analytical standards for PFAS. Nearly all PFAS that were intentionally added to Sample A (Table 1) were reported by greater than 70 % of participating laboratories, except for 5:3 fluorotelomer betaine (NISTPFAS003794, 26 %). Due to its quaternary amine functional group, 5:3 fluorotelomer betaine can only be detected in positive ionization mode and its detection may have been limited to those laboratories that used positive ionization in their methods. The reporting rate of the top twenty compounds ranged from 96 % (26 out of 27 laboratories) to 19 % (5 out of 27 laboratories). No individual PFAS in Sample A was detected by all the participating laboratories.

Of the top twenty reported PFAS identifications (Table 3), fifteen were reported at a Level 1a confidence by at least one laboratory. As the sample was developed using commercially available analytical standards, many of the intentionally added PFAS were reported with Level 1a confidence if laboratories used matching analytical standards as part of their NTA identification workflow. Overall, the solution was a dilution of analytical-grade standards containing only thirteen PFAS and there were 133 individual PFAS reported by at least two laboratories. While the other 120 PFAS cannot be proven absent without additional targeted analysis, it is unlikely that many of these compounds would be present in the sample at detectable concentrations. There were 152 compounds reported once among all participating

laboratories (**Fig. 2**) with Level 3 confidence or higher, suggesting that there were reported PFAS detections that could not be reproduced by more than one laboratory. As laboratories used their own PFAS detection methods and workflows this may be difficult to examine in a systematic manner to find the cause of this discrepancy or disprove a reported identification.

Table 3. Table of the top 20 most reported PFAS in Sample A. For each laboratory (column) that reported the specific PFAS (row), the identification confidence reported is presented. If a value is blank, the participant did not report the PFAS as present. The final two columns show the highest reported confidence among all labs for each PFAS and the reporting rate for each PFAS among all laboratories. Rows with bold text and highlighted gray represent compounds known to be present in the sample through internal (NIST) preliminary analysis.

