SERDP

FINAL REPORT

# Improving Access and Utility of Analytical Data for the Confident Discovery and Identification of PFAS in Environmental Matrices

Benjamin Place
Jared Ragland
Jessica Reiner
***National Institute of Standards & Technology, U.S. Department of Commerce***

**November 2024**

**SERDP Project ER20-1056**

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*

**1. REPORT DATE** *(DD-MM-YYYY)*
17-04-2025

**2. REPORT TYPE**
SERDP Final Report

**3. DATES COVERED** *(From - To)*

**4. TITLE AND SUBTITLE**
Improving Access and Utility of Analytical Data for the Confident Discovery and Identification of PFAS in Environmental Matrices

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
Benjamin Place, Jared Ragland, Jessica Reiner

**5d. PROJECT NUMBER**
ER20-1056

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Chemical Sciences Division, National Institute of Standards & Technology
100 Bureau Dr., Mail Stop
8392
Gaithersburg, MD 20899

**8. PERFORMING ORGANIZATION REPORT NUMBER**
ER20-1056

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
Office of the Deputy Assistant Secretary of Defense (Energy Resilience & Optimization)
3500 Defense Pentagon, RM 5C646
Washington, DC 20301-3500

**10. SPONSOR/MONITOR'S ACRONYM(S)**
SERDP

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**
ER20-1056

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
DISTRIBUTION STATEMENT A. Approved for public release: distribution unlimited.

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
Analytical data regarding PFAS is widely produced by research laboratories but is not widely available for analytical laboratories to utilize for the identification of unknown PFAS. The work research will improve access and utility of PFAS analytical data through the development of a readily available reference database. Using a combination of R statistical language and SQL database structure, a mass spectral database of PFAS was developed. Then a quality assurance program (QAP) was developed to engage and educate analytical laboratories about the use of the database and to evaluate and refine the ability of analytical laboratories to use the database for PFAS identification. Through the technical approach, a database infrastructure was developed and populated with nearly 5,000 individual PFAS structures and consensus reference mass spectra representing 132 PFAS. Following its deployment, an interlaboratory study was conducted with 34 participating laboratories to identify PFAS in three unknown solutions. Participating laboratories were given access to DIMSpec and the associated data analysis tools. This research resulted in a novel database (DIMSpec) that is accessible to all PFAS analysts with a range of useful tools and information.

**15. SUBJECT TERMS**
Per- and polyfluoroalkyl substances; mass spectrometry; non-targeted analysis

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | Benjamin Place |
| UNCLASS | UNCLASS | UNCLASS | UNCLASS | 76 | **19b. TELEPHONE NUMBER** *(include area code)*<br>401-975-3941 |

**Standard Form 298 (Rev. 8-98)**
**Prescribed by ANSI Std. Z39.18**

# SERDP FINAL REPORT

Project: ER20-1056

## TABLE OF CONTENTS

# LIST OF FIGURES

**Page**

# LIST OF TABLES

**Page**

## ACRONYMS AND ABBREVIATIONS

AFFF: aqueous film-forming foams
CERCLA: Comprehensive Environmental Response, Compensation and Liability Act
DIMSpec: Database Infrastructure for Mass Spectrometry
DOI: digital object identifier
ECF: electrochemical fluorination
InChI: International Chemical Identifier
ISBN: International Standard Book Number
LC-HRMS: liquid chromatography – high resolution mass spectrometry
MTA: material transfer agreement
NIST: National Institute of Standards and Technology
NTA: non-targeted analysis
NTA-MRT: Non-Targeted Analysis Method Reporting Tool
NISTIR: National Institute of Standards and Technology Internal Report
OECD: Organization for Economic Co-operation and Development
PFAS: per- and polyfluoroalkyl substance
PFCA: perfluoroalkyl carboxylic acid
PFSA: perfluoroalkyl sulfonic acid
SQL: Structured Query Language
QAP: quality assurance program
USEPA: United States Environmental Protection Agency

## KEYWORDS

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

# ACKNOWLEDGEMENTS

We would like to thank Drs. Elin Ulrich and Antony Williams (US Environmental Protection Agency) for their collaboration with the PFAS150 material transfer agreement and the USEPA Chemicals Dashboard. In addition, we would like to thank Drs. Jennifer Field and Christopher Higgins, and their respective laboratory members, for their support developing the initial PFAS suspect list and non-targeted analysis data.

Finally, we would like to recognize the entire NIST PFAS Program research team for their work in support of this project, including Alix Rodowa, Amy Cuthbertson, Niksa Blonder, Carolyn Burdette, Catherine Rimmer, John Kucklick, Nathan Mahynski, Carlos Gonzalez, and Katherine Peter (former NIST postdoctoral fellow).

# DISCLAIMER

Portions of this report, including text, figures, and tables, are reproduced without change from previously published NIST Internal Report 8544 (NISTIR 8544). This includes Sections 3.3, 4.2, 8.3, 9.2, and 10.2. The citation for NISTIR 8544 is:

Place, B. , Reiner, J. , Ragland, J. , Rodowa, A. , Burdette, C. , Cuthbertson, A. , Rimmer, C. , Kucklick, J. and Mahynski, N. (2024), Per- and Polyfluoroalkyl Substances - Non-Targeted Analysis Interlaboratory Study Final Report, NIST Interagency/Internal Report (NISTIR), National Institute of Standards and Technology, Gaithersburg, MD, [online], https://doi.org/10.6028/NIST.IR.8544 (Accessed October 17, 2024)

# ABSTRACT

## INTRODUCTION AND OBJECTIVES

The use of spectral libraries is essential to the success of a non-targeted analysis (NTA) protocol to identify unknown per- and polyfluoroalkyl substances (PFAS) in environmental samples. Due to the lack of standard data formats and database structures current mass spectral libraries have limited interoperability between analytical laboratories and even different platforms within laboratories. The objective of this research is to develop a data infrastructure to contain PFAS mass spectral information and metadata of detected PFAS. With an open database structure, analytical laboratories will be able to include this database in their NTA workflow to identify unknown PFAS.

## TECHNICAL APPROACH

Analytical data, specifically fragmentation mass spectra, regarding PFAS is widely produced by research laboratories, but is not widely available for analytical laboratories to utilize for the identification of unknown PFAS. This research will improve access and utility of PFAS analytical data through the development of a readily available reference database. Using a combination of R statistical language and SQL database structure, a mass spectral database of PFAS was developed (Task 1A-C). Then a quality assurance program (QAP) was developed to engage and educate analytical laboratories about the use of the database (Task 2A) and to evaluate and refine the ability of analytical laboratories to use the database for PFAS identification (Task 2B).

## RESULTS

Through the technical approach, a database infrastructure was developed and populated with nearly 5,000 individual PFAS structures and 351 consensus reference mass spectra representing 132 PFAS. This database, called Database Infrastructure for Mass Spectrometry (DIMSpec), was deployed with this reference data in the beginning of 2024. Following its deployment, an interlaboratory study was conducted with 34 participating laboratories to identify PFAS in three unknown solutions. Participating laboratories were given access to DIMSpec and the associated data analysis tools. Of the participants, 27 laboratories returned results and the reporting rate of PFAS identities ranged from 26 – 96 % of the known PFAS in the samples.

## BENEFITS

This research resulted in a novel database (DIMSpec) that is accessible to all PFAS analysts with a range of useful tools and information. This database was designed to allow additional data analysis tools to be developed by the research community or the United States Department of Defense (DOD). The database will continue to be populated with PFAS mass spectral information; each update will improve its accuracy and utility for the PFAS measurement community. As the scope reference spectra database fits within NIST's mission, the database will continue to be freely available to all users. At the conclusion of the interlaboratory study, all participating analytical laboratories were given hands-on experience with the application of the DIMSpec and were provided with an evaluation of their PFAS-NTA method performance.

# EXECUTIVE SUMMARY

# 1.0 INTRODUCTION

## 1.0 PRESENCE OF PFAS IN THE ENVIRONMENT FROM UNKNOWN SOURCES

Per- and polyfluoroalkyl substances (PFAS) are a large class of anthropogenic chemicals with unique properties (Kissa, 2001), a subset of PFAS are known to be persistent, bioaccumulative, and/or toxic to humans and the environment (see Bartell & Vieira, 2021; Evich et al., 2022; and Fenton et al., 2021 and sources therein). Depending on the definition, there can be over 10,000 chemicals that are considered PFAS.(Buck et al., 2011; Gaines et al., 2023; OECD, 2021) Currently, there are regulations for six PFAS in drinking water (40 CFR § 141 (2024), 40 CFR § 142 (2024) and two PFAS are designated as hazardous substances by through the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) (40 CFR § 302 (2024)).

Over a decade of research has demonstrated the existence of PFAS beyond perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonic acids in environmental materials due to a range of sources, including the release of aqueous film-forming foams used for fighting liquid fuel-based fires.(Backe et al., 2013; Barzen-Hanson et al., 2017) While the other PFAS may not be directly regulated, there is evidence demonstrating that many of these PFAS can transform into PFAS of concern in the environment. This brought about the rise of the term “PFAS Precursors” to indicate chemicals that transform into perfluoroalkyl acids through abiotic and biological processes.(Ruyle et al., 2023)

## 1.1 ROLE OF NON-TARGETED ANALYSIS FOR THE IDENTIFICATION OF PFAS

Non-targeted analysis (NTA) is a category of techniques that intend to identify chemicals in a sample with limited-to-no *a priori* knowledge regarding the chemical composition and quantities within the sample. This includes the identification of previously unknown chemicals in environmental samples where no chemical standards exist for performing conventional targeted, quantitative analysis. As there can be thousands of PFAS that could exist in the environment, and chemical standards are limited to less than 100 PFAS, the use of NTA is well-suited for the detection and identification of PFAS in a wide range of samples. In fact, NTA techniques have been routinely applied to materials containing PFAS and PFAS-impacted environmental samples over the past decade, resulting in the identification of previously unknown PFAS in the environment.(Barzen-Hanson et al., 2017; Place & Field, 2012)

Previous studies have used NTA to identify PFAS by elucidating the chemical structure through interpretation of analytical data and other supporting information (such as patents). While this approach can be used to identify truly novel PFAS, it is time-consuming, and the confidence of the identification can be limited. Comparison of measured fragmentation mass spectra with reference mass spectra of known compounds provides one of the highest levels of confidence in compound identification. But this approach is limited to the availability of reference mass spectra. Conventionally, libraries containing reference mass spectra are developed based on generated mass spectra using chemical standards, but there are not many chemical standards for the number of possible PFAS.

### 1.2 IMPROVING DATA ACCESS TO ENABLE NON-TARGETED ANALYSIS FOR PFAS

The mass spectral data produced from individual laboratories analytical instrumentation are generated in a vendor-specific proprietary data format. This can include lists of chemical suspects and reference mass spectra. This condition limits the ability of individual laboratories to share their data with other laboratories, especially those that have different vendor instruments. It is important to understand the current needs and predict future needs for data use, which includes the systematic collection of metadata that is relevant for current data use or could be relevant for future data investigations. The database developed through the first objective of this study needed to be interoperable and accessible to provide impactful data and tools that improve NTA for PFAS identification.

## 2.0 OBJECTIVES

The primary objective of this research was to enable analytical laboratories to better identify novel PFAS by improving access to high-quality reference mass spectra and providing a comprehensive quality assurance program (QAP) for PFAS identification via NTA. These objectives were accomplished through two overarching tasks:

> *Task 1*: Develop a common database structure for the detection and identification of PFAS.
>
> *Task 2*: Establish a world-class QAP aimed at developing and qualifying analytical laboratories for the detection and identification of PFAS in environmental matrices.

## 3.0 TECHNICAL APPROACH

### 3.0 INTERNAL GENERATION OF REFERENCE MASS SPECTRA

Internal generation of reference mass spectra for PFAS was performed using two different sources of materials: commercially available standard mixtures and synthesized single component solutions (acquired through the US EPA). Standard mixture solutions were analyzed directly without additional sample preparation. Single component standard solutions were combined into a single solution by 1:50 (by volume) dilution in methanol. Additional solutions were acquired as part of a material transfer agreement (MTA) with the U.S. Environmental Protection Agency, entitled “Per- and Polyfluoroalkyl Substance (PFAS) Standards”. The set of PFAS solutions will be referred to as the EPA PFAS150 standard set in this report. The EPA PFAS150 standard set contains 141 individual PFAS in solution that were generated by a chemical manufacturer, many of the PFAS are not commercially available otherwise.

Reference mass spectra for these samples were generated using a standardized LC-HRMS workflow and quality control procedures that enabled high quality and reproducible mass spectra. Generally, the LC-HRMS method included a reversed phase separation using specific mobile phases for the ionization polarity (0.1 % formic acid in water and methanol for positive ionization and 10 mM ammonium acetate in water and methanol for negative ionization). Mass spectrometric analysis was performed using a Thermo Fisher Scientific Q-Exactive hybrid mass spectrometer (Waltham, MA) and full scan MS1/data-dependent MS2 experiments for all compounds; this method allowed for the production of high-quality mass spectra with minimal interferences from co-eluting compounds.

For quality control, a novel approach was developed using standard solutions containing known compounds that were analyzed within the above-described workflow at least twice within an analytical sequence. Quality control parameters, including mass accuracy, retention time stability, chromatographic peak parameters, and mass spectrum reproducibility, were analyzed using in-house developed R scripts to verify that the within-sequence accuracy and precision with within tolerable limits.

All reference mass spectra generated through the above-described internal procedures were inspected manually, annotated using the Non-Targeted Analysis Method Reporting Tool (NTA-MRT), and stored until they could be incorporated into the developed database. The NTA-MRT is available at https://github.com/usnistgov/NISTPFAS.

## 3.1 EXTERNAL ACQUISITION OF REFERENCE MASS SPECTRA

Additional reference mass spectra were generated using data from published peer-reviewed literature. Specifically, the Dr. Jennifer Field laboratory at Oregon State University provided the data from Barzen-Hanson et al. (2017). Only data produced from AFFFs and technical solutions were used to generate the reference mass spectra.

## 3.2 TEST MATERIAL DEVELOPMENT FOR A NON-TARGETED ANALYSIS INTERLABORATORY STUDY

For the administration of the PFAS non-targeted analysis interlaboratory study (PFAS-NTAILS), a set of three test solutions were created that contained both known and unknown PFAS. The solutions include:

- **Sample A**, which 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.
- **Sample B**, which 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.
- **Sample C**, which was a methanolic extract of an AFFF-impacted soil amended with an analytical standard of a single PFAS in methanol.

All solutions were ampuled in 2023 and placed into boxes for storage. Boxes of ampoules were stored at 4 °C until safety labeling and shipment.

# 4.0 RESULTS AND DISCUSSION

## 4.0 TASK 1: DEVELOPMENT OF A COMMON DATABASE STRUCTURE FOR THE DETECTION AND IDENTIFICATION OF PFAS

### 4.0.1 Establishment of the NIST PFAS Suspect List of Possible Per- and Polyfluoroalkyl Substances

To address the need for a well-curated list of PFAS structures that could be detected using NTA workflows, NIST developed a suspect list of structures of the possible PFAS, which included empirically observed and *in silico* predicted structures. NIST developed an automated protocol that required specified input values to address this data gap and provide a more robust, and easily updated, suspect list for PFAS, called the "NIST List of Possible Per- and Polyfluorinated Alkyl Substances (PFAS)" or the "NIST PFAS Suspect List" for short. The suspect list is provided to the community via the NIST public data repository (B. Place, 2021) that is maintained by the NIST PFAS Program so that it may be easily available, updateable, and version controlled.

Aggregation of the initial suspect list used two primary sources of data: the Organisation for Economic Co-operation and Development PFAS (PFASOECD) list from the EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/) and the PFAS XIC (extracted ion chromatogram) List provided by Dr. Christopher Higgins (Colorado School of Mines; ORCID: 0000-0001-6220-8673; chiggins@mines.edu). Since the initial release of the PFAS Suspect List, several additional PFAS structures have been contributed by individual laboratories. As of release of this report, the current PFAS Suspect List (v1.7, https://data.nist.gov/od/id/mds2-2387) contains 4,967 individual PFAS structures.