Compound Name	DIM001	DIM002	DIM003	DIM004	DIM005	DIM006	DIM011	DIM014	DIM017	DIM018	DIM020	DIM021	DIM023	DIM027	DIM029	DIM032	DIM033	DIM035	DIM037	DIM038	DIM040	DIM043	DIM044	DIM045	DIM047	DIM048	DIM049	Maximum Confidence ID	Reporting Rate (%)
NISTPFAS002640 - Perfluoroheptanoic acid	2a	2b	2a	2c	1a	1a	1a	1a	1a	2b	3d	1a	1a	1a	1a	2a		1a	1a	1a	1a	2b	1a	1a	2a	1a	1a	1a	96%
NISTPFAS003043 - Perfluorohexanesulfonic acid	2a	3d	2a	2b	1a	1a	1a	1a	1a	2b	2a	1a	1a	1a	1a	2a		1a	1a	1a	1a	1a	1a	3b	2a	1a	1a	1a	96%
NISTPFAS002635 - Perfluorononanoic acid	2a	2c	2a	2c	1a	1a	1a	1a	1a	2b	2a	1a	1a	1a	1a			1a	1a	1a	1a	1a	1a	1a	2a	1a	1a	1a	93%
NISTPFAS002643 - Perfluorohexanoic acid	2a	2c	2a	2c	1a		1a	1a	1a	2b	3d	1a	1a	1a	1a	4	1a	1a	1a	1a	1a	2c	1a		2a	1a	1a	1a	93%
NISTPFAS003045 - Perfluorobutanesulfonic acid	2a	2b	2a	2b	1a		1a	1a	1a	2b	2a	1a	1a	1a	1a		2b	1a	1a	1a	1a	3b	1a	1a	2a	1a	1a	1a	93%
NISTPFAS002637 - Perfluorooctanoic acid	2a	2c	2a	2c	1a	1a	1a	1a	1a	2b	2a	1a	1a	1a	1a			1a	1a	1a	1a	1a	1a		2a	1a	1a	1a	89%
NISTPFAS003041 - Perfluorooctanesulfonic acid	2a	2b	2a	2b	1a	1a	1a	1a	1a	2b	2a	1a	1a	1a	1a			1a		1a	1a	1a	1a	3b	2a	1a	1a	1a	89%
NISTPFAS000878 - Perfluorohexane sulfomido amine			2a	3c	1a	4	2b	3c	2a	2b	2b	4	4	2b	3b		2b	2a	1a	2a	1a	2a	1a	5a	2b		1a	1a	85%
NISTPFAS002632 - Perfluorodecanoic acid	2a		2a	2c		1a	1a	1a	1a	2b	2a	1a	1a	1a	1a			1a	1a	1a	1a	1a	1a	1a	2a	1a	1a	1a	85%
NISTPFAS003238 - Chloro-perfluorooctane sulfote	2a		2a	3b	1b		2c	3c	2a		2b	1a	2a	1b	2a	2a	2b		2a	1a	2c	1b	3d	2a	2a	2a	2a	1a	81%
NISTPFAS002646 - Perfluoropentanoic acid			2a	2c	1a		1a	1a	1a	2b	3d	1a	1a	1a	1a			1a	1a	1a	1a	3b	1a		2a	1a	1a	1a	78%
NISTPFAS002649 - Perfluorobutanoic acid	2a		2a	2c	1a		1a	1a	2a		2a	1a	1a	1a	1a			1a	1a	1a	1a	4			2a	1a	1a	1a	74%
NISTPFAS000852 - Perfluorohexane sulfomido amine oxide		2c	4	2b		2c			4			1a			4			3a		3c	3b						1a	1a	41%
NISTPFAS002560 - Perfluorohexanesulfomide		2b		1b		2b		1a	2b	2a			1a					1a	1b					2a				1a	37%
NISTPFAS003794 - 5:3 Fluorotelomer betaine		2a							3c									1a	2a		4			2a			2a	1a	26%
NISTPFAS004053 - Dihydrogen-substituted fluoro triether tridecanoic acid		4			4			2c			4	4												3d		4		2c	26%
NISTPFAS004634 - Dihydrogen-substituted fluoro triether undecanoic acid		4			4			2c			4	4	5a															2c	22%
NISTPFAS002338 - 1H-Perfluoronone		4													5b		2b					2a		3d				2a	19%
NISTPFAS002342 - 8H-Perfluorooctane		4			4										5b							2a		3d				2a	19%
NISTPFAS002350 - 1,1,1,2,2,3,3,4,4,5,5-Undecafluoropentane		4													5b		2c					2c		3d				2c	19%
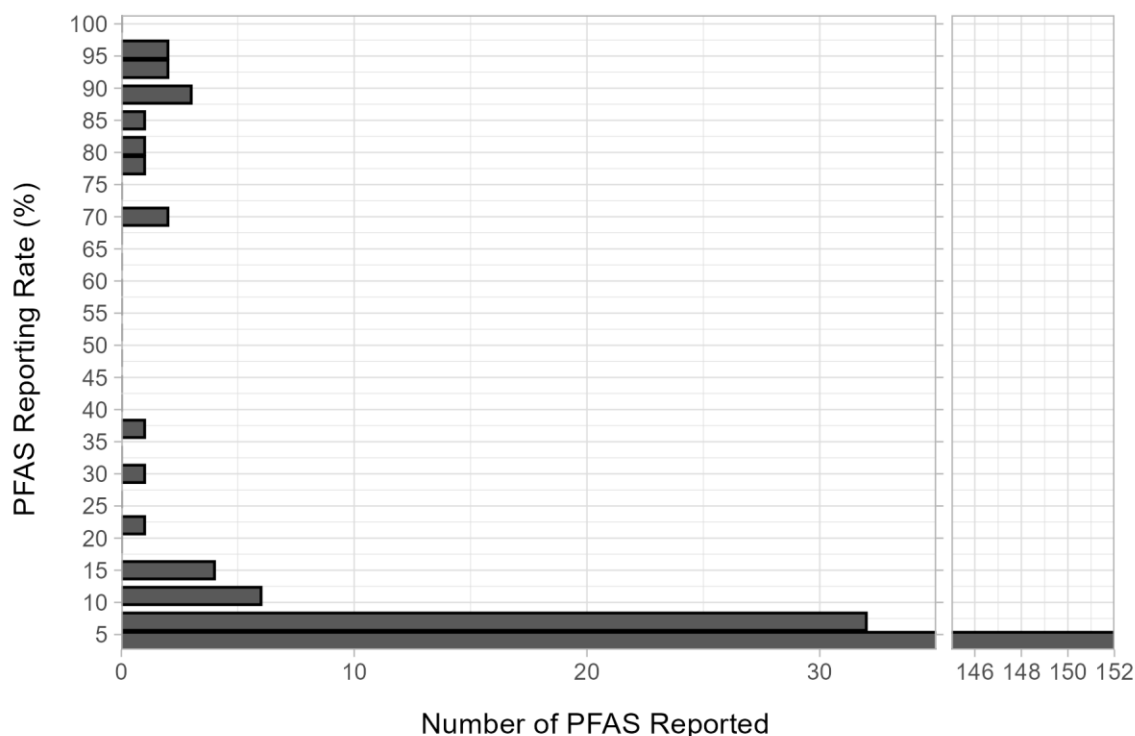
Fig. 2. Histogram of PFAS reported with Level 3 or higher confidence in Sample A by individual laboratories, showing the number of PFAS (x-axis) at specific reporting rates (y-axis). For example, 152 PFAS identities were reported only once among the 27 participating laboratories (least reporting rate of 3.7 %), while 2 PFAS identities were reported 26 times among the 27 participating laboratories (greatest reporting rate of 96.3 %).

2.3.2. Sample B

Sample B was a mixture of two different AFFF commercial formulations diluted in methanol. It contained PFAS synthesized using ECF and telomerization processes, therefore there are multiple classes of PFAS that could be present. Of the top twenty reported PFAS (Table 4), ten were identified at a Level 1a confidence by at least one laboratory. No individual PFAS in Sample B was detected by all the participating laboratories.

Overall, there were 143 individual PFAS reported by at least two laboratories for Sample B. Ten of the PFAS in the top twenty most reported PFAS were known to be present in the AFFF formulations prior to dilution. The reporting rate of the top twenty identified PFAS ranged from 96 % (26 out of 27 laboratories) to 44 % (12 out of 27 laboratories). As this material was a dilution of two commercial AFFF formulations, many PFAS could be present as the active components, or as impurities or transformation products of the active components. Additional targeted analyses would be required to confirm the identity of these additional PFAS.

Based on **Fig. 3**, 116 PFAS were reported once among all participating laboratories with Level 3 confidence or higher. Notably, this is less than the number of PFAS that were reported once in Sample A (152 PFAS), which was a much less complex material than Sample B. Further investigation into this observation is warranted.

Table 4. Table of the top 20 most reported PFAS in Sample B. For each laboratory (column) that reported the specific PFAS (row), the identification confidence reported is presented. If a value is blank, the participant did not report the PFAS as present. The final two columns show the highest reported confidence among all labs for each PFAS and the reporting rate for each PFAS among all laboratories. Rows with bold text and highlighted gray represent compounds known to be present in the sample through internal (NIST) preliminary analysis.

| Compound Name	DIM001	DIM002	DIM003	DIM004	DIM005	DIM006	DIM011	DIM014	DIM017	DIM018	DIM020	DIM021	DIM023	DIM027	DIM029	DIM032	DIM033	DIM035	DIM037	DIM038	DIM040	DIM043	DIM044	DIM045	DIM047	DIM048	DIM049	Maximum Confidence ID	Reporting Rate (%)
NISTPFAS003043 - Perfluorohexanesulfonic acid	2a	2b	2a	2c	1a	1a	1a	1b	1a	2b	2a	1a	1a	1a	1a	2a		1a	1a	1a	1a	1a	1a	1a	2a	1a	1a	1a	96%
NISTPFAS003041 - Perfluorooctanesulfonic acid	2a	2c	2a	2c	1a	1a	1a	1a	1a	2b	2a	1a	1a	1a	1a			1a		1a	1a	1a	1a	1a	2a	1a	1a	1a	89%
NISTPFAS003044 - Perfluoropentanesulfonic acid	2a	2b	2a	2c	1a	4	1a	5a	1a	2b	2a	1a	1a	1a	1a			1a	1a	1a	1a		1a	5a	2a			1a	85%
NISTPFAS003052 - 6:2 Fluorotelomer sulfonic acid	2a		2a	2c	1a	1a	1a		1a	2b	2a	4	1a		1a	2a	2b	1a		1a	1a	1a	1a	2a	1a	1a	1a	1a	85%
NISTPFAS000878 - Perfluorohexane sulfomido amine		2c	2a	2b	2a		2b	3c	2c	2b	2b	4	4	2b	2a			2a	1a	2a	1a	2a	1a	5a	2b		1a	1a	81%
NISTPFAS003045 - Perfluorobutanesulfonic acid	2a	2b	2a	2b	1a		1a	1b	1a	2b		1a	1a	1a	1a			1a	1a	1a	1a		1a	1a	2a	1a	1a	1a	81%
NISTPFAS003285 - 6:2 Fluorotelomer thia propanoamido dimethyl ethyl sulfote	2a	2b	2a	3b		2a		2a	2a	2b	2b	4		2a	2a		2b		2b	2a	2a		4	3d	2a	2a	2a	2a	78%
NISTPFAS000880 - Perfluorobutane sulfomido amine		2c	2a	2b	3d		2c		2c	2c	5b	4	4	2a	3c			2c		2a	2a		3d	5a	2b		2a	2a	70%
NISTPFAS002643 - Perfluorohexanoic acid	2a	2c	2b	2b	1a		1a	1b	1a	2b	3d	4	1a		1a			3d	1a	1a	1a				2a			1a	70%
NISTPFAS000879 - Perfluoropentane sulfomido amine		2c	2a	2c	3c	4	2b		2b	2c	2b			2a	2c			2c		2a	2a	4	3d		2b		2a	2a	67%
NISTPFAS002637 - Perfluorooctanoic acid	2a	2c	2a	2c	1a		1a			2b	2a	1a	1a	1a	1a			3d	1a	1a	1a				2a			1a	67%
NISTPFAS003042 - Perfluoroheptanesulfonic acid		3d	2a	2b	1a	4	1a		1a	2b	2a	1a			2a			1a	1a	1a	1a	3a	1a		2a			1a	67%
NISTPFAS003492 - Perfluoropropane sulfote	4	3b	3d	2b	2c		2b		1a	2b	3b		2a			4		1a	1a	2a	1a		1a		2a		1a	1a	67%
NISTPFAS003407 - 6:2 Fluorotelomer sulfinyl propamido dimethyl ethyl sulfote			2a			2b		2a	2b	2b		2b		2a		2b		2b	3c	2a	2a		5a	2a		2b		2a	56%
NISTPFAS003430 - 3-(N-(3-(dimethylamino)propyl)-perfluoropentylsulfomido)propanoic acid	4	2c	2a	2c		2b			2a	3d			2c		4			2c		2a	2a	4		5a			2a	2a	56%
NISTPFAS003455 - N-Dimethyl ammonio propyl perfluoropropane sulfomide		2c	3d	2b	3c		2b		2a	2c	2b		4		2c	4				3d			5a	2b		2b		2a	56%
NISTPFAS000881 - 6:2 Fluorotelomer sulfomide amine			2a	2b					2b		2b	4	3c		2a				2b	2a	2a	4		5a	2a		2b	2a	52%
NISTPFAS004390 - 6:2 Fluorotelomer thia ammoniohydroxypropyl ethanoic acid		5a				2b		3a			4	4	5a	4		2b		3a	3c	4	3a		5a			3b		2b	52%
NISTPFAS000861 - N-[3-(Dimethylamino)propyl]-N-[(tridecafluorohexyl)sulfonyl]-beta-alanine	2a	2c	2a	2c					3b	2c		4						2c	3b		2a	4		5a			2a	2a	48%
NISTPFAS000862 - N-[3-(Dimethylamino)propyl]-N-[(nofluorobutyl)sulfonyl]-beta-alanine	4	2c	2a	2c		4			3a	2c	2b							2c			2a	4					2a	2a	44%