### 4.0.2 Development of a Database Infrastructure for Mass Spectrometry (DIMSpec)

A database toolkit was developed to provide an infrastructure for the management and use of mass spectrometry data and associated metadata. This toolkit was named the Database Infrastructure for Mass Spectrometry (DIMSpec), with this project's specific application for PFAS, although the database will be solely referred to as DIMSpec for the rest of this report. In addition, as part of a NIST-wide effort to make data more compliant with the FAIR (Findable, Accessible, Interoperable, and Reusable) principles (Wilkinson et al., 2016), the database and affiliated tools were designed using open-source formats that can be easily shared and reused by laboratories within and outside of NIST. The information provided in this report includes an overview of guidance for the setup, population, and use of DIMSpec databases and its affiliated toolkit. Complete instructions and more detail are available in the User Guide (https://pages.nist.gov/dimspec/docs), which will be updated as the project continues.

The database schema for DIMSpec databases is described in detail in the User Guide. The utility of DIMSpec for NTA was greatly enhanced by providing controlled vocabulary in terms of normalization tables, and all chemical entities are cross-linked with additional validated names (where available) such as database identifiers from CompTox and PubChem, common acronyms, and machine interpretable structure notation in the form of InChI and SMILES to prevent ambiguity in chemical identifier.(Place & Ragland, 2022)

Additional information regarding the design and infrastructure of the database is provided in more detail in Ragland & Place 2024 and in the User Guide (https://pages.nist.gov/dimspec/docs).

A cartoon diagramming the overall conceptual structure of the DIMSpec project is provided (**Figure 1**), as is the full entity relationship diagram of the underlying database schema in the supporting documentation.

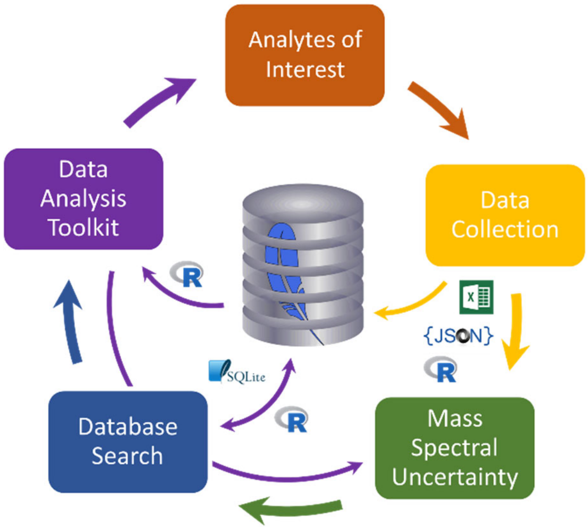


**Figure 1. Conceptual Diagram of the DIMSpec Toolkit**

### 4.0.3 Non-Targeted Analysis Method Reporting Tool (NTA-MRT)

To assist with annotation of chemical identity and observed fragmentation patterns, a macro-enabled Microsoft Excel workbook was developed that "allows for the controlled ontology of method data reporting and the export of the data into a single concise, human-readable file, written in a standard JavaScript Object Notation (JSON)." To annotate the data intended for inclusion in a DIMSpec database, users fill out this workbook by annotating features of interest and associated fragmentation identities. This tool is freely available via GitHub and instructions for completing it are contained within the workbook itself (https://github.com/usnistgov/NISTPFAS/tree/main/methodreportingtool).

### 4.0.4 Quality Assessment of Imported Data (DIMSpec-QC)

A DIMSpec mass spectral database incorporates empirical mass spectral data from analytical standards and complex mixtures with relevant analytical method metadata and mass spectral annotation. Algorithms were developed in R to validate the quality of new experimental data for import into a DIMSpec database, specifically to ensure that annotations "made sense" when applied to the spectra collected. For ease of use, the DIMSpec Quality Control (DIMSpec-QC) application was developed that incorporates the R functions into a web application powered by the Shiny package.(Chang et al., 2024) Scripts for automated setup are included, and DIMSpec-QC installs along with the project. The primary purpose of the DIMSpec-QC application is to apply quality control checks to NTA-MRT-generated files which may then be imported using functions from the toolkit.

### 4.0.5 Development of Data Analysis Tools for DIMSpec (MSMatch)

The Mass Spectral Match for Non-Targeted Analysis (MSMatch) application was built to accelerate non-targeted analysis projects by searching experiment result data in mzML format for matches against a curated mass spectral library of compounds and annotated fragments. MSMatch is a web application built using the Shiny package in R and installs alongside DIMSpec. Every effort has been made to make MSMatch as intuitive for users as possible. The data analysis tools include two specific approaches to identifying PFAS: the compound match tool and the fragment match tool.

*Compound match*

The compound matching function of MSMatch examines experimental mass spectra (unknowns) against mass spectra known to the attached DIMSpec database. The compound matching algorithm can be used to search against all mass spectra or against mass spectra with precursor $m/z$ values within an acceptable range. An evaluation of match score uncertainty is also provided. The calculation of mass spectral uncertainty and estimation of the distribution of the match scores is described in Place 2021.

*Fragment match*

In addition to the compound match function, the fragment matching function provides information regarding the individual fragments of the input mass spectra (unknowns). This can be used in combination with the compound match function; in addition, this application can be used when there are no good compound matches to provide substructure information. Fragments measured within the feature of interest will be matched against database fragments with known annotations. Population of the Database with Reference Mass Spectra

Data produced internally and acquired externally were incorporated into the database. As of this report, 351 consensus reference mass spectra representing 132 individual PFAS are included. This list is accurate only as of this report, as community members are beginning to submit spectra for consideration; DIMSpec databases are intended to grow over time to provide increased value.

## 4.1 TASK 2: ESTABLISHMENT OF A WORLD-CLASS QAP AIMED AT DEVELOPING AND QUALIFYING ANALYTICAL LABORATORIES FOR THE DETECTION AND IDENTIFICATION OF PFAS IN ENVIRONMENTAL MATRICES.

### 4.1.1 Development of Education and Training Tools for DIMSpec

With the public release of DIMSpec and the publication of the Ragland & Place 2024, NIST researchers generated a document to provide users with an understanding on the installation and development of the DIMSpec infrastructure and the use of the associated applications.

The first form of education and training was presented as a detailed user guide, which is publicly available at https://pages.nist.gov/dimspec/docs/index.html. This user guide includes a general introduction and installation instructions for DIMSpec applications. In addition, detailed information for the associated applications (Table Viewer, DIMSpec-QC, and MSMatch) are provided as part of the user guide. In addition to this user guide, a set of quick start guides were developed for DIMSpec and the associated applications to enable more efficient use of the tools.

*Training videos*

While the documentation provided for reference, users identified the value in having additional resources for training on the use of DIMSpec and its associated applications. There were five videos (called "episodes") that were created to communicate the installation and use of DIMSpec and the associated applications. The videos are located on the NIST site at: https://www.nist.gov/programs-projects/and-polyfluoroalkyl-substances-pfas/research/reference-data-and-tools/dimspec. The videos were made available prior to the initiation of the interlaboratory study, to enable the participating laboratories to use the guides and videos to operate DIMSpec and its associated applications for their NTA workflows.

### 4.1.2 Results of PFAS NTAILS

Recruitment of participants (up to 50 laboratories) for the ILS started on February 1, 2024, and ran until March 1, 2024. The three test samples (described previously) were shipped to 34 participating laboratories. Of the participating laboratories, 27 submitted NTA results. Of the laboratories that submitted data, 11 laboratories were from academic institutions, three laboratories were analytical instrument vendors, six laboratories were commercial/contract analytical laboratories, and seven were from government organizations (US Federal, State, and non-US).

For all samples, summary tables include the reporting rates of the top twenty compounds, which is defined as the twenty most frequently reported compounds (highest reporting rates) in each respective sample.

*Sample A*

Sample A was a solution of multiple analytical standards for PFAS. Nearly all PFAS that were intentionally added to Sample A were reported by greater than 70 % of participating laboratories, except for 5:3 fluorotelomer betaine (NISTPFAS003794, 26 % reporting rate). 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.

*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, ten were identified at a Level 1a confidence by at least one laboratory (the confidence levels are described by Charbonnet et al., 2022). No individual PFAS in Sample B was detected by all the participating laboratories.

*Sample C*

Sample C was a methanolic extract of an AFFF-impacted soil, with a single added compound. Of the top twenty identified PFAS, 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. Further targeted analysis of this material would be needed to verify the majority of the PFAS identified in this sample.

*Overall Results*

There is a wide distribution in the number of PFAS identities reported by individual laboratories (**Figure 2**). Notably, for all three samples, no single PFAS was reported by every lab (100 % reporting rate). This result could be due to interlaboratory differences in detecting PFAS or in reporting their identities. Generally, most laboratories reported the presence of those PFAS known to NIST to be present in the samples. All known PFAS were reported at Level 1a confidence by at least one laboratory.

Sample A had the broadest range of reporting rates within the top twenty most commonly 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; where the samples with more PFAS present at detectable concentrations resulted in a greater number of frequently reported PFAS.

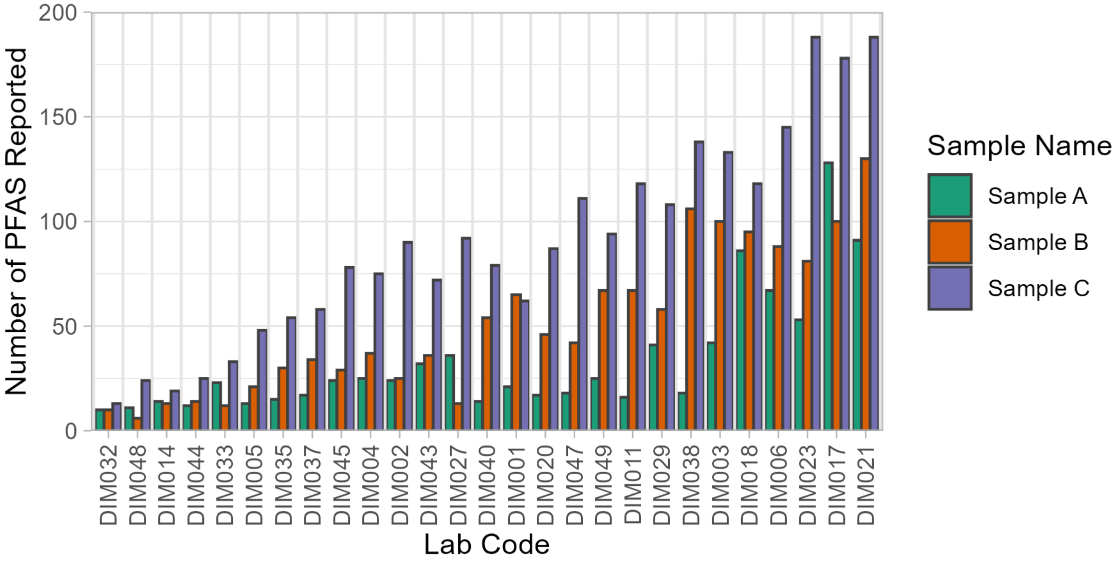


**Figure 2. Total number of PFAS identified (y-axis) for each participating laboratory (x-axis) by the individual samples. Labs are ordered by increasing mean number of PFAS identified.**

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 **Figure 3**. 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 majority of the participating laboratories.

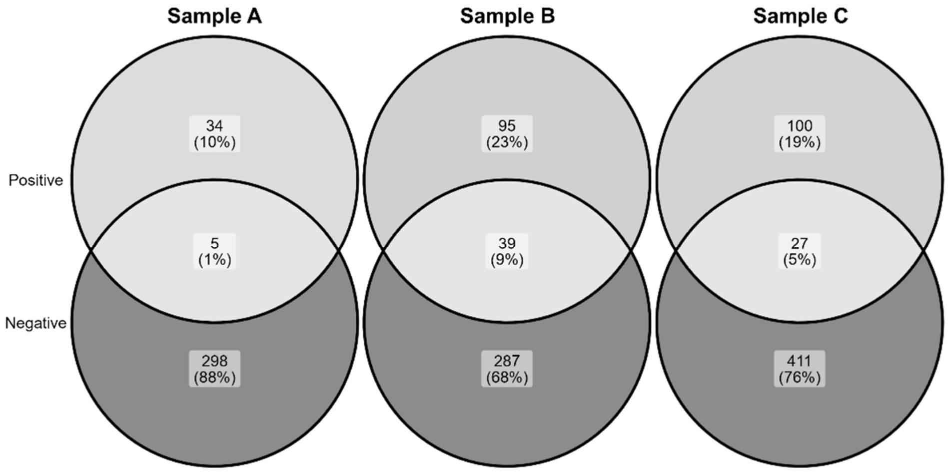


**Figure 3. Venn diagram showing the number of individual PFAS that were reported in each sample and the respective ionization polarities with which they were detected. Top: PFAS identified by positive polarity only; Bottom: PFAS identified by negative polarity only; Middle: PFAS identified by both positive and negative polarity.**

# 5.0 IMPLICATIONS FOR FUTURE RESEARCH AND BENEFITS

## 5.1 DEVELOPMENT OF A COMMON DATABASE STRUCTURE FOR THE DETECTION AND IDENTIFICATION OF PFAS

The presented work resulted in the successful development of an accessible and interoperable database for the access to non-targeted analytical data for the identification of PFAS. As of 15 October 2024, the NIST Suspect List of Possible PFAS has been downloaded 1,373 times by 1,154 unique users and the DIMSpec database has been downloaded 441 times by 430 unique users. These statistics are gathered from the NIST Public Data Repository and do not include downloads directly from Github which does not track such metrics; therefore, it is expected that these values underestimate the total number of downloads and unique users. This work included frequent interactions between NIST and multiple mass spectrometer vendors, which shows promise for the continued interoperability of this database.

## 5.2 ESTABLISHMENT OF A WORLD-CLASS QAP AIMED AT DEVELOPING AND QUALIFYING ANALYTICAL LABORATORIES FOR THE DETECTION AND IDENTIFICATION OF PFAS IN ENVIRONMENTAL MATRICES.

There were 27 laboratories that were able to participate in this interlaboratory study and provide results. Generally, most laboratories were able to identify PFAS that were present in the samples (as confirmed by internal, targeted measurements). Some laboratories reported a larger number of PFAS identities in all samples than most laboratories, including a solution containing a limited number of spiked compounds. While the additional identities cannot be confirmed or denied, it is unlikely that all these compounds are truly present in the sample. The disparity of the number of PFAS identities reported between laboratories may indicate quality assurance/quality control (QA/QC) issues, such as background contamination or a poor understanding of accuracy of individual laboratories' NTA methods. Currently, there are no broadly accepted QA/QC protocols for NTA methods, although community working groups, such as BP4NTA (Place BJ, 2021), are working to identify best practices for QA/QC of NTA methods.

Future work should examine the possibility of developing metrics for estimating true positive rates in unknown samples and the use of blank controls to account for within-laboratory contamination. The intention of this study was to evaluate the NTA workflow, therefore samples were provided as solvent extracts of environmentally relevant matrices. Future work could evaluate laboratories' abilities to extract environmental materials and identify PFAS in the extracts.

## 5.3 IMPLICATIONS FOR FUTURE RESEARCH AND IMPLEMENTATION

For the foreseeable future DIMSpec, the infrastructure itself and the PFAS database, will continue to be freely available on the NIST Public Data Repository and Github. Future updates to the database, including additions of mass spectra, will require continued support of the database, which could occur through internal or external (non-NIST) administration and maintenance.

There could be new applications of DIMSpec to answer additional research questions, including forensic source attribution of PFAS pollution or novel non-PFAS contaminants in environmental matrices. The infrastructure was designed to be flexible and enable a wide variety of analytical and sample information to address new research questions and measurement challenges. The use of DIMSpec demonstrates the value of open and free data to support government, academia, and industry laboratories and should encourage other database developers to adopt similar approaches for data accessibility.

# 6.0 OBJECTIVE

A relatively new capability to many laboratories, non-targeted analysis (NTA) is a general category of analytical chemistry techniques aimed at identifying chemicals in complex mixtures without the use of purified chemical standards. This allows for richer chemical analysis and greater characterization of samples, offering likely identities on a scale several orders of magnitude greater than classical targeted techniques where chemicals are identified with direct matches to purified standards. However, significant barriers to implementation have been identified as analytical laboratories attempt to rapidly include NTA techniques into existing analytical workflows. These barriers include limited availability of high-quality reference mass spectra and poor between-vendor interoperability of generated mass spectra, among others. Together, these two specific barriers significantly limit identification and reporting of probable PFAS. In addition, there is limited information on the comparability of individual laboratory performance using NTA for the identification of PFAS.