9
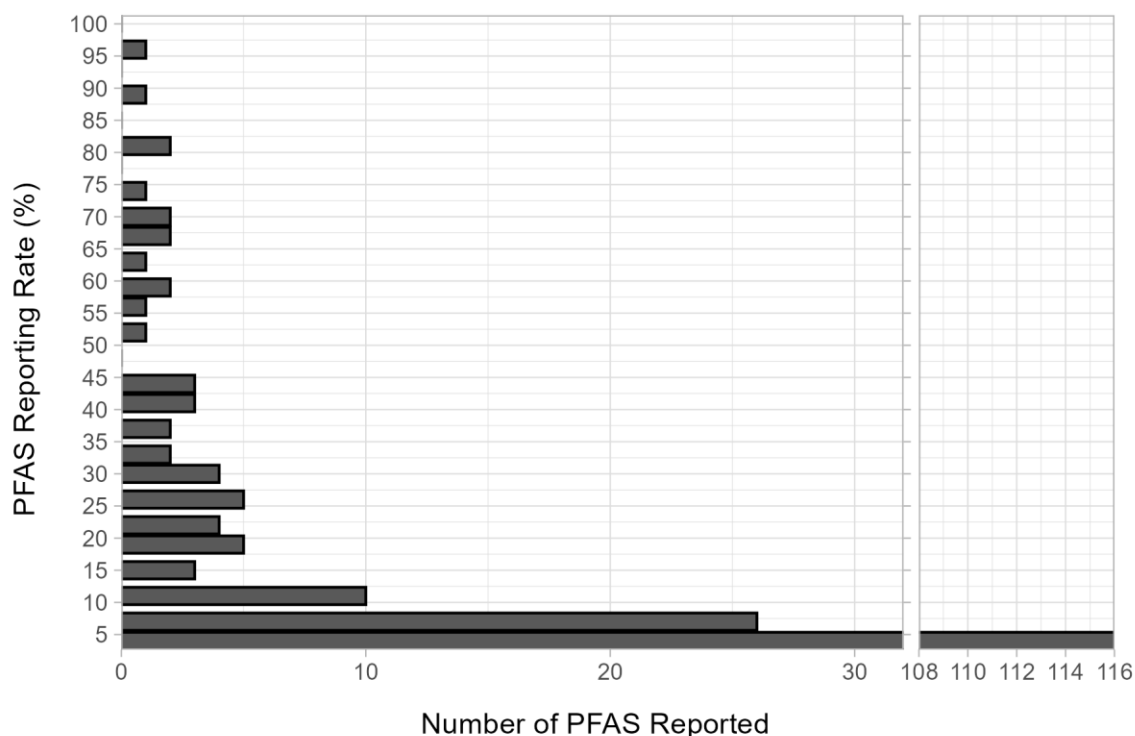
Fig. 3. Histogram of PFAS reported with Level 3 or higher confidence in Sample B by individual laboratories, showing the number of PFAS (x-axis) at specific reporting rates (y-axis). For example, 116 PFAS identities were reported once among the 27 participating laboratories (reporting rate of 3.7 %), while 1 PFAS identity were reported 26 times among the 27 participating laboratories (reporting rate of 96.3 %).

2.3.3. Sample C

Sample C was a methanolic extract of an AFFF-impacted soil, with a single added compound. Of the top twenty identified PFAS (Table 5), seventeen were reported at a Level 1a confidence by at least one laboratory. No compound in Sample C was reported by all the participating laboratories. In general, there were more compounds reported by multiple laboratories than in Samples A and B, as shown in **Fig. 4**. Further targeted analysis of this material would be needed to verify the majority of the PFAS identified in this sample.

Overall, there were 237 individual PFAS reported by at least two laboratories. Ten of the PFAS in the top twenty most reported PFAS were known to be present in the sample. The single analytical standard (N-AP-FHxSA; NISTPFAS000878) added to the soil extract (nominally 0.1 µg/g) was detected within the top twenty reported PFAS by 85 % of the laboratories (23 out of 27 laboratories). In contrast to the previous two samples, the top twenty most frequently reported PFAS were reported at rates above 75 % (identification by more than 20 out of 27 laboratories). This could be due to the greater complexity of the material with more PFAS detectable and identifiable by a broader number of laboratories.

Based on **Fig. 4**, there were 147 PFAS reported once among all participating laboratories with Level 3 confidence or higher. Notably, this is fewer than in Sample A (152 PFAS), but greater

than in Sample B (116 PFAS). As an extract of an environmental sample, Sample C is most likely more complex than Sample A, though there is no prior data to compare differences in complexity between Sample B and C.

Table 5. Table of the top 20 most reported PFAS in Sample C. For each laboratory (column) that reported the specific PFAS (row), the identification confidence reported is presented. If a value is blank, the participant did not report the PFAS as present. The final two columns show the highest reported confidence among all labs for each PFAS and the reporting rate for each PFAS among all laboratories. Rows with bold text and highlighted gray represent compounds known to be present in the sample through internal (NIST) preliminary analysis.