The primary objective of this research was to enable analytical laboratories to better identify novel PFAS by improving access to high-quality reference mass spectra and providing a comprehensive quality assurance program (QAP) for PFAS identification via NTA. These objectives were accomplished through two overarching tasks:

> *Task 1*: Development of a common database structure for the detection and identification of PFAS.
>
> *Task 2*: Establishment of a world-class QAP aimed at developing and qualifying analytical laboratories for the detection and identification of PFAS in environmental matrices.

This research addressed the SERDP 2020 Statement of Need number ERSON-20-C5, "Forensic Methods for Source Tracking and Allocation of Per- and Polyfluoroalkyl Substances" by supplying "spectral libraries of PFAS to include both AFFF-derived PFAS as well as PFAS derived from other sources (i.e., consumer products, utilization in industrial manufacturing processes, landfill leachate, etc.)." Through use of the database, analytical laboratories will have enhanced capabilities to confidently identify unknown PFAS and provide evidence of material origins. With continued support, this effort can be consistently maintained and administered in support of the statement of need.

# 7.0 BACKGROUND

## 7.0 PRESENCE OF PFAS IN THE ENVIRONMENT FROM UNKNOWN SOURCES

Per- and polyfluoroalkyl substances (PFAS) are a large class of anthropogenic chemicals with unique properties (Kissa, 2001), a subset of PFAS are known to be persistent, bioaccumulative, and/or toxic to humans and the environment (see Bartell & Vieira, 2021; Evich et al., 2022; and Fenton et al., 2021 and sources therein). Depending on the definition, there can be over 10,000 chemicals that are considered PFAS.(Buck et al., 2011; Gaines et al., 2023; OECD, 2021) Currently, there are regulations for six PFAS in drinking water (40 CFR § 141 (2024), 40 CFR § 142 (2024) and two PFAS are designated as hazardous substances by CERCLA (40 CFR § 302 (2024)).

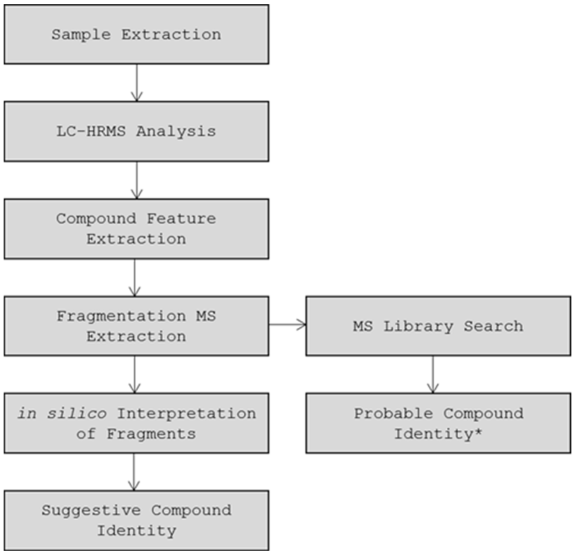
Most research on the environmental and human health relevance of PFAS has been limited to a subset of PFAS, including perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). Proposed regulations within the European Union consider a broader range of PFAS beyond those regulated in the US. The possibility of class and/or structure-based regulations can drive the need for more non-specific detection tools to detect a larger number of chemicals.

Over a decade of research has demonstrated the existence of PFAS beyond PFCAs and PFSAs in environmental materials due to a range of sources, including the release of aqueous film-forming foams used for fighting liquid fuel-based fires.(Backe et al., 2013; Barzen-Hanson et al., 2017) While the other PFAS may not be directly regulated, there is evidence demonstrating that many PFAS can transform into PFAS of concern in the environment. This brought about the rise of the term “PFAS Precursors” to indicate chemicals that transform into perfluoroalkyl acids (PFAAs) through abiotic and biological processes.(Ruyle et al., 2023)

## 7.1 ROLE OF NON-TARGETED ANALYSIS FOR THE IDENTIFICATION OF PFAS

Non-targeted analysis (NTA) is a category of techniques that intend to identify chemicals in a sample with limited-to-no *a priori* knowledge regarding the chemical composition and quantities within the sample. This includes the identification of previously unknown chemicals in environmental samples where no chemical standards exist for performing conventional targeted, quantitative analysis. There could be thousands of PFAS that could exist in the environment, and chemical standards are limited to around100 PFAS, therefore the use of NTA can be useful for the detection and identification of PFAS in a wide range of samples. In fact, NTA techniques have been routinely applied to materials containing PFAS and PFAS-impacted environmental samples over the past decade, resulting in the identification of PFAS previously unknown in the environment.(Barzen-Hanson et al., 2017; Place & Field, 2012)

Previous studies using NTA to identify PFAS elucidated the chemical structure through interpretation of analytical data and other supporting information (such as patents). While this approach can be used to identify truly novel PFAS, it is time-consuming and can be limited in confidence of identification, structural elucidation by HRMS typically is limited to suggestive or possible chemical identities. Comparison of fragmentation mass spectra between reference mass spectra of known compounds provides one of the highest levels of confidence in compound identification. **Figure 4** demonstrates an example NTA workflow for the identification of a chemical compound. This approach is limited to the availability of reference mass spectra. Conventionally, libraries containing reference mass spectra are developed based on generated mass spectra using chemical standards, but there are not as chemical standards as the number of possible PFAS.



*Probable Compound Identity is dependent upon the library containing a mass spectrum for the compound to be identified.

**Figure 4. Example workflow for NTA. *In silico* interpretation involves computational approaches to predict the possible chemical compound structure and/or identity of an unknown.**

## 7.2 IMPROVING DATA ACCESS TO ENABLE NON-TARGETED ANALYSIS FOR PFAS

Open access to data has been a priority within the federal government; the Federal Chief Data Officer Council has presented specific recommendations to making data more usable by other federal agencies and the public (https://www.cdo.gov/data-sharing/). This effort includes improving the interoperability of data – the ability to use the data between different users (and instruments) – and the accessibility of the data by reducing the barriers for other users to utilize the data.

The mass spectral data produced from individual laboratories' analytical instrumentation are generated in a vendor-specific proprietary data format. This can include lists of chemical suspects and reference mass spectra. This condition limits the ability of individual laboratories to share their data for use by other laboratories, especially those that have different vendor instruments. It is important to understand the current needs and predict future needs for data use, which include the systematic collection of metadata that is relevant for current data use or could be relevant for future data investigations. A future database, the first objective of the presented study, will need to be interoperable and accessible to provide impactful data and tools that enables better NTA for PFAS identification.

# 8.0 MATERIALS AND METHODS

## 8.0 INTERNAL GENERATION OF REFERENCE MASS SPECTRA

### 8.0.1 Standards and Materials

All solvents (LCMS grade) and reagents used for sample preparation and analysis were purchased from Thermo Fisher Scientific (Waltham, MA).

*Standard mixture solutions*

Standards used for all standard mixture solutions were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Mixture solutions, PFAC30PAR, PFAC-MXG, and FTA-MXA, were analyzed directly without additional sample preparation. Single component solutions containing 5:3 FTB, 5:1:2, FTB, N-AP-FHxSA, N-TAmP-FHxSA, and N-CMAmP-6:2FOSA were combined into a single solution by 1:50 (by volume) dilution in methanol. All reference mass spectra generated through analysis of the standard mixtures are listed in **Table 17** in the **Appendix A**.

*PFAS150 single component solutions*

Additional solutions were acquired as part of a material transfer agreement (MTA) with the U.S. Environmental Protection Agency (USEPA), entitled "Per- and Polyfluoroalkyl Substance (PFAS) Standards". The set of PFAS solutions will be referred to as the EPA PFAS150 standard set in this report. The USEPA PFAS150 standard set contains 141 individual PFAS in solution that were generated by a chemical manufacturer, many of the PFAS are not commercially available otherwise.

Through the agreement, the EPA PFAS150 samples were shipped overnight and were received on November 16, 2022. The plastic boxes with vials were immediately transferred to a -20 °C freezer until sample preparation. There were approximately 150 µL of sample in each of 142 vials (one of the vials was a blank ethanol solution). Each vial was weighed and 1 mL (exact mass known) of methanol was added to the vial. As the mass of the solution in the vial was not known, the concentrations of the stock solutions are considered nominal and could not be used for quantitative analysis. For analysis by LC-HRMS, all samples were volumetrically diluted 1:21 (by volume) in methanol.

While all 141 individual PFAS solutions were screened using a preliminary flow-injection analysis method with no chromatographic column, not all PFAS were detectable by the ionization modes available and therefore only a subset of the 141 individual PFAS solutions were used to generate reference mass spectra. All reference mass spectra generated through analysis of the PFAS150 solutions are listed in **Table 17** in the **Appendix A**.

### 8.0.2 Analytical Methods

*Liquid chromatography*

Chromatographic separation was performed using an UltiMate 3000 Liquid Chromatograph (Thermo Fisher Scientific, Waltham, MA). The autosampler was maintained at 7 °C, the injection volume was 1 µL for QC samples and 5 µL for standards and methanol blanks. The liquid chromatography column was an Agilent Poroshell EC-C18 (Agilent Technologies, Santa Clara, CA) with dimensions 3.0 mm inner diameter, 50 mm length, and 2.1 µm particle diameter and the column was maintained at 40 °C for the entirety of the run. Separation was performed with two different mobile phase sets, 0.1 % (by volume) formic acid in water (A) and 0.1 % (by volume) formic acid in methanol (B) for positive ionization mode and 10 mM (by mass) ammonium acetate in water (A) and 10 mM (by mass) ammonium acetate in methanol (B). The flow rate was 0.450 mL/min for the entirety of the run.

*Chromatographic separation of standard mixture solutions*

As the standards were mixtures of PFAS, a long chromatographic separation was used. The column was equilibrated for 5 min prior to injection at 5 % B. After injection, the mobile phase was held at 5 % B for 1 min before it was increased linearly to 95 % B over 15 min, held at 95 % B for 10 min before returning to the starting composition. The post-column flow was diverted to waste for the first 2 min of the run.

*Chromatographic separation of PFAS150 single component solutions*

As the standards were single component solutions, a short chromatographic separation was used. The column was equilibrated for 2.5 min prior to injection at 5 % B. After injection, the mobile phase was held at 5 % B for 1 min before it was increased linearly to 95 % B over 9 min, held at 95 % B for 5 min before returning to the starting composition. The post-column flow was diverted to waste for the first 0.1 min of the run.

*Mass spectrometry*

Mass spectrometric analysis was performed using a Thermo Fisher Scientific Q-Exactive hybrid mass spectrometer. Data was collected using MS1/DD-MS2 experiment for all ionization types using similar settings (**Table 1**). For the MS1 scan, the resolution was 70,000, AGC target 3e6 ions, Maximum IT 100 ms, scan range 100 *m/z* to 1500 *m/z*. For the data-dependent scan, the resolution was 17,500, AGC target 1e5, maximum IT 50 ms, TopN count 5, isolation window of 0.7, minimum AGC target 5e3 ions, and intensity threshold 1e5. For each ionization combination, an inclusion list was added to only fragment those compounds observed in the specified ionization mode.

**Table 1. Relevant mass spectrometer parameters for the generation of reference mass spectra.**

| **Ionization Type** | **Electrospray Ionization** | | **Atmospheric Pressure Chemical Ionization** | |
|---|---|---|---|---|
| **Ionization Polarity** | Positive | Negative | Positive | Negative |
| **Spray Voltage (kV)** | 5 | -5 | | |
| **Spray Current (µA)** | | | 5 | -5 |
| **Probe Heater Temperature (°C)** | 350 | | | |

| Ionization Type | Electrospray Ionization | Atmospheric Pressure Chemical Ionization |
|---|---|---|
| **Capillary Temperature (°C)** | 380 | |
| **Sheath Gas** | 60 | |
| **Auxiliary Gas** | 20 | |
| **Spare Gas** | 10 | |

### 8.0.3 Quality Control Protocols

To verify instrument performance and data quality, a standard quality control protocol was developed and applied throughout the generation of the reference mass spectra. Quality control solutions consisting of volumetric dilutions of RM 8446 (vial 1) and RM 8447 in methanol (for negative ionization) and known gravimetric preparation of 9 compounds in methanol (for positive ionization) were analyzed at least twice within an analytical sequence during reference mass spectra generation. Analysis of quality control parameters (**Table 2**) was performed using an R Markdown document specifically made for analysis of the NIST instrumental data.

**Table 2. Quality control parameters for the generation of reference mass spectra.**

| Parameter | Description | Metric Calculation | Recommended Boundary |
|---|---|---|---|
| mass accuracy | the accuracy of the measured *m/z* value. | Mean mass accuracy error (ppm) between known and measured *m/z* of each individual compound in all QC samples. | 5 ppm |
| retention time | time since injection that the chromatographic peak apex passes the detector. | Standard deviation of retention times of each individual compound in all QC samples | 0.1 min |
| peak width | Width of the chromatographic peak. | Standard deviation of the peak width of each individual compound in all QC samples. | 0.1 min |
| peak area | Integrated area under the chromatographic peak | Standard deviation of the peak area of each of the individual compounds in all QC samples. | 30 % |
| peak asymmetry | Asymmetry of the chromatographic peak, as determined by the | Standard deviation of peak asymmetry values of each individual compound in all QC samples. | 3 |

| Parameter | Description | Metric Calculation | Recommended Boundary |
|---|---|---|---|
| | ratio of the left (A) and right (B) peak width. | | |
| MS1 matchscore | Match score between measured precursor ion isotopic pattern and *in silico* isotopic pattern. | Standard deviation of the MS1 match score of each individual compound in all QC samples. Match scores are calculated as the dot product of the two mass spectra (measured and predicted isotopic patterns). | 0.01 |
| MS2 matchscore | Match score between measured fragmentation mass spectrum and mean fragmentation mass spectrum. | Standard deviation of the MS2 match score of each individual compound in all QC samples. Match scores are calculated as the dot product of the two mass spectra (measured and mean fragmentation mass spectra). | 0.05 |

### 8.0.4 Generation of Reference Mass Spectra

Chromatographic peaks for the specific PFAS in each analyzed solution were manually inspected using Thermo Fisher Scientific Xcalibur Qual Browser to determine retention time, start time, end time, height, and area.

For database searching and fragment annotation, all raw data files were converted from vendor-specific proprietary data format (*.raw) to an open-source format (*.mzML) using Proteowizard msConvert (Adusumilli & Mallick, 2017) with the following settings: peak picking (vendor) for MS levels 1-2, and thresholding (absolute) for values greater than 1.

All sample, method, compound, and fragment annotation information for each sample was recorded using the Non-Targeted Analysis-Method Reporting Tool (NTA-MRT) to generate Sample JSON files. The NTA-MRT is further described in **Section 9.0.4** and the current version is publicly available at https://github.com/usnistgov/NISTPFAS under the “methodreportingtool” directory.

## 8.1 EXTERNAL ACQUISITION OF REFERENCE MASS SPECTRA

Additional reference mass spectra were generated using data from previously published, peer-reviewed literature. Specifically, the Dr. Jennifer Field laboratory at Oregon State University provided the data from Barzen-Hanson et al., (2017). Only data produced from AFFFs and technical solutions were used to generate the reference mass spectra. All reference mass spectra generated through this dataset are listed in **Table 17** in **Appendix A**.

## 8.2 TEST MATERIAL DEVELOPMENT FOR A NON-TARGETED ANALYSIS INTERLABORATORY STUDY

### 8.2.1 Standards and Materials

LCMS grade methanol was purchased from Fisher Scientific (Burdick & Jackson Brand LC-MS; Hampton, NH). PFAS calibration solutions used for the preparation of two test solutions (**Table 3**) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Historic commercial formulations of two aqueous film-forming foams (AFFFs) were acquired through the US Naval Research Laboratory, the individual identities of these AFFFs are not included in this report. AFFF-impacted soil was acquired from DoD locations with known AFFF releases. Pre-scored 2-mL clear glass ampoules were used for the packaging of the materials (Duran Wheaton Kimble 2 mL Gold Band Ampule Pre-scored, PN: 176776, Millville, NJ).