| Compound me	DIM001	DIM002	DIM003	DIM004	DIM005	DIM006	DIM011	DIM014	DIM017	DIM018	DIM020	DIM021	DIM023	DIM027	DIM029	DIM032	DIM033	DIM035	DIM037	DIM038	DIM040	DIM043	DIM044	DIM045	DIM047	DIM048	DIM049	Maximum Confidence ID	Reporting Rate (%)
NISTPFAS003043 - Perfluorohexanesulfonic acid	2a	2b	2a	2b	1a	1a	1a	1a	1a	2b	2a	1a	1a	1a	1a	2a		1a	1a	1a	1a	1a	1a	1a	2a	1a	1a	1a	96%
NISTPFAS002560 - Perfluorohexanesulfomide	2a	3d	2a	2b	2a	2b	2b	2a	1a	2b	2a	1a		1a	2c	2a	1a	1a	1a	1a	1a	2b	1a	5a	2a		1a	1a	93%
NISTPFAS003041 - Perfluorooctanesulfonic acid	2a	3d	2b	2c	1a	1a	1a	1a	1a	2b	2a	1a	1a	1a	1a			1a	1a	1a	1a	1a	1a	1a	2a	1a	1a	1a	93%
NISTPFAS002559 - Perfluorooctanesulfomide	2a	2c	2a	2b	1a	1a	1a	1b	1a	2b	2a	1a	1a		1a			1a	1a	1a	1a	2c	1a	1a	2a	1a	1a	1a	89%
NISTPFAS003042 - Perfluoroheptanesulfonic acid	2a	2b	2a	2b	1a	1a	1a		1a	2b	2a	1a	1a		1a		1a	1a	1a	1a	1a	1a	1a	1a	2a	2a	1a	1a	89%
NISTPFAS003044 - Perfluoropentanesulfonic acid	2a	2b	2a	2b	1a	3d	1a	5a	1a	2b	2a	1a	1a	1a	1a			1a	1a	1a	1a	3a	1a	3b	2a		1a	1a	89%
NISTPFAS000878 - Perfluorohexane sulfomido amine		3d	2a	2c	2a	4	2b	3c	2a	2b	2b	4	2a	2b	2a			2a	1a	2a	1a	2a	1a	5a	2b		1a	1a	85%
NISTPFAS002630 - Perfluoroundecanoic acid	2a	2b	2a	2b	1a	2b	1a	1b	1a	2b	3d	1a	1a	1a	1a			1a	1a	1a	1a		1a	2a	1a	1a		1a	85%
NISTPFAS002632 - Perfluorodecanoic acid	2a	2b	2a	2b	1a		1a	1b	1a	2b	2a	1a	1a	1a	1a			1a	1a	1a	1a	1a		1a	2a	1a	1a	1a	85%
NISTPFAS002643 - Perfluorohexanoic acid	2a	3d	2a	2c	1a		1a	1b	1a	2b	2a	1a	1a	1a	1a	4	1a	1a	1a	1a	1a		1a		2a		1a	1a	85%
NISTPFAS003039 - Perfluorodecanesulfonic acid	2a	3d	2a	2b	1a	1a	1a		1a	3d	2a	1a	1a	1a	1a			1a	1a	1a	1a	2c	1a		2a	1a	1a	1a	85%
NISTPFAS003040 - Perfluorononesulfonic acid	2a	3d	2a	2b	1a		1a		1a	2b	2a	1a	1a	1a	1a		2b	1a	1a	1a	1a	3b	1a		2a	2b	1a	1a	85%
NISTPFAS003045 - Perfluorobutanesulfonic acid	2a	2b	2a	2b	1a		1a	1b	1a	2b	2a	1a	1a	1a	1a			1a	1a	1a	1a		1a	1a	2a	2a	1a	1a	85%
NISTPFAS003085 - Pentafluorosulfide perfluorooctane sulfate	2a	2b	2a			2b	2b	2a	2a	2b	2b	4	2a	2b	2a		2b	2c	2b	2b	2a	2a	4		2b	2a	2a	2a	85%
NISTPFAS003086 - Pentafluorosulfide perfluoronone sulfate		2b	2b			4	2b	2b	2a	2b	2b	4	2b	3b	4	2a		2c	2b	2b	2b	2a	4	5a	2b	2a	2c	2a	85%
NISTPFAS000009 - Perfluorobutylsulfomide	2a	2c	2a	2c	2a	3d	2b		1a	2c	2a		1a		3b			1a	1a	1a	1a	3a	1a	3d	2a	2a	1a	1a	81%
NISTPFAS000708 - N-Ethyl-N-((tridecafluorohexyl)sulfonyl)glycine	2a	2c	2b	2b	4	4		5b		3d	2b	4	4	2b	3b		2b	2c	3c	2c		4	4		5a	5a	4	2a	81%
NISTPFAS002629 - Perfluorododecanoic acid	2a	2c	2a	2b	1a	1a	1a		1a	3d	3d	1a	1a	1a	1a		3d	1a	1a	1a	3d		3b	2a	2a			1a	81%
NISTPFAS003038 - Perfluorododecanesulfonic acid		3d	2b	2b	1a	1a	1a		2b	2b	2b	1a	1a	2a	2a			1a	1a	2c	1a	1a	1a	5a	2a		1a	1a	81%
NISTPFAS002637 - Perfluorooctanoic acid	2a	2b	2a	2c	1a		1a	1a		2b	2a	1a	1a	1a	1a			1a	1a	1a	1a	1a	1a		2a		1a	1a	78%
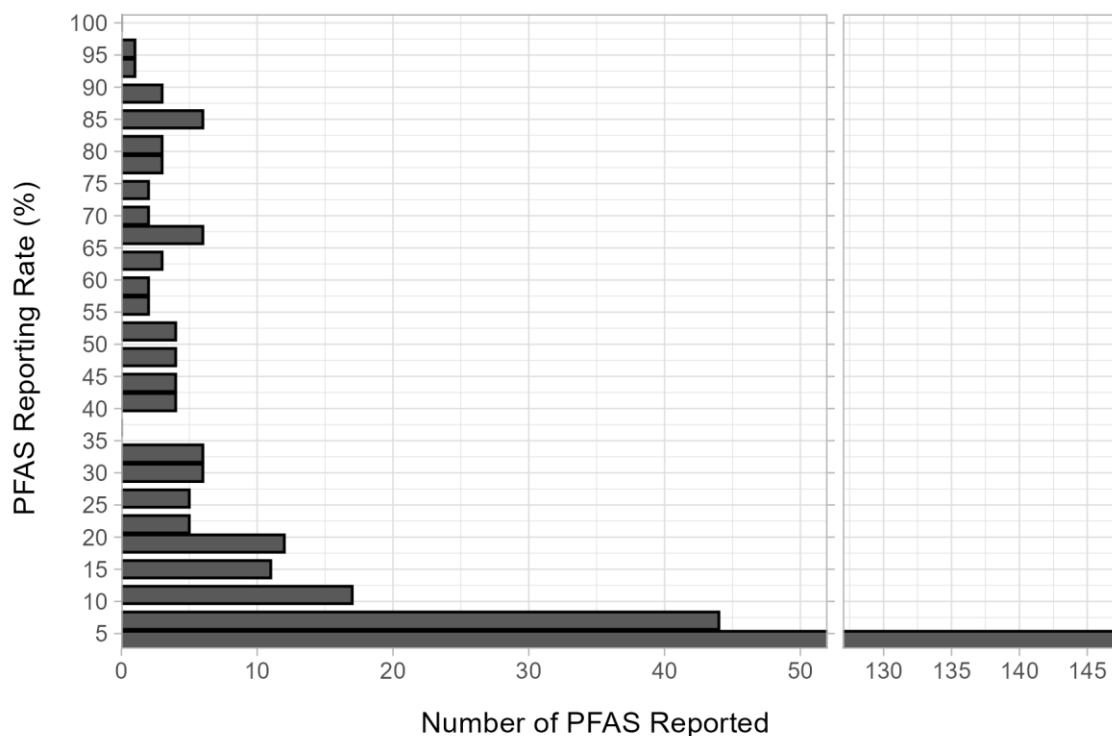
Fig. 4. Histogram of PFAS reported with Level 3 or higher confidence in Sample C by individual laboratories, showing the number of PFAS (x-axis) at specific reporting rates (y-axis). For example, 147 PFAS identities were reported once among the 27 participating laboratories (reporting rate of 3.7 %), while 1 PFAS identity was reported 26 times among the 27 participating laboratories (reporting rate of 96.3 %).