**Table 3. List of calibration solutions used to generate the test solutions.**

| Solution | Components | Lot number | Nominal concentration in solution (µg/mL) |
|---|---|---|---|
| 5:3 FTB | 2-[(4,4,5,5,6,6,7,7,8,8,8-Undecafluorooctyl)dimethylammonio]acetate (5:3FTB), CASRN: 171184-14-8 | 53FTB1121 | 50 |
| 8Cl-PFOS | Sodium 8-chloroperfluoro-1-octanesulfonate, CASRN: 2481740-05-8 | 8CLPFOS1118 | 50 |
| N-AP-FHxSA | N-(3-dimethylamino)propyl)-perfluorohexyl-1-sulfonamide, CASRN: 50598-28-2 | NAPFHXSA1121 | 50 |
| PFAC-MXA | mixed perfluoroalkyl acids (C4-C10 perfluorocarboxyclic acids (PFCA); C4, C6, C8 perfluorosulfonic acids (PFSA)) | PFACMXA0922 | 5 |

### 8.2.2 Test Material Preparation

Three test solutions were prepared gravimetrically. The first solution (**Sample A**) was created using gravimetric dilutions of PFAS calibration solutions in methanol; the masses of solutions and final concentrations of the solutions are shown in **Table 4**. The solution was stored in a 250 mL polypropylene bottle at 4 °C until packaging.

**Table 4.** **Mass of specific solutions and solvents added for Sample A, including nominal concentrations of specific components.**

| Solution/Solvent | Mass added (g) | Nominal concentration in solution (µg/g) |
|---|---|---|
| 5:3 FTB | 0.0974 | 0.1013 |
| 8Cl-PFOS | 0.1059 | 0.1101 |
| N-AP-FHxSA | 0.1146 | 0.1192 |
| PFAC-MXA | 0.8910 | 0.0927 |
| methanol | 46.8710 | N/A |

The second solution (**Sample B**) was created using gravimetric dilution of two AFFF formulations in methanol; the masses of the formulations and final concentrations of the solutions are shown in **Table 5**. The solution was stored in a 250 mL polypropylene bottle at 4 °C overnight and was filtered using a Whatman ashless 40 filter (150 mm diameter, P/N 1440-150, GE Healthcare Life Sciences, Marlborough, MA) into a new 250 mL polypropylene bottle. In previous experiments, storage at 4 °C of AFFF solution was found to precipitate out salts. Therefore, filtration was used as a preventative measure. The filtered solution was stored at 4 °C until packaging.

**Table 5.** **Mass of AFFF formulations and solvents added for Sample B, including nominal concentrations of specific components.**

| Formulation/Solvent | Mass added (g) | Nominal concentration of AFFF in solution (µg/g) |
|---|---|---|
| AFFF #1 | 0.18436 | 939.8 |
| AFFF #2 | 0.21958 | 1119.4 |
| methanol | 195.76 | N/A |

The third solution (**Sample C**) was created by extracting an AFFF-impacted soil into methanol. A grab sample comprised of a random sub-sample, of the bulk soil materials. Approximately 400 g (exact mass known) of the soil was added to a 2 L polypropylene bottle. Then 600 mL (exact mass known) of methanol was added to the bottle. The bottle was shaken briefly to mix thoroughly and sonicated for 30 min using VWR Symphony ultrasonicator (Radnor, PA). The solution was allowed to settle for 30 min at room temperature and then was filtered using a Whatman ashless 40 filter into a new 2 L polypropylene bottle. The solution was stored at 4 °C until further processing. On June 1, 2023, the solution was transferred to two Turbovap vessels and concentrated under nitrogen gas in a Turbovap II (Zymark, Hopkinton, MA, NIST Property Number 558101) set to 60 °C to approximately 20 mL in each vessel (40 mL total). The concentrated solution was transferred to a new 250 mL polypropylene bottle and additional methanol was added first to the turbovap vessel then added to the new bottle to create a total volume of approximately 60 mL (exact mass known). In addition, a 200 µL aliquot (exact mass known) of PFAS calibration solution N-AP-FHxSA was added to the bottle. As there were visible solids in this solution, the solution was filtered using a Whatman ashless 40 filter into a new 250 mL bottle. The filtered solution was stored at 4 °C until packaging. The measured masses of soil and solutions used to create Sample C are shown in **Table 6** and **Table 7**.

**Table 6. Mass of soil and solvents added for Sample C for extraction.**

| Sample/Solvent | Mass added (g) |
|---|---|
| AFFF-impacted soil | 402.10 |
| methanol | 457.65 |

**Table 7. Final mass of Sample C and mass of solution added, including nominal concentrations of specific components.**

| Formulation/Solvent | Mass added (g) | Nominal concentration of components in solution (µg/g) |
|---|---|---|
| soil extract | 44.46911 | N/A |
| N-AP-FHxSA | 0.12190 | 0.1367 |

### 8.2.3 Test Material Packaging

All solutions were ampuled on June 1, 2023. Using the Ampulmatic-10 filling and sealing machine (Monera Technologies Corporation, Allentown, PA), the ampoules were filled with approximately 1 mL of solution, then the headspace was purged with argon (10 psi or 69 kPa) and flame sealed. Due to solution volume limitations, ampoules of Sample A and Sample C were filled by hand using an automatic pipettor set to 1 mL. Ampoules of Sample B were filled using the Ampulmatic-10 filling system, set to fill with approximately 1 mL of solution. All ampoules were placed into boxes in order of filling (front to back, left to right). Boxes of ampoules were stored in the refrigerator (4 °C) until labeling and shipment.

# 9.0 RESULTS AND DISCUSSION

## 9.0 TASK 1: DEVELOPMENT OF A COMMON DATABASE STRUCTURE FOR THE DETECTION AND IDENTIFICATION OF PFAS

### 9.0.1 Establishment of the NIST PFAS Suspect List of Possible Per- and Polyfluoroalkyl Substances

Within the conceptual umbrella of non-targeted analysis (NTA), the use of a list or database of possible chemical targets (*suspects*) is known as suspect screening analysis (SSA). There are a wide variety of chemical databases that contain a multitude of suspect compounds, including PubChem (https://pubchem.ncbi.nlm.nih.gov/; Kim et al., 2023), ChemSpider (https://www.chemspider.com/; Pence & Williams, 2010), and the United States Environmental Protection Agency (EPA; https://ror.org/03tns0030) CompTox Chemicals Dashboard v2.4.1 (CompTox) (https://comptox.epa.gov/dashboard/; Williams et al., 2017). While these databases can be useful for SSA, they are often limited to well-studied chemical compounds or are dependent upon users to submit chemical compounds, which often leads to inconsistent data entries. In the realm of SSA for PFAS, there are several thousand chemical compounds that are theoretically possible, but most have limited information or have not been observed empirically. Many of these compounds are therefore not included in well-curated databases due to a lack of information. An automated protocol requiring limited input values was developed to address this data gap and provide a more robust, and easily updated, suspect list for PFAS, called the “NIST List of Possible Per- and Polyfluorinated Alkyl Substances (PFAS)” or the “NIST PFAS Suspect List” for short. The suspect list is provided to the community via a NIST public data repository (Place, 2021) that is maintained by the NIST PFAS Program so that it may be easily available, updateable, and version controlled.

Aggregation of the initial suspect list used two primary sources of data: the Organization for Economic Co-operation and Development PFAS (PFASOECD) list from the EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/chemical-lists/PFASOECD) and the PFAS XIC (extracted ion chromatogram) List provided by Dr. Christopher Higgins (Colorado School of Mines; ORCID: 0000-0001-6220-8673; chiggins@mines.edu).

The first source was the EPA CompTox Chemicals Dashboard list of PFASOECD chemicals (accessed on March 1, 2021), which has been curated by the EPA from the OECD Global Database for PFAS (OECD, 2018). To suit the purposes of building a mass spectral list of unambiguous, individual PFAS with known chemical structures, it was refined by removing all instances with entries deemed multicomponent, isotopically heavy, or lacking chemical structures. Properties were extracted from that list to obtain DTXSID (a CompTox-specific database identifier), Chemical Name, and MS-Ready SMILES structure (McEachran et al., 2018). All compounds lacking MS-Ready SMILES structures represented compounds without a single defined structure (e.g., a mixture of two or more compounds) and therefore were removed. When stereoisomers with matching MS-Ready SMILES were identified, the entry with no stereospecificity was preferred and other instances were removed. If there was no compound without stereospecificity, a new entry was created with the stereospecificity removed. This was done as the MS-Ready SMILES structures do not support notation of stereospecificity. This resulted in 3,098 PFAS compounds with structures. For the initial suspect list, the MS-Ready SMILES structures were converted to

InChI using OpenBabel (https://openbabel.org/). Compounds from this list have SOURCE_TYPE designated as “C” (Curated; see below).

The PFAS XIC List was a Microsoft Excel workbook curated by the Higgins Lab at the Colorado School of Mines (https://ror.org/04raf6v53). Source information was systematically replaced with DOI, ISBN, or websites and had SOURCE_TYPE values reflecting the provided source data. Some structures were generated internally using ChemDraw (version 19.1; Revvity Signals Software, Inc., Waltham, MA) other structures were provided via email from Drs. P. Lee Ferguson and Gordon Getzinger of Duke University.

The merged list contained a variety of information regarding 2,155 compounds but only 1,038 with assigned chemical structures applicable to these purposes. Of retained information (**Table 8**), the minimum input information required for inclusion in the NIST Suspect List (column names from the NIST Suspect List are in parentheses) included:

1) Compound name (*NAME*) with no regulation on format;
2) InChI structural notation (*INCHI*);
3) Source information for the structure, such as DOI, ISBN, or website link (*SOURCE*); and
4) Single letter representing the type of source used for structure (*SOURCE_TYPE*), as described in **Table 9**.

Using the combined PFASOECD Chemicals List from CompTox and the PFAS XIC List, there were 3,895 compounds that passed validation and were included in the suspect list. An additional 241 compounds required expert review; most were duplicate occurrences or contained local negative charges. Duplicate occurrences were inspected to verify duplication and duplicates were removed. Structures with local negative charges were redrawn in ChemDraw to remove the local negative charge and the InChI, SMILES, InChI Key, elemental formula, and monoisotopic mass were adjusted accordingly. After this review process, the initial suspect list contained 4,041 individual PFAS structures.

Since the initial release of the PFAS Suspect List, several additional PFAS structures have been contributed by individual laboratories. As of release of this report, the current PFAS Suspect List (v1.7, https://data.nist.gov/od/id/mds2-2387) contains 4,967 individual PFAS structures.

### Table 8. Retained Properties in the NIST List of Possible PFAS

| **Property** | Description | Required |
|---|---|---|
| NAME | User input name of the compound, there are no formal rules for nomenclature | X |
| INCHI | IUPAC International Chemical Identifier (InChI) notation for the chemical structure | X |
| SOURCE | Citation for the source of the compound structural information | X |
| SOURCE_TYPE | Type of citation used for the source of the compound structural information | X |
| ADDITIONAL | An aggregation of all additional columns provided by the input user | |
| SMILES | Simplified molecular-input line-entry system (SMILES) structural notation | |
| INCHIKEY | Hashed form of the InChI structure | |
| FIXEDHINCHI | InChI Key notation with fixed hydrogen layer (non-standard) | |
| LOCAL_POS | Number of atoms with a fixed positive charge state | |
| LOCAL_NEG | Number of atoms with a fixed negative charge state | |
| FORMULA | Elemental formula of the structure | |
| FIXEDMASS | Exact mass of the fixed elemental formula, including charge state | |
| NETCHARGE | Formal charge of the structure | |
| DTXSID | EPA DSS Tox Substance Identifier | |
| DTXCID | EPA DSS Tox Compound Identifier | |
| CASRN | Chemical Abstracts Service registry number | |
| PUBCHEMID | PubChem ID for the InChI Key structure, multiple IDs are separated by semi-colons (;) | |
| INSPECTEDBY | The initials of the user that manually evaluated the structure and the associated data, NA indicates that the structure has not been manually evaluated | |

**Table 9. Controlled Vocabulary for the SOURCE_TYPE column in Table 8**

| Value | Type | Definition |
|---|---|---|
| C | Curated | Compound structure, name, and other properties have been evaluated through a documented process for a database or library. |
| E | Empirical | Structure has been measured and derived empirically through a peer-reviewed process. |
| I | Inferred | Structure that is a homolog (differing in CF2 chain lengths) of an empirically derived structure but has not been observed empirically. |
| S | In Silico | Structure has been predicted through documented in silico processes but has not been observed empirically. |
| D | Documented | Structure has been reported in documentation (e.g., patent, safety data sheet), but has not been observed empirically. |
| L | Limited | Limited information to support structure, only a formula exists |

### 9.0.2 Development of a Database Infrastructure for Mass Spectrometry (DIMSpec)

A database toolkit was developed to provide an infrastructure for the management and use of mass spectrometry data and associated metadata. This toolkit was named the Database Infrastructure for Mass Spectrometry (DIMSpec), with this project's specific application for PFAS, although the database will be solely referred to as DIMSpec for the rest of this report. In addition, as part of a NIST-wide effort to make data more compliant with the FAIR (Findable, Accessible, Interoperable, and Reusable) principles (Wilkinson et al., 2016), the database and affiliated tools were designed using open-source formats that can be easily shared and reused by laboratories within and outside of NIST. The information provided in this report includes an overview of guidance for the setup, population, and use of DIMSpec databases and its affiliated toolkit. Complete instructions and more detail are available in the User Guide (https://pages.nist.gov/dimspec/docs), which will be updated as the project continues.

This toolkit is freely and openly available from GitHub (https://github.com/usnistgov/dimspec) either by fork, clone, or download. The currently published version (v1.1.0-202409) includes the described database as part of an R project, as the bulk of the functionality is built in the R language. Initial installation does require an internet connection to download software installers and dependencies; on a system which does not contain any required software components this can take a considerable amount of time. The underlying database engine is SQLite (https://www.sqlite.org/index.html; v3.43+ as deployed at NIST as of this report), a lightweight relational database engine which has been broadly supported and available to a wide array of data tools for more than two decades.

At the time of this report, the DIMSpec toolkit is based on R (v4.3+) and includes all functionality necessary to create and interact with DIMSpec databases connecting mass spectral data and metadata regarding the samples and instrument conditions under which those spectra were collected. It includes:

1. an example mass spectral database, in this case the DIMSpec for PFAS (described below);
2. functions necessary to create and reuse such databases;
3. functions necessary to interact with such databases from the R programming language;
4. integration of a Python environment containing a chemometrics platform (RDKit v2021.09.4 https://www.rdkit.org) for chemical informatics and expanded integration with existing workflows;
5. a RESTful application programming interface (API) to allow additional programmatic communication over a network for expanded utility and interactive API documentation;
6. three web applications to assist with use of the toolkit; and
7. full documentation, including of R functions provided as part of the toolkit.

The database schema for DIMSpec databases is described in detail in the User Guide, and a simplified version is provided in this report in **Appendix A**. The utility of DIMSpec for NTA was greatly enhanced by providing controlled vocabulary in terms of normalization tables, and all chemical entities are cross-linked with additional validated names (where available) such as database identifiers from CompTox and PubChem, common acronyms, and machine interpretable structure notation in the form of InChI and SMILES to prevent ambiguity in chemical identifier (Place & Ragland, 2022).

Additional information regarding the design and infrastructure of the database is provided in more detail in (Ragland & Place, 2024) and in the User Guide (https://pages.nist.gov/dimspec/docs).

The first database created using the DIMSpec toolkit was seeded with compounds on the NIST Suspect List described in **Section 9.0.1**. Mass spectra for these compounds (generation of which is described in **Sections 8.0** and **8.1**) were then annotated using the Non-Targeted Analysis Method Reporting Tool (NTA-MRT, described in **Section 9.0.3**). Quality control checks were applied using the DIMSpec-QC application (described in **Section 9.0.5**) and resulting JSON files used to populate the database.

A cartoon diagramming the overall conceptual structure of the DIMSpec project is provided (**Figure 5**), as is the full entity relationship diagram of the underlying database schema in the supporting documentation.

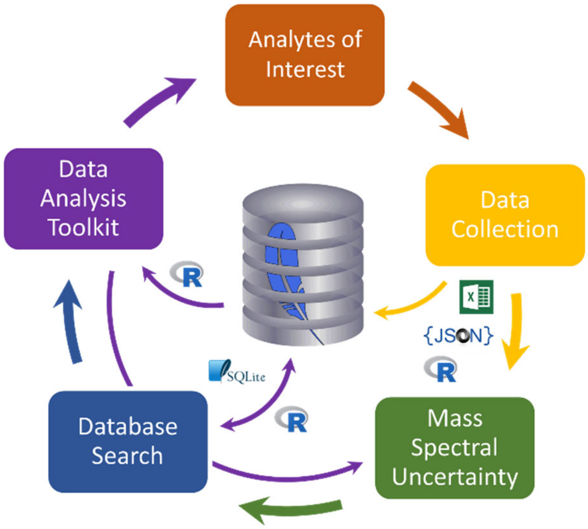


Figure 5. Conceptual Diagram of the DIMSpec Toolkit

### 9.0.3 Conversion of Instrument Files to an Open Format

For use with the DIMSpec toolkit, instrument vendor produced data files must first be converted into an open format. Currently, the open source mzML format is the most widely applicable, and Proteowizard’s msConvert software (Adusumilli & Mallick, 2017) can convert most current vendor formats into mzML. This provides an ability to apply a single transformation platform for files from multiple vendor formats into a single open format. Most aspects of DIMSpec projects rely first on conversion of vendor files into the mzML format.

### 9.0.4 Non-Targeted Analysis Method Reporting Tool (NTA-MRT)

To assist with laborious annotation of chemical identity and observed fragmentation patterns, a macro-enabled Microsoft Excel workbook was developed that “allows for the controlled ontology of method data reporting and the export of the data into a single concise, human-readable file, written in a standard JavaScript Object Notation (JSON).” To annotate data for inclusion in a DIMSpec database, users fill out this workbook by annotating features of interest and associated fragmentation identities. Generated method files in JavaScript Object Notation (JSON) format are then submitted alongside the mzML file containing measured mass spectra. After quality control checks are performed, the resulting JSON object contains all necessary data to perform an import into a DIMSpec database.

This tool is freely available via GitHub and instructions for completing it are contained within the workbook itself (https://github.com/usnistgov/NISTPFAS/tree/main/methodreportingtool). After a user has completed the NTA-MRT, clicking the “Export to JSON file output” button on the first tab creates JSON files for each sample describing the annotations, sample metadata, and analytical metadata stored by a DIMSpec database.

Note: the file name entered in the NTA-MRT under the Sample tab must **exactly match** (case-sensitive) the paired mzML file name.

### 9.0.5 Quality Assessment of Imported Data (DIMSpec-QC)

A DIMSpec mass spectral database incorporates empirical mass spectral data from analytical standards and complex mixtures with relevant analytical method metadata and mass spectral annotation. Algorithms were developed in R to validate the quality of new experimental data for import into a DIMSpec database, specifically to ensure that annotations "made sense" when applied to the spectra collected. For ease of use, the DIMSpec Quality Control (DIMSpec-QC) application was developed that incorporates the R functions into a web application powered by the Shiny package (Chang et al., 2024). Scripts for automated setup are included, and DIMSpec-QC installs along with the project. The primary purpose of the DIMSpec-QC application is to apply quality control checks to NTA-MRT-generated files which may then be imported using functions from the toolkit.

*Application of DIMSpec-QC*

There are two types of JavaScript Object Notation (JSON) files used for the data import and quality control process:

**Sample JSON:** these files (conventionally labeled "[mzmlfilename]_mzml_sample.JSON") are exported from the NTA-MRT and contain sample, method, and compound information related to a paired mzML file.

**Peak JSON:** these files are exported from the DIMSpec-QC application (conventionally labeled "[mzmlfilename]_mzml_cmpd[compoundreferencenumber].JSON") and extend the sample JSON files with mass spectral data related to the specified compound.

Seven individual quality control checks are included in the current version. Different checks are represented by different parameter names. The QC checks with their named parameters are as follows:

> measurederror: is the reported precursor ion *m/z* value within the reported instrumental error of the calculated precursor ion *m/z* of the designated compound? This calculation uses the instrument relative mass error contained in Sample JSON file, the absolute minimum mass error (default: 0.01 Da) in the QC Settings of the DIMSpec-QC application, and the monoisotopic mass of the designated compound in the DIMSpec database.
>
> ms1_isotopepattern: does the MS1 isotopic pattern of the submitted data match the calculated isotopic pattern with a match score above an expected value? This calculation uses the Minimum MS1 isotopic match score (default: 0.5), the lower MS1 window value (default: 1), and the upper MS1 window value (default: 4) in the QC Settings of the DIMSpec-QC application.
>
> ms1precursor_detected: is the reported precursor ion *m/z* value present in the MS1 mass spectrum of the submitted data? This calculation uses the instrument relative mass error contained in Sample JSON file and the absolute minimum mass error (default: 0.01 Da) in the QC Settings of the DIMSpec-QC application.

annfragments_detected: are the reported annotated fragment ion *m/z* values present in the MS1 mass spectrum of the submitted data? This calculation uses the instrument relative mass error and annotated fragment ion list contained in Sample JSON file and the absolute minimum mass error (default: 0.01 Da) in the QC Settings of the DIMSpec-QC application.

annfragment_accuracy: are the reported annotated fragment ion *m/z* values value within the reported instrumental error of the fragment ion *m/z* of the designated fragment, calculated from the elemental formula? This calculation uses the instrument relative mass error and fragment elemental formulas contained in the Sample JSON file and the absolute minimum mass error (default: 0.01 Da) in the QC Settings of the DIMSpec-QC application.

annfragments_subset: are the reported annotated fragment elemental formulas a subset of the elemental formula of the designated compound? For example, is the fragment "C3F7" a subset of the designated compound elemental formula "C8F15O2H"? In this example, the result would be true. This calculation uses the elemental formula contained in the Sample JSON file and the elemental formula of the designated compound in the DIMSpec database.

annfragment_elementalmatch: if there is a SMILES structure provided for an annotated fragment, does the elemental formula of the SMILES structure match the elemental formula provided for the same annotated fragment. This calculation uses the SMILES structure and the elemental formula contained in the Sample JSON file.

optimized_ums_parameters: this is not a quality check but occurs during the same data processing step. The optimized settings for the uncertainty mass spectrum of the MS1 and MS2 data is calculated for import into the DIMSpec database. The parameters used for this calculation are set on the DIMSpec-QC application under the QC Settings page and use the function optimal_ums to determine the optimized parameters.

After assessment of quality using DIMSpec-QC, the quality control parameters are associated with the individual compounds via the Peak JSON file. These Peak JSON files can be imported, with all affiliated metadata, into the DIMSpec database. Data are not rejected based on any quality criteria, but the result of each check is stored in the database, associated with each peak imported.

### 9.0.6 Importing Data into a DIMSpec Database

Once data are collected, converted to the .mzML format, annotated by the NTA-MRT, and quality control flags are applied by DIMSpec-QC, mass spectra can be imported to a DIMSpec database using functions defined in the toolkit. As of this report, and in recognition that data workflows may vary extensively between investigating laboratories, the only import routine currently provided is a command line tool, and it requires files generated by the NTA-MRT, mentioned above.

Field mapping is defined in a comma-separated-value file which contains a list of import file elements and their properties, with connections for each to their destination tables and columns within the database schema; individual elements are resolved by toolkit functions which perform much of the transformation. This allows for programmatic manipulation of the associated database schema mapping between input files and the database. New maps can be created and used in support of other import formats in the future, and though the import functions are heavily parameterized they may need to be customized for other workflows.

### 9.0.7 Development of Data Analysis Tools for DIMSpec (MSMatch)

The Mass Spectral Match for Non-Targeted Analysis (MSMatch) application was built to accelerate non-targeted analysis projects by searching experiment result data in mzML format for matches against a curated mass spectral library of compounds and annotated fragments. MSMatch is a web application built using the Shiny package in R and installs alongside DIMSpec. It is a prime example tools that can built on top of the DIMSpec tool set.

Every effort has been made to make MSMatch as intuitive for users as possible. Hints in the form of tooltips are available throughout; hover over question mark icons or controls to see them. These can be toggled on and off at any time using the “Show Tooltips” toggle button at the bottom left of the application window. If enabled, advanced search settings can be similarly toggled on and off for the session (see Application Settings for instructions on how to set default accessibility and settings for tooltips and advanced settings). The “hamburger” (≡) icon at the top left of the screen will collapse the left-hand navigation panel to provide more horizontal room on smaller screens, though the application will rearrange itself when screens are smaller than a minimum width.

For now, this application is distributed for demonstration and evaluation with an implementation of NIST DIMSpec containing high resolution accurate mass spectrometry data for per- and polyfluorinated alkyl substances (PFAS).

Users can upload experiment mzML data files and input features of interest for searching against the DIMSpec PFAS Database. The MSMatch application serves two primary functions, compound match and fragment match.

*Compound match*

The compound matching function of MSMatch examines experimental mass spectra (unknowns) against mass spectra known to the attached DIMSpec database. The compound matching algorithm can be used to search against all mass spectra (Search Type: All) or against mass spectra with precursor *m/z* values within an acceptable range (Search Type: Precursor Search). In most cases the “Precursor Search” option should remain selected; the “All” option takes a considerable amount of time and may yield poor matches as every known spectrum is compared. The “Use Optimized Search Parameters” checkbox will utilize a set of predefined properties for known compounds to accelerate the search; uncheck this box to perform a wider search.

Narrative results are provided regarding the top match and the match currently being viewed, including a method summary for how the reference was measured. The spectral comparison is visualized in a "butterfly plot" showing measurements in black and the comparison (database) spectrum in red; toggle the different fragmentation levels (e.g. MS1 vs MS2) to view those independently.

Evaluation of match score uncertainty is also provided. Results from a bootstrapped version of the match algorithm are displayed as boxplots for both forward and reverse matches. The calculation of mass spectral uncertainty and estimation of the distribution of the match scores is described in Place, 2021.

*Fragment match*

In addition to the compound match function, the fragment matching function provides information regarding the individual fragments of the input mass spectra (unknowns). This can be used in combination of the compound match function; in addition, this application can be used when there are no good compound matches to provide substructure information. Fragments measured within the feature of interest will be matched against database fragments with known annotations. Mass spectrum with a high number of measured m/z values generally completes within 30 seconds, yielding results to indicate possible compound identity.

Matched fragment annotations and associated metadata are provided below this output. Match records are in the expandable table to the left. As matches may have structural annotation or not, these are separated to indicate confidence and annotations with structural notation are displayed at the top. The results of the match fragment algorithm provide the following information regarding specific annotated fragments:

- A human readable measurement narrative about the known fragment.
- If structural notation is present a molecular model is displayed
- Compounds and peaks within which this fragment has been previously annotated appear in the table to the right. Select the tab to switch between compounds and peaks.

The MSMatch application provides a new way to make NTA tools developed at NIST, using DIMSpec, more accessible to stakeholders. This application is intended to supplement current laboratories' workflows rather than replace them entirely.

### 9.0.8 Population of the Database with Reference Mass Spectra

Data produced internally and acquired externally (see Sections 8.0 and 8.1) were incorporated into the database. As of this report, 351 consensus reference mass spectra representing 132 individual PFAS are included. The list of PFAS and number of reference mass spectra are shown in **Table 17** in the Supporting Data. This list is accurate only as of this report, as community members are beginning to submit spectra for consideration; DIMSpec databases are intended to grow over time to provide increased value.

## 9.1 TASK 2: ESTABLISHMENT OF A WORLD-CLASS QAP AIMED AT DEVELOPING AND QUALIFYING ANALYTICAL LABORATORIES FOR THE DETECTION AND IDENTIFICATION OF PFAS IN ENVIRONMENTAL MATRICES.

### 9.1.1 Development of Education and Training Tools for DIMSpec (Task 2A)

*User guide to DIMSpec and its associated applications*

With the public release of DIMSpec and the publication of the DIMSpec paper (Ragland & Place, 2024), NIST researchers generated a document to provide users with an understanding on the installation and development of the DIMSpec infrastructure and the use of the associated applications.

The first form of education and training was presented as a detailed user guide, which is publicly available at https://pages.nist.gov/dimspec/docs/index.html. This user guide includes a general introduction and installation instructions for DIMSpec applications. In addition, detailed information for the associated applications (Table Viewer, DIMSpec-QC, and MSMatch) are provided as part of the user guide. All functions and algorithms that utilize R were documented within the scripts. This information is also accessible within the User Guide documentation at: https://pages.nist.gov/dimspec/docs/appendix-function-reference.html. As DIMSpec was designed to be extensible to many research questions outside of PFAS, this User Guide is generalized for a broad number of applications.

In addition to this user guide, a set of quick start guides were developed for DIMSpec and the associated applications to enable more efficient use of the tools. These quick start guides are easily available for download from the User Guide and include:

- DIMSpec Quick Guide – Installation
  - https://pages.nist.gov/dimspec/docs/quick_install.pdf
- DIMSpec Quick Guide – Plumber
  - https://pages.nist.gov/dimspec/docs/quick_plumber.pdf
- DIMSpec Quick Guide - Web Applications
  - https://pages.nist.gov/dimspec/docs/quick_apps.pdf
- DIMSpec Quick Guide - Advanced Use
  - https://pages.nist.gov/dimspec/docs/quick_advanced.pdf
- DIMSpec Quick Guide - Importing Data
  - https://pages.nist.gov/dimspec/docs/quick_import.pdf
- File Conversion using msconvert
  - https://pages.nist.gov/dimspec/docs/file_convert.pdf

*Training videos*

The documentation noted above was provided for reference; NIST researchers created additional resources for training on the use of DIMSpec and its associated applications. There were five videos (called “episodes”) that were created to communicate the installation and use of DIMSpec and the associated applications. The videos are located on the NIST site at:

https://www.nist.gov/programs-projects/and-polyfluoroalkyl-substances-pfas/research/reference-data-and-tools/dimspec. As of August 27, 2024, the introduction video (Episode 1: Download and Setup) had 129 plays since its initial release on February 29, 2024.

The videos were made available prior to the administration of the interlaboratory study, to enable the participating laboratories to use the guides and videos to operate DIMSpec and its associated applications for their NTA workflows.

### 9.1.2 Interlaboratory Study: Application of DIMSpec (Task 2B)

NIST has been administering interlaboratory studies (ILS) for over 30 years for a wide variety of programs, including environmental measurements. For a successful ILS, there are three parts: (1) development of the test samples; (2) sample shipment to and analysis by analytical laboratories; (3) submission and analysis of the results. Where Task 2A focuses on the development of a program to engage and educate analytical laboratories on the use of DIMSpec, Task 2B focuses on demonstrating the application of DIMSpec by running an ILS. The content of the following sections has been previously published in a NIST Interagency Report and is available at: https://doi.org/10.6028/NIST.IR.8544.

Three samples were created to represent an increasing amount of material complexity and were comprised of a variable number of PFAS (Section 3.3.2). Samples were shipped to participants around March 6, 2024. During the execution of the PFAS NTAILS, the NIST PFAS Team routinely responded to inquiries which ranged from general operation of DIMSpec to interpretation of result data.

Participating laboratories were required to submit results using a Microsoft Excel workbook that included method information and a standardized format for reporting identified PFAS, which included reporting the PFAS identity (using the NIST Suspect List of Probable PFAS identifier), instrumental analysis information (e.g., retention time and measured *m/z*), and level of confidence using the PFAS Confidence of Identification scale (Charbonnet et al., 2022). The results reporting workbook is available at https://data.nist.gov/od/id/mds2-3518 with the file name “PFASNTAILS_Reporting Form.xlsx”.

- **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 10**.