2.3.4. Overall Results

There is a wide distribution in the number of PFAS identities reported by individual laboratories (**Fig. 5**). Notably, for all three samples, no single PFAS was reported by every lab (100 % identification rate). This result could be due to interlaboratory differences in detecting PFAS or in reporting their identities. Generally, most laboratories reported PFAS that were known to be present in the samples. All known PFAS were reported at Level 1a confidence by at least one laboratory; most of the known PFAS were identified using analytical standards by internal targeted measurements.

Sample A had the broadest range of reporting rates within the top twenty identified PFAS (96 % to 19 %), while Sample C had the smallest (96 % to 78 %). This result could be due to the increasing complexity from Sample A to Sample B to Sample C; the samples with more PFAS present at detectable concentrations resulted in a greater number of frequently reported PFAS.
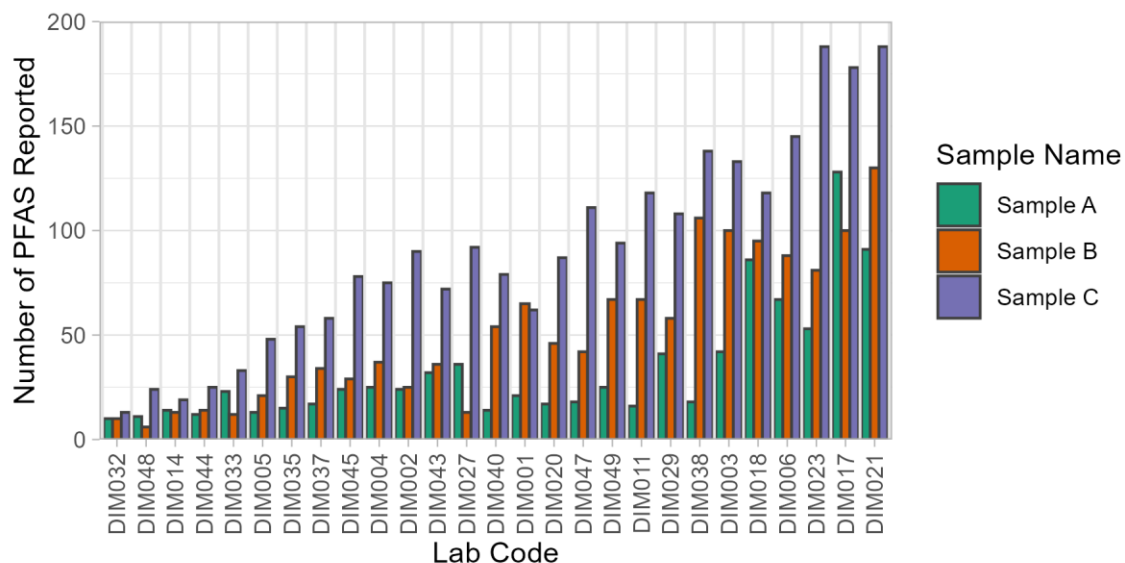
Fig. 5. Total number of PFAS reported (y-axis) for each participating laboratory (x-axis) by the individual samples. Labs are ordered by increasing mean number of PFAS identified.

Participating laboratories either used negative ionization only or both positive and negative ionization for the analysis of the samples. The distribution of PFAS detected in positive ionization mode, negative ionization mode, and both ionization modes are shown in **Fig. 6**. For all samples, the majority of identified PFAS were detected using negative ionization, although many PFAS were detected using positive ionization only. As less than half of the participating laboratories (44 %) used both positive and negative ionization modes, the compounds detectable only by positive ionization (such as 5:3 fluorotelomer betaine) would have been missed by the majority of participating laboratories.
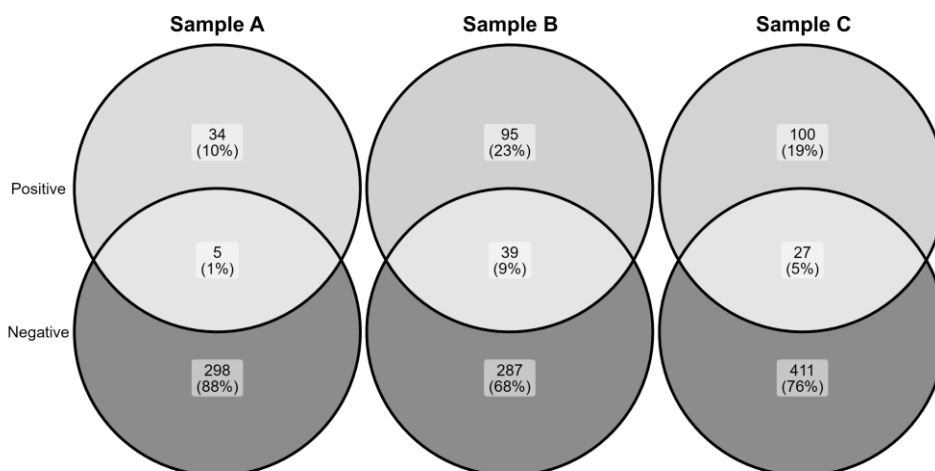