**Table 10. 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 |
| 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. Due to previous targeted analysis of the AFFF formulations, the known PFAS in Sample B are listed in **Table 11.**

**Table 11. PFAS known to be present in Sample B prior to the ILS.**

| PFAS Preferred Name | Acronym | NIST ID |
|---|---|---|
| Perfluorohexane sulfonamido amine | N-AP-FHxSA | NISTPFAS000878 |
| Perfluorobutanoic acid | PFBA | NISTPFAS002649 |
| Perfluorohexanoic acid | PFHxA | NISTPFAS002643 |
| Perfluoroheptanoic acid | PFHpA | NISTPFAS002640 |
| Perfluorooctanoic acid | PFOA | NISTPFAS002637 |
| Perfluoropropanesulfonic acid | PFPrS | NISTPFAS003492 |
| Perfluorobutanesulfonic acid | PFBS | NISTPFAS003045 |
| Perfluoropentanesulfonic acid | PFPeS | NISTPFAS003044 |
| Perfluorohexanesulfonic acid | PFHxS | NISTPFAS003043 |
| Perfluoroheptanesulfonic acid | PFHpS | NISTPFAS003042 |
| Perfluorooctanesulfonic acid | PFOS | NISTPFAS003041 |
| 6:2 fluorotelomer sulfonic acid | 6:2 FTS | NISTPFAS003052 |
| Perfluorobutanesulfonamide | FBSA | NISTPFAS000009 |
| Perfluorohexanesulfonamide | FHxSA | NISTPFAS002560 |

| | | |
|---|---|---|
| N,N,N-trimethyl-3-[[(1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluorohexyl)sulfonyl]amino]-1-Propanaminium | N-TAmP-FHxSA | NISTPFAS003633 |
| 6:2 Fluorotelomer sulfonamide betaine | 6:2 FTAB | NISTPFAS003736 |

- **Sample C** was a methanolic extract of an AFFF-impacted soil amended with an analytical standard of a single PFAS in methanol. Due to previous targeted analysis of the soil material and the added PFAS solution, the known PFAS in Sample C are listed in **Table 12.**

**Table 12. PFAS known to be present in Sample C prior to the ILS.**

| PFAS Preferred Name | Acronym | NIST ID |
|---|---|---|
| Perfluorohexane sulfonamido amine | N-AP-FHxSA | NISTPFAS000878 |
| Perfluorohexanoic acid | PFHxA | NISTPFAS002643 |
| Perfluoroheptanoic acid | PFHpA | NISTPFAS002640 |
| Perfluorooctanoic acid | PFOA | NISTPFAS002637 |
| Perfluoropentanesulfonic acid | PFPeS | NISTPFAS003044 |
| Perfluorohexanesulfonic acid | PFHxS | NISTPFAS003043 |
| Perfluoroheptanesulfonic acid | PFHpS | NISTPFAS003042 |
| Perfluorooctanesulfonic acid | PFOS | NISTPFAS003041 |
| Perfluorononanesulfonic acid | PFNS | NISTPFAS003040 |
| Perfluorodecanesulfonic acid | PFDS | NISTPFAS003039 |
| Perfluorohexanesulfonamide | FHxSA | NISTPFAS002560 |
| Perfluorooctanesulfonamide | FOSA | NISTPFAS002559 |

### 9.1.3 Description of Interlaboratory Study Participating Laboratories

Recruitment of participants (up to 50 laboratories) for the ILS started on February 1, 2024, and ran until March 1, 2024. During this time, 32 laboratories registered for the ILS. After the recruitment period ended two additional laboratories asked to participate in the ILS. Since there were still openings for the ILS, these laboratories were allowed to participate. Laboratories were asked to define their research sector (Academic Institutions, Analytical Instrument Vendors, Commercial/Contract Analytical Laboratory, or Governmental Organizations). Laboratories from all four research sectors registered for the ILS.

Overall, samples were shipped to 34 participating laboratories, 27 of which submitted results. Of the laboratories that submitted data, eleven laboratories were from academic institutions, three laboratories were analytical instrument vendors, six laboratories were commercial/contract analytical laboratories, and seven were from government organizations (US Federal, State, and non-US) (**Figure 6**). 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. Any updates or corrections needed to be submitted by July 19, 2024, for inclusion in the final report. Results in the following sections were analyzed as provided by participants.

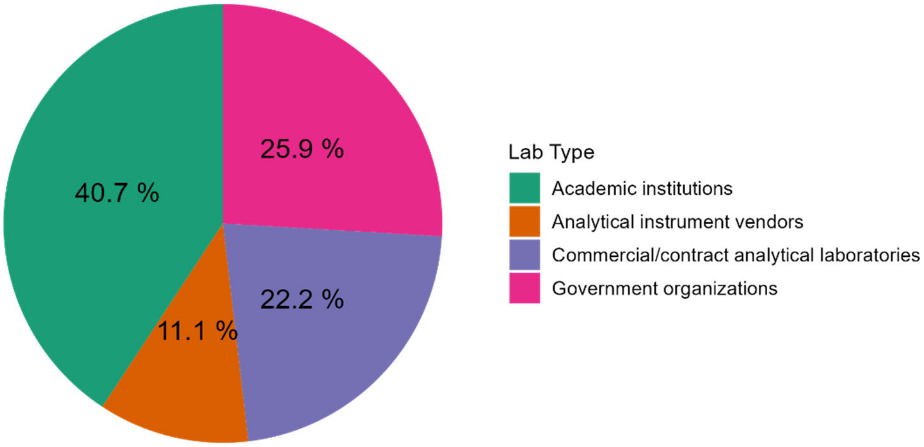


**Figure 6. Composition of the participating laboratories that submitted results, values within each sector are the percentage of the total.**

Most 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 13**).

**Table 13. 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.

### 9.1.4 Results of PFAS NTAILS

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:

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

For all samples, summary tables include the reporting rates of the top twenty compounds, which is defined as the twenty most frequently reported compounds (highest reporting rates) in each respective sample.

#### 9.1.4.1 Sample A

Sample A was a solution of multiple analytical standards for PFAS. Nearly all PFAS that were intentionally added to Sample A (**Table 14**) 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 (**Table 14**).

Of the top twenty reported PFAS identifications (**Table 14**), 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 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 (**Figure 7**) 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 14. Top 20 most frequently 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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **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 |
| **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 |
| **NISTPFAS002635 - Perfluorononoic 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 |
| **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 |
| **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 |
| **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 |
| **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 |
| **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 |  |
| **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 |
| **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 |
| **NISTPFAS002646 - Perfluoropentanoic acid** |  |  | 2a | 2c | 1a |  | 1a | 1a | 1a | 2b | 3d | 1a | 1a | 1a | 1a |  |  | 1a | 1a | 1a | 1a | 3b | 1a |  | 2a | 1a |
| **NISTPFAS002649 - Perfluorobutanoic acid** | 2a |  | 2a | 2c | 1a |  | 1a | 1a | 2a |  | 2a | 1a | 1a | 1a | 1a |  |  | 1a | 1a | 1a | 1a | 4 |  |  | 2a | 1a |
| NISTPFAS000852 - Perfluorohexane sulfomido amine oxide |  | 2c | 4 | 2b |  | 2c |  |  | 4 |  |  |  | 1a |  | 4 |  |  |  | 3a |  | 3c | 3b |  |  |  |  |
| NISTPFAS002560 - Perfluorohexanesulfomide |  |  | 2b |  | 1b |  | 2b |  | 1a | 2b | 2a |  |  | 1a |  |  |  | 1a | 1b |  |  |  |  |  | 2a |  |
| **NISTPFAS003794 - 5:3 Fluorotelomer betaine** |  |  | 2a |  |  |  |  |  |  |  | 3c |  |  |  |  |  |  |  | 1a | 2a |  | 4 |  |  | 2a |  |
| NISTPFAS004053 - Dihydrogen-substituted fluoro triether tridecanoic acid |  |  | 4 |  |  | 4 |  |  | 2c |  | 4 | 4 |  |  |  |  |  |  |  |  |  |  |  | 3d |  |  |
| NISTPFAS004634 - Dihydrogen-substituted fluoro triether undecanoic acid |  |  | 4 |  |  | 4 |  |  | 2c |  | 4 | 4 |  | 5a |  |  |  |  |  |  |  |  |  |  |  |  |
| NISTPFAS002338 - 1H-Perfluoronone |  |  | 4 |  |  |  |  |  |  |  |  |  |  |  | 5b |  | 2b |  |  |  |  | 2a |  | 3d |  |  |
| NISTPFAS002342 - 8H-Perfluorooctane |  |  | 4 |  |  | 4 |  |  |  |  |  |  |  |  | 5b |  |  |  |  |  |  | 2a |  | 3d |  |  |
| NISTPFAS002350 - 1,1,1,2,2,3,3,4,4,5,5-Undecafluoropentane |  |  | 4 |  |  |  |  |  |  |  |  |  |  |  | 5b |  | 2c |  |  |  |  | 2c |  | 3d |  |  |

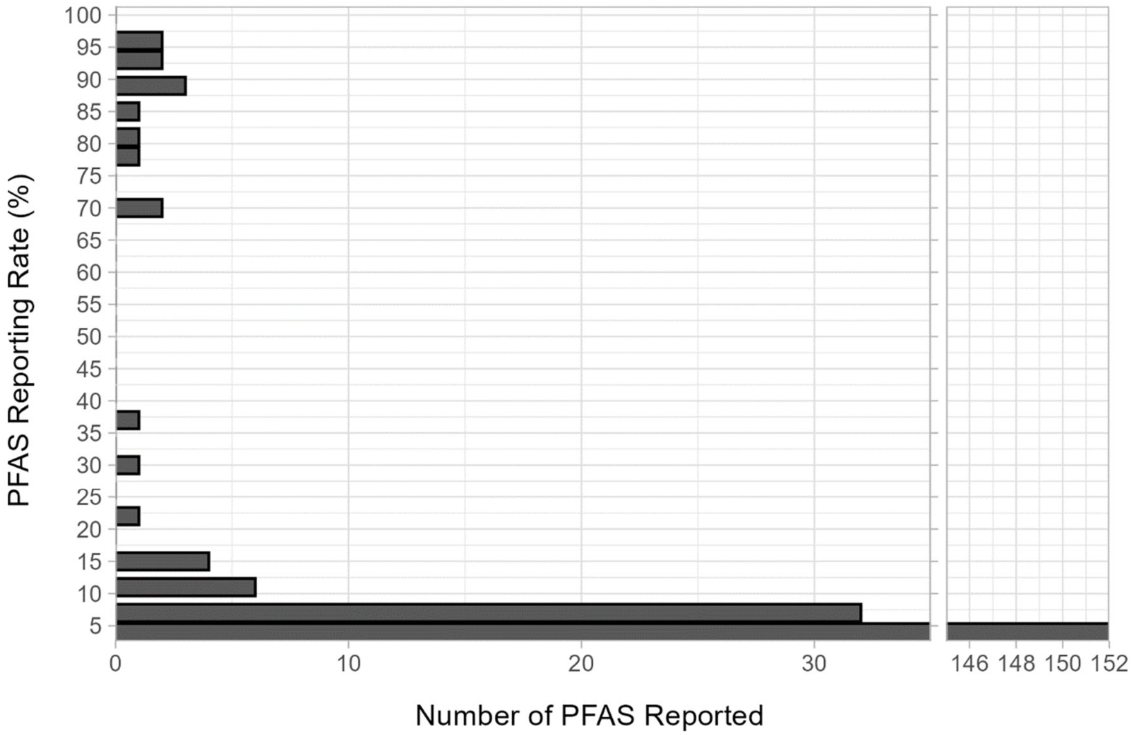


**Figure 7.** **Histogram of PFAS reported with Level 3 or higher confidence in Sample A by individual laboratories, showing the number of PFAS (x-axis) at specific reporting rates (y-axis). For example, 152 PFAS identities were reported only once among the 27 participating laboratories (least reporting rate of 3.7 %), while 2 PFAS identities were reported 26 times among the 27 participating laboratories (greatest reporting rate of 96.3 %).**

#### 9.1.4.2 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 15**), 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.

There were 116 PFAS reported once among all participating laboratories with Level 3 confidence or higher (**Figure 8**). 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.

able 15.   Top 20 most frequently reported PFAS in Sample B. For each laboratory (column) that reported the specif
AS (row), the identification confidence reported is presented. If a value is blank, the participant did not report the PFAS
esent. The final two columns show the highest reported confidence among all labs for each PFAS and the reporting rate
h PFAS among all laboratories. Rows with bold text and highlighted gray represent compounds known to be present in
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** | **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** | **1a** | **2a** | **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** | **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** | **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% |

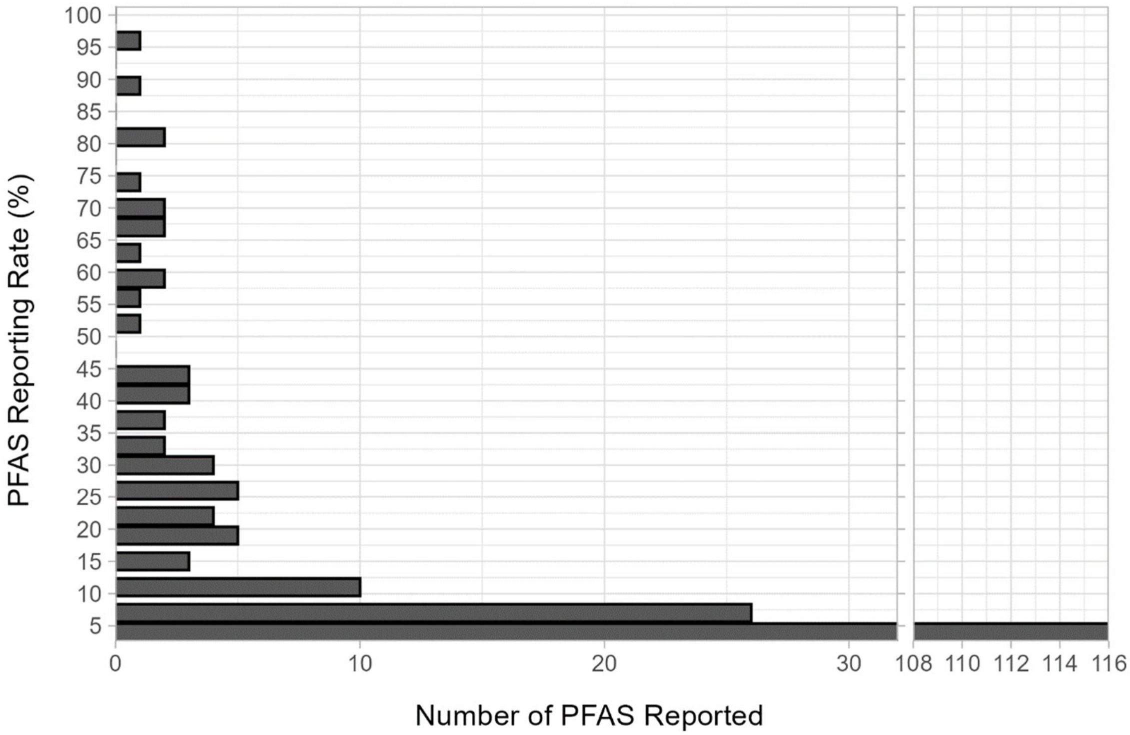


**Figure 8. Histogram of PFAS reported with Level 3 or higher confidence in Sample B by individual laboratories, showing the number of PFAS (x-axis) at specific reporting rates (y-axis). For example, 116 PFAS identities were reported once the 27 participating laboratories (reporting rate of 3.7 %), while 1 PFAS identity were reported 26 times among the 27 participating laboratories (reporting rate of 96.3 %).**

### 9.1.4.3 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 **Figure 9**. 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.

There were 147 PFAS reported once among all participating laboratories with Level 3 confidence or higher (**Table 16**). 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.

**able 16. Top 20 most frequently reported PFAS in Sample C. For each laboratory (column) that reported the specif AS (row), the identification confidence reported is presented. If a value is blank, the participant did not report the PFAS esent. The final two columns show the highest reported confidence among all labs for each PFAS and the reporting rate h PFAS among all laboratories. Rows with bold text and highlighted gray represent compounds known to be present in 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% |

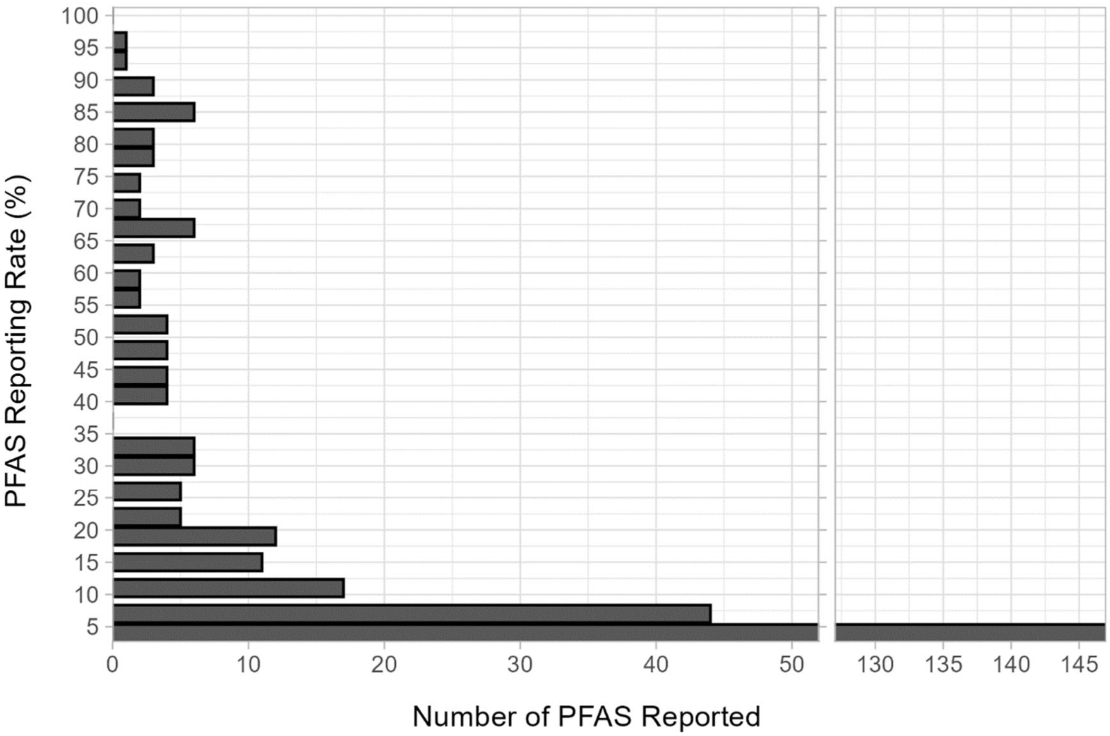


**Figure 9. Histogram of PFAS reported with Level 3 or higher confidence in Sample C by individual laboratories, showing the number of PFAS (x-axis) at specific reporting rates (y-axis). For example, 147 PFAS identities were reported once the 27 participating laboratories (reporting rate of 3.7 %), while 1 PFAS identity was reported 26 times among the 27 participating laboratories (reporting rate of 96.3 %).**

### 9.1.4.4 Overall Results

There is a wide distribution in the number of PFAS identities reported by individual laboratories (**Figure 10**). 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 the presence of those PFAS known to NIST to be present in the samples. All known PFAS were reported at Level 1a confidence by at least one laboratory. For a complete list of all PFAS identified at all confidence levels, see the table provided at: https://doi.org/10.18434/mds2-3518.

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.

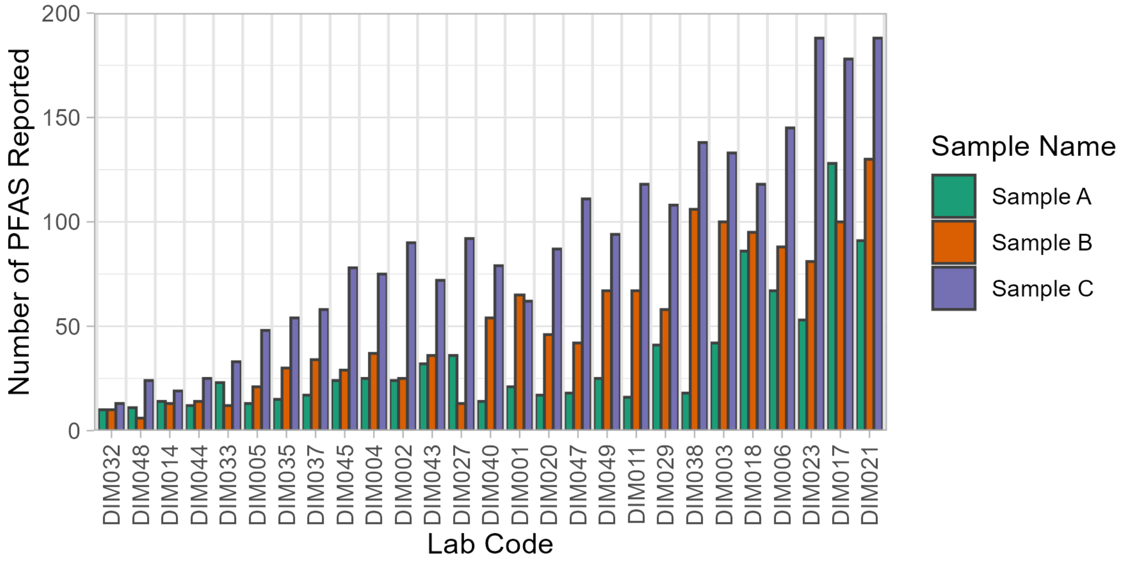


**Figure 10. Total number of PFAS identified (y-axis) for each participating laboratory (x-axis) by the individual samples. Labs are ordered by increasing mean number of PFAS identified.**

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 **Figure 11**. 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 56 % of the participating laboratories.

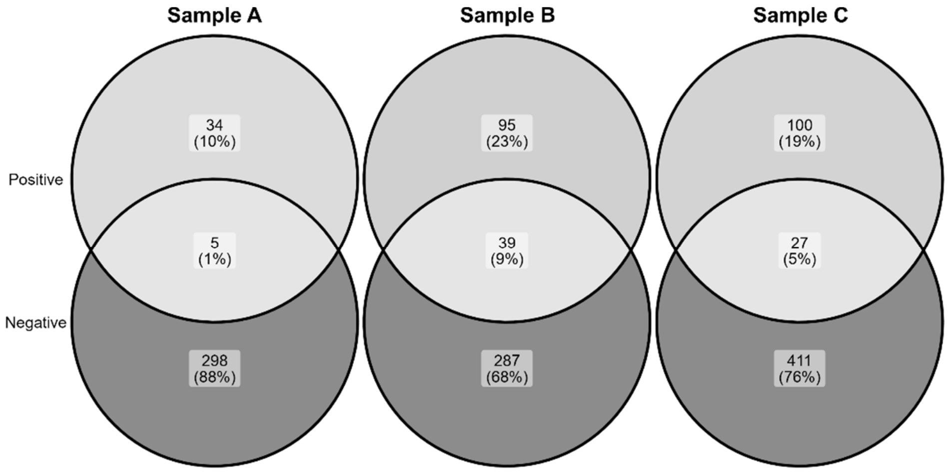


**Figure 11.** **Venn diagram showing the number of individual PFAS that were reported in each sample and the respective ionization polarities with which they were detected. Top: PFAS identified by positive polarity only; Bottom: PFAS identified by negative polarity only; Middle: PFAS identified by both positive and negative polarity.**

# 10.0 CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH / IMPLEMENTATION

## 10.0 DEVELOPMENT OF A COMMON DATABASE STRUCTURE FOR THE DETECTION AND IDENTIFICATION OF PFAS

The presented work resulted in the successful development of an accessible and interoperable database for the access to non-targeted analytical data for the identification of PFAS. As of Friday, 13 September 2024, the NIST Suspect List of Possible PFAS has been downloaded 1,331 times by 1,112 unique users and the DIMSpec database has been downloaded 417 times by 407 unique users. These statistics are gathered from the NIST Public Data Repository and do not include downloads directly from Github which does not track such metrics; therefore, it is expected that these values underestimate the total number of downloads and unique users. This work included frequent interactions between NIST and multiple mass spectrometer vendors, which shows promise for the continued interoperability of this database.

## 10.1 ESTABLISHMENT OF A WORLD-CLASS QAP AIMED AT DEVELOPING AND QUALIFYING ANALYTICAL LABORATORIES FOR THE DETECTION AND IDENTIFICATION OF PFAS IN ENVIRONMENTAL MATRICES.

The successful administration of the PFAS NTAILS demonstrated the interlaboratory comparability of NTA results and allowed for an initial testing of the function of DIMSpec and its associated functions. Participating analytical laboratories had the opportunity to receive hands-on experience with the application of DIMSpec and its associated applications.

There were 27 laboratories that were able to participate in this interlaboratory study and provide results. Generally, most laboratories were able to identify PFAS that were present in the samples (as confirmed through internal, targeted measurements). Some laboratories reported a significant number of PFAS identities in all samples, including a solution containing a limited number of spiked compounds. While the additional identities cannot be confirmed or denied, it is unlikely that all these compounds are present in the sample. The disparity of the number of PFAS identities reported between laboratories may indicate quality assurance/quality control (QA/QC) issues, such as background contamination or a poor understanding of accuracy of individual laboratories' NTA methods. Currently, there are no broadly accepted QA/QC protocols for NTA methods, although community working groups, such as BP4NTA (Place BJ, 2021), are working to identify best practices for QA/QC of NTA methods.

Future work should examine the possibility of developing metrics for estimating true positive rates in unknown samples. The intention of this study was to evaluate the NTA workflow, therefore samples were provided as solvent extracts of environmentally relevant matrices. Future work could evaluate laboratories' abilities to extract environmental materials and identify PFAS in the extracts.

## 10.2 IMPLICATIONS FOR FUTURE RESEARCH AND IMPLEMENTATION

For the foreseeable future DIMSpec, the infrastructure itself and the PFAS database, will continue to be freely available on the NIST Public Data Repository and Github. Future updates to the database, including additions of mass spectra, will require continued support of the database, which could occur through internal or external (non-NIST) administration and maintenance.

There could be new applications of DIMSpec to answer additional research questions, including forensic source attribution of PFAS pollution or novel non-PFAS contaminants in environmental matrices. The infrastructure was designed to be flexible and enable a wide variety of analytical and sample information to address new research questions and measurement challenges. The use of DIMSpec demonstrates the value of open and free data to support government, academia, and industry laboratories and should encourage other database developers to adopt similar approaches for data accessibility.

# 11.0 LITERATURE CITED

Adusumilli, R., & Mallick, P. (2017). Data Conversion with ProteoWizard msConvert. *Methods in Molecular Biology (Clifton, N.J.)*, *1550*, 339–368. https://doi.org/10.1007/978-1-4939-6747-6_23

Backe, W. J., Day, T. C., & Field, J. A. (2013). Zwitterionic, Cationic, and Anionic Fluorinated Chemicals in Aqueous Film Forming Foam Formulations and Groundwater from U.S. Military Bases by Nonaqueous Large-Volume Injection HPLC-MS/MS. *Environmental Science & Technology*, *47*(10), 5226–5234. https://doi.org/10.1021/es3034999

Bartell, S. M., & Vieira, V. M. (2021). Critical review on PFOA, kidney cancer, and testicular cancer. *Journal of the Air & Waste Management Association*, *71*(6), 663–679. https://doi.org/10.1080/10962247.2021.1909668

Barzen-Hanson, K. A., Roberts, S. C., Choyke, S., Oetjen, K., McAlees, A., Riddell, N., McCrindle, R., Ferguson, P. L., Higgins, C. P., & Field, J. A. (2017). Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater. *Environmental Science & Technology*, *51*(4), 2047–2057. https://doi.org/10.1021/acs.est.6b05843

Buck, R. C., Franklin, J., Berger, U., Conder, J. M., Cousins, I. T., de Voogt, P., Jensen, A. A., Kannan, K., Mabury, S. A., & van Leeuwen, S. P. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integrated Environmental Assessment and Management*, *7*(4), 513–541. https://doi.org/10.1002/ieam.258

Chang, W., Cheng, J., Allaire, J. J., Sievert, C., Schloerke, B., Xie, Y., Allen, J., McPherson, J., Dipert, A., & Borges, B. (2024). *shiny: Web Application Framework for R*. https://shiny.posit.co/

Charbonnet, J. A., McDonough, C. A., Xiao, F., Schwichtenberg, T., Cao, D., Kaserzon, S., Thomas, K. V., Dewapriya, P., Place, B. J., Schymanski, E. L., Field, J. A., Helbling, D. E., & Higgins, C. P. (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

Evich, M. G., Davis, M. J. B., McCord, J. P., Acrey, B., Awkerman, J. A., Knappe, D. R. U., Lindstrom, A. B., Speth, T. F., Tebes-Stevens, C., Strynar, M. J., Wang, Z., Weber, E. J., Henderson, W. M., & Washington, J. W. (2022). Per- and polyfluoroalkyl substances in the environment. *Science*, *375*(6580), eabg9065. https://doi.org/10.1126/science.abg9065

Fenton, S. E., Ducatman, A., Boobis, A., DeWitt, J. C., Lau, C., Ng, C., Smith, J. S., & Roberts, S. M. (2021). Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. *Environmental Toxicology and Chemistry*, *40*(3), 606–630. https://doi.org/10.1002/etc.4890

Gaines, L. G. T., Sinclair, G., & Williams, A. J. (2023). A proposed approach to defining per- and polyfluoroalkyl substances (PFAS) based on molecular structure and formula. *Integrated*

*Environmental Assessment and Management*, *19*(5), 1333–1347. https://doi.org/10.1002/ieam.4735

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2023). PubChem 2023 update. *Nucleic Acids Research*, *51*(D1), D1373–D1380. https://doi.org/10.1093/nar/gkac956

Kissa, E. (2001). *Fluorinated Surfactants and Repellents*. Dekker. https://books.google.com/books?id=xQ1lvQEACAAJ

McEachran, A. D., Mansouri, K., Grulke, C., Schymanski, E. L., Ruttkies, C., & Williams, A. J. (2018). "MS-Ready" structures for non-targeted high-resolution mass spectrometry screening studies. *Journal of Cheminformatics*, *10*(1), 45. https://doi.org/10.1186/s13321-018-0299-2

OECD. (2018). *Summary report on the new comprehensive global database of Per- and Polyfluoroalkyl Substances (PFASs)*. OECD. https://doi.org/10.1787/1a14ad6c-en

OECD. (2021). *Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances: Recommendations and Practical Guidance* (61; p. 45). Organisation for Economic Co-operation and Development. https://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/terminology-per-and-polyfluoroalkyl-substances.pdf

Pence, H. E., & Williams, A. (2010). ChemSpider: An Online Chemical Information Resource. *Journal of Chemical Education*, *87*(11), 1123–1124. https://doi.org/10.1021/ed100697w

Place, B. (2021, April). *Suspect List of Possible Per- and Polyfluoroalkyl Substances (PFAS)*. National Institute of Standards and Technology. https://doi.org/10.18434/MDS2-2387

Place, B. J. (2021). Development of a Data Analysis Tool to Determine the Measurement Variability of Consensus Mass Spectra. *Journal of the American Society for Mass Spectrometry*, *32*(3), 707–715. https://doi.org/10.1021/jasms.0c00423

Place, B. J., & Field, J. A. (2012). Identification of Novel Fluorochemicals in Aqueous Film-Forming Foams Used by the US Military. *Environmental Science & Technology*, *46*(13), 7120–7127. https://doi.org/10.1021/es301465n

Place, B.J., Ulrich, E.M., Challis, J.K., Chao, A., Du, B., Favela, K., Feng, Y.L., Fisher, C.M., Gardinali, P., Hood, A. & Knolhoff, A.M., 2021. An introduction to the benchmarking and publications for non-targeted analysis working group. *Analytical chemistry*, *93*(49), pp.16289-16296. https://doi.org/10.1021/acs.analchem.1c02660

Place, B. J., & Ragland, J. M. (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

Ragland, J. M., & Place, B. J. (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*. https://doi.org/10.1021/jasms.4c00073

Ruyle, B. J., Thackray, C. P., Butt, C. M., LeBlanc, D. R., Tokranov, A. K., Vecitis, C. D., & Sunderland, E. M. (2023). Centurial Persistence of Forever Chemicals at Military Fire Training Sites. *Environmental Science & Technology*, *57*(21), 8096–8106. https://doi.org/10.1021/acs.est.3c00675

Wilkinson, M. D., Dumontier, M., Aalbersberg, Ij. J., Appleton, G., Axton, M., Baak, A., Blomberg, N., Boiten, J.-W., da Silva Santos, L. B., Bourne, P. E., Bouwman, J., Brookes, A. J., Clark, T., Crosas, M., Dillo, I., Dumon, O., Edmunds, S., Evelo, C. T., Finkers, R., … Mons, B. (2016). The FAIR Guiding Principles for scientific data management and stewardship. *Scientific Data*, *3*(1), 160018. https://doi.org/10.1038/sdata.2016.18