Fig. 6. Venn diagram showing the number of individual PFAS that were reported in each sample and the respective ionization polarities with which they were detected. Top: PFAS identified by positive polarity only; Bottom: PFAS identified by negative polarity only; Middle: PFAS identified by both positive and negative polarity.

3. Discussion

This study represents one of the first attempts to characterize the interlaboratory performance of NTA methods to identify PFAS in complex samples. True positive rates are difficult to calculate for individual laboratories, as the identities of all PFAS in the samples cannot be validated without additional targeted analyses, even for the intentionally constructed Sample A. Generally, most laboratories were able to identify those PFAS previously known through NIST targeted analyses. This result suggests that most of the participating labs can provide true positive identifications of some PFAS.

The number of compounds reported by individual labs covered a broad range, as shown in **Fig. 7**, and there may have been cases of laboratories over-reporting or under-reporting the identities of PFAS due to their own internal criteria for identification validation. Future NTA interlaboratory studies may need to incorporate additional method information or metrics to improve comparability of labs with different reporting criteria. In addition, laboratories were asked to submit a single PFAS identity for each feature (chromatographic retention time with a specific mass-to-charge (m/z)), where there may have been instances of more than one candidate PFAS. This limited the ability of laboratories to communicate uncertainty and therefore may have caused a laboratory to report an incorrect single PFAS identity when the correct PFAS identity was just as likely. In future studies, consideration should be given to enable laboratories to submit additional potential identities.

This report represents an abbreviated review of the interlaboratory results for the identification of PFAS through NTA workflows. The complexity of the submitted results will require more in-depth analysis to draw additional conclusions or inferences, including comparisons of the measured results (retention time and m/z) as well as the interpreted results (compound identification) across participating laboratories. Further analysis will be performed and presented in a separate report.

References

[1]   Williams AJ (2022) Assembly and Curation of Lists of Per- and Polyfluoroalkyl Substances (PFAS) to Support Environmental Science Research. *Frontiers in Environmental Science* 10: 850019. https://doi.org/10.3389/fenvs.2022.850019

[2]   Ulrich EM (2019) EPA's non-targeted analysis collaborative trial (ENTACT): genesis, design, and initial findings. *Analytical and Bioanalytical Chemistry* 411:853-866. https://doi.org/10.1007/s00216-018-1435-6

[3]   Ragland JM (2024) A Portable and Reusable Database Infrastructure for Mass Spectrometry, and Its Associated Toolkit (The DIMSpec Project). *Journal of the American Society for Mass Spectrometry* 35(6):1282-1291. https://doi.org/10.1021/jasms.4c00073

[4]   Place BJ (2021) Suspect List of Possible Per- and Polyfluoroalkyl Substances (PFAS). National Institute of Standards and Technology, https://doi.org/10.18434/mds2-2387

[5]   Place BJ (2022) Speaking the Same Language: The Need for Accurate and Consistent Reporting of Novel Per- and Polyfluoroalkyl Substances. *Environmental Science & Technology* 56(15):10564-10566. https://doi.org/10.1021/acs.est.2c04273

[6]   Charbonnet JA (2022) Communicating Confidence of Per- and Polyfluoroalkyl Substance Identification via High-Resolution Mass Spectrometry. *Environmental Science & Technology Letters* 9(6):473-481. https://doi.org/10.1021/acs.estlett.2c00206

Appendix A. Supporting Information

Supporting information is available at the NIST Public Data Repository at
https://doi.org/10.18434/mds2-3518. Specific items provided include:

sample_a_table.csv – Summary table of all PFAS detected in Sample A, including individual laboratory reported PFAS identities.

sample_b_table.csv – Summary table of all PFAS detected in Sample B, including individual laboratory reported PFAS identities.

sample_c_table.csv – Summary table of all PFAS detected in Sample C, including individual laboratory reported PFAS identities.

all_pfas_table.csv – Summary table of all PFAS detected in all samples, does not include individual laboratory reported PFAS identities.

PFASNTAILS_Reporting Form.xlsx – Example form provided to laboratories for reporting results.