Williams, A. J., Grulke, C. M., Edwards, J., McEachran, A. D., Mansouri, K., Baker, N. C., Patlewicz, G., Shah, I., Wambaugh, J. F., Judson, R. S., & Richard, A. M. (2017). The CompTox Chemistry Dashboard: A community data resource for environmental chemistry. *Journal of Cheminformatics*, *9*(1), 61. https://doi.org/10.1186/s13321-017-0247-6

# APPENDIX A: SUPPORTING DATA

**Table 17. List of PFAS With Mass Spectra in the Current DIMSpec for PFAS Database. The number of individual samples that have mass spectra for the respective compound are listed in the *n* column.**

| NIST Suspect ID | CAS # | Chemical Name | *n* |
|---|---|---|---|
| NISTPFAS003041 | 1763-23-1 | Perfluorooctanesulfonic acid | 14 |
| NISTPFAS002637 | 335-67-1 | Perfluorooctanoic acid | 13 |
| NISTPFAS003043 | 355-46-4 | Perfluorohexanesulfonic acid | 13 |
| NISTPFAS003042 | 375-92-8 | Perfluoroheptanesulfonic acid | 12 |
| NISTPFAS003045 | 375-73-5 | Perfluorobutanesulfonic acid | 12 |
| NISTPFAS003044 | 2706-91-4 | Perfluoropentanesulfonic acid | 11 |
| NISTPFAS002643 | 307-24-4 | Perfluorohexanoic acid | 10 |
| NISTPFAS003040 | 68259-12-1 | Perfluorononanesulfonic acid | 7 |
| NISTPFAS003492 | 423-41-6 | Perfluoropropane sulfonate | 7 |
| NISTPFAS003736 | 34455-29-3 | 6:2 Fluorotelomer sulfonamido propyl betaine | 7 |
| NISTPFAS003110 |  | (6E)-1,1,2,2,3,3,4,4,5,5,6,7,8,8,8-pentadecafluorooct-6-ene-1-sulfonic acid | 6 |
| NISTPFAS002559 | 754-91-6 | Perfluorooctanesulfonamide | 5 |
| NISTPFAS002640 | 375-85-9 | Perfluoroheptanoic acid | 5 |
| NISTPFAS003039 | 335-77-3 | Perfluorodecanesulfonic acid | 5 |
| NISTPFAS003052 | 27619-97-2 | 6:2 Fluorotelomer sulfonic acid | 5 |
| NISTPFAS003295 | NOCAS_881085 | Keto-perfluorooctane sulfonate | 5 |
| NISTPFAS003633 |  | N-Trimethylammoniopropyl perfluorohexane sulfonamide | 5 |
| NISTPFAS000706 | 2991-50-6 | 2-(N-Ethylperfluorooctanesulfonamido)acetic acid | 4 |
| NISTPFAS000861 | 141607-32-1 | N-[3-(Dimethylamino)propyl]-N-[(tridecafluorohexyl)sulfonyl]-beta-alanine | 4 |
| NISTPFAS000862 | 172616-04-5 | N-[3-(Dimethylamino)propyl]-N-[(nonafluorobutyl)sulfonyl]-beta-alanine | 4 |
| NISTPFAS000879 | 68555-78-2 | Perfluoropentane sulfonamido amine | 4 |
| NISTPFAS002560 | 41997-13-1 | Perfluorohexanesulfonamide | 4 |
| NISTPFAS002652 | 863090-89-5 | 2,2,3,3,4,4-Hexafluoro-4-(trifluoromethoxy)butanoic acid | 4 |
| NISTPFAS002656 | 377-73-1 | 2,2,3,3,4,4-Hexafluoro-4-(trifluoromethoxy)butanoic acid, Perfluoro Ether pentanoic acid | 4 |
| NISTPFAS002664 | 151772-58-6 | Perfluoro-3,6-dioxaheptanoic acid | 4 |
| NISTPFAS003051 | 39108-34-4 | 8:2 Fluorotelomer sulfonic acid | 4 |
| NISTPFAS003053 | 757124-72-4 | 4:2 Fluorotelomer sulfonic acid | 4 |
| NISTPFAS003416 |  | 5:1:2 Fluorotelomer betaine | 4 |
| NISTPFAS003429 |  | N-Dimethyl ammonio propyl perfluoropropane sulfonamido propanoic acid | 4 |

| NIST Suspect ID | CAS # | Chemical Name | *n* |
|---|---|---|---|
| NISTPFAS003430 | 1383438-83-2 | 3-(N-(3-(dimethylamino)propyl)-perfluoropentylsulfonamido)propanoic acid | 4 |
| NISTPFAS003794 |  | 5:3 Fluorotelomer betaine | 4 |
| NISTPFAS003962 |  | Hydrogen-substituted perfluorooctane sulfonate | 4 |
| NISTPFAS000009 | 30334-69-1 | Perfluorobutylsulfonamide | 3 |
| NISTPFAS000878 | 50598-28-2 | Perfluorohexane sulfonamido amine | 3 |
| NISTPFAS000880 | 68555-77-1 | Perfluorobutane sulfonamido amine | 3 |
| NISTPFAS000883 | 2355-31-9 | 2-(N-Methylperfluorooctanesulfonamido) acetic acid | 3 |
| NISTPFAS002627 | 376-06-7 | Perfluorotetradecanoic acid | 3 |
| NISTPFAS002628 | 72629-94-8 | Perfluorotridecanoic acid | 3 |
| NISTPFAS002630 | 2058-94-8 | Perfluoroundecanoic acid | 3 |
| NISTPFAS002632 | 335-76-2 | Perfluorodecanoic acid | 3 |
| NISTPFAS002635 | 375-95-1 | Perfluorononanoic acid | 3 |
| NISTPFAS002646 | 2706-90-3 | Perfluoropentanoic acid | 3 |
| NISTPFAS002657 | 919005-14-4 | 4,8-Dioxa-3H-perfluorononanoic acid | 3 |
| NISTPFAS003047 | 756426-58-1 | Perfluoro(2-((6-chlorohexyl)oxy)ethanesulfonic acid) | 3 |
| NISTPFAS003048 | 113507-82-7 | Perfluoro(2-ethoxyethane)sulfonic acid | 3 |
| NISTPFAS003085 |  | Pentafluorosulfide perfluorooctane sulfonate | 3 |
| NISTPFAS003121 |  | (6E)-1,1,2,2,3,3,4,4,5,6,7,8,8,8-tetradecafluorooct-6-ene-1-sulfonic acid | 3 |
| NISTPFAS003238 | 1651215-26-7 | Chloro-perfluorooctane sulfonate | 3 |
| NISTPFAS004703 | 763051-92-9 | 2-[(8-Chloro-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-hexadecafluorooctyl)oxy]-1,1,2,2-tetrafluoroethane-1-sulfonic acid | 3 |
| NISTPFAS000756 | 4151-50-2 | N-Ethylperfluorooctanesulfonamide | 2 |
| NISTPFAS002629 | 307-55-1 | Perfluorododecanoic acid | 2 |
| NISTPFAS002667 | 13252-13-6 | Perfluoro-2-methyl-3-oxahexanoic acid | 2 |
| NISTPFAS003084 |  | Pentafluorosulfide perfluoroheptane sulfonate | 2 |
| NISTPFAS003108 |  | Unsaturated perfluorohexane sulfonate | 2 |
| NISTPFAS003112 |  | Unsaturated perfluorodecane sulfonate | 2 |
| NISTPFAS003113 |  | Unsaturated perfluoroundecane sulfonate | 2 |
| NISTPFAS003131 |  | Hydrogen-substituted perfluoropropane sulfonate | 2 |
| NISTPFAS003285 | 62880-95-9 | 6:2 Fluorotelomer thia propanoamido dimethyl ethyl sulfonate | 2 |
| NISTPFAS003296 |  | Keto-perfluorononane sulfonate | 2 |
| NISTPFAS003297 |  | Keto-perfluorodecane sulfonate | 2 |
| NISTPFAS003491 | 354-88-1 | Perfluoroethane sulfonate | 2 |
| NISTPFAS003611 |  | N-Hydroxyethyldimethylammoniopropyl perfluorohexanesulfonamide | 2 |
| NISTPFAS003644 |  | N-Trimethylammoniopropyl perfluorohexane sulfonamido propanoic acid | 2 |

| NIST Suspect ID | CAS # | Chemical Name | *n* |
|---|---|---|---|
| NISTPFAS003727 | | N-Hydroxyethyldimethylammoniopropyl perfluorohexanesulfonamidoethanol | 2 |
| NISTPFAS000707 | 68957-63-1 | Glycine, N-ethyl-N-[(pentadecafluoroheptyl)sulfonyl]- | 1 |
| NISTPFAS000708 | 68957-32-4 | N-Ethyl-N-((tridecafluorohexyl)sulfonyl)glycine | 1 |
| NISTPFAS000709 | 68957-31-3 | Glycine, N-ethyl-N-[(undecafluoropentyl)sulfonyl]- | 1 |
| NISTPFAS000710 | 68957-33-5 | N-Ethyl-N-[(nonafluorobutyl)sulfonyl]glycine | 1 |
| NISTPFAS000876 | 13417-01-1 | Perfluorooctane sulfonamido amine | 1 |
| NISTPFAS000881 | 34455-22-6 | 6:2 Fluorotelomer sulfonamide amine | 1 |
| NISTPFAS000953 | 31506-32-8 | N-Methylperfluorooctanesulfonamide | 1 |
| NISTPFAS001199 | 132958 | 1H,1H,6H,6H-Perfluorohexane-1,6-diol diacrylate | 1 |
| NISTPFAS001339 | 559-94-4 | (Perfluorobutyryl)-2-thenoylmethane | 1 |
| NISTPFAS001662 | 34143-74-3 | 2-(Perfluorooctyl)ethanthiol | 1 |
| NISTPFAS002339 | 4180-26-1 | 1H,1H,9H-Perfluorononyl acrylate | 1 |
| NISTPFAS002462 | 307-31-3 | Perfluorooctanamidine | 1 |
| NISTPFAS002470 | 423-54-1 | Perfluorooctanamide | 1 |
| NISTPFAS002475 | 355-66-8 | Octafluoroadipamide | 1 |
| NISTPFAS002476 | 13485-61-5 | Nonafluoropentanamide | 1 |
| NISTPFAS002477 | 355-81-7 | Perfluoropentanamide | 1 |
| NISTPFAS002478 | 662-50-0 | Heptafluorobutyramide | 1 |
| NISTPFAS002540 | 355-27-1 | 1H,1H-Perfluoropentylamine | 1 |
| NISTPFAS002623 | 16517-11-6 | Perfluorostearic acid | 1 |
| NISTPFAS002625 | 67905-19-5 | Perfluorohexadecanoic acid | 1 |
| NISTPFAS002645 | 336-08-3 | Octafluoroadipic acid | 1 |
| NISTPFAS002649 | 375-22-4 | Perfluorobutanoic acid | 1 |
| NISTPFAS002650 | 801212-59-9 | Perfluoro-4-isopropoxybutanoic acid | 1 |
| NISTPFAS002725 | 812-70-4 | 3-Perfluoroheptylpropanoic acid | 1 |
| NISTPFAS002729 | 356-02-5 | 3:3 Fluorotelomer carboxylic acid | 1 |
| NISTPFAS002825 | 423-65-4 | 11:1 Fluorotelomer alcohol | 1 |
| NISTPFAS002835 | 375-82-6 | 6:1 Fluorotelomer alcohol | 1 |
| NISTPFAS002836 | 335-99-9 | 1H,1H,7H-Dodecafluoro-1-heptanol | 1 |
| NISTPFAS002840 | 355-80-6 | 1H,1H,5H-Perfluoropentanol | 1 |
| NISTPFAS002841 | 376-90-9 | Hexafluoroamylene glycol | 1 |
| NISTPFAS002872 | 94159-84-9 | 1-(Perfluorofluorooctyl)propane-2,3-diol | 1 |
| NISTPFAS002874 | 125070-38-4 | 3-(Perfluoro-2-butyl)propane-1,2-diol | 1 |
| NISTPFAS003021 | 80220-63-9 | ((Perfluorooctyl)ethyl)phosphonic acid | 1 |
| NISTPFAS003022 | 252237-40-4 | 2-(Perfluorohexyl)ethylphosphonic acid | 1 |
| NISTPFAS003046 | 70259-86-8 | 1,1,2,2,3,3,4,4-Octafluorobutane-1-sulphonic acid | 1 |
| NISTPFAS003082 | | Pentafluorosulfide perfluoropentane sulfonate | 1 |
| NISTPFAS003086 | | Pentafluorosulfide perfluorononane sulfonate | 1 |
| NISTPFAS003109 | | Unsaturated perfluoroheptane sulfonate | 1 |
| NISTPFAS003115 | | Unsaturated perfluorotridecane sulfonate | 1 |

| **NIST Suspect ID** | **CAS #** | **Chemical Name** | ***n*** |
|---|---|---|---|
| NISTPFAS003119 | | Hydrogen-substituted unsaturated perfluorohexane sulfonate | 1 |
| NISTPFAS003236 | 1651215-29-0 | Chloro-perfluorohexane sulfonate | 1 |
| NISTPFAS003286 | 755698-73-8 | 8:2 Fluorotelomer thia propanoamido dimethyl ethyl sulfonate | 1 |
| NISTPFAS003287 | 690947-60-5 | 10:2 Fluorotelomer thia propanoamido dimethyl ethyl sulfonate | 1 |
| NISTPFAS003293 | | Keto-perfluorohexane sulfonate | 1 |
| NISTPFAS003294 | | Keto-perfluoroheptane sulfonate | 1 |
| NISTPFAS003334 | | N-Ethylperfluoropropane sulfonamido acetic acid | 1 |
| NISTPFAS003346 | | N-Methylperfluorohexane sulfonamido acetic acid | 1 |
| NISTPFAS003407 | 1513864-10-2 | 6:2 Fluorotelomer sulfinyl propanamido dimethyl ethyl sulfonate | 1 |
| NISTPFAS003418 | | 7:1:2 Fluorotelomer betaine | 1 |
| NISTPFAS003420 | | 10:2 Hydrogen-substituted fluorotelomer betaine | 1 |
| NISTPFAS003489 | 422-64-0 | Perfluoro-n-propanoic acid | 1 |
| NISTPFAS003493 | 749786-16-1 | Perfluoroundecane sulfonate | 1 |
| NISTPFAS003610 | | N-Hydroxyethyldimethylammoniopropyl perfluoropentanesulfonamide | 1 |
| NISTPFAS003613 | | N-Hydroxyethyldimethylammoniopropyl perfluorooctanesulfonamide | 1 |
| NISTPFAS003615 | | N-Hydroxyethyldimethylammoniopropyl perfluorodecanesulfonamide | 1 |
| NISTPFAS003635 | 70225-25-1 | 3-[[(Heptadecafluorooctyl)sulfonyl]amino]-N,N,N-trimethyl-1-propanaminium | 1 |
| NISTPFAS003726 | | N-Hydroxyethyldimethylammoniopropyl perfluoropentanesulfonamidoethanol | 1 |
| NISTPFAS003728 | | N-Hydroxyethyldimethylammoniopropyl perfluoroheptanesulfonamidoethanol | 1 |
| NISTPFAS003729 | | N-Hydroxyethyldimethylammoniopropyl perfluorooctanesulfonamidoethanol | 1 |
| NISTPFAS003744 | 34455-23-7 | 8:2 Fluorotelomer sulfonamido propyl dimethyl amine | 1 |
| NISTPFAS003807 | 88992-46-5 | 6:2 Fluorotelomer thia hydroxy propyl trimethyl ammonium | 1 |
| NISTPFAS003808 | 727351-53-3 | 8:2 Fluorotelomer thia hydroxy propyl trimethyl ammonium | 1 |
| NISTPFAS003906 | 17587-22-3 | (Heptafluorobutanoyl)pivaloylmethane | 1 |
| NISTPFAS003919 | 34455-21-5 | 8:2 Fluorotelomer sulfonamide betaine | 1 |
| NISTPFAS003960 | | Hydrogen-substituted perfluorohexane sulfonate | 1 |
| NISTPFAS003961 | | Hydrogen-substituted perfluoroheptane sulfonate | 1 |
| NISTPFAS003993 | | N-Sulfo propyl dimethyl ammonio propyl perfluorohexane sulfonamide | 1 |
| NISTPFAS004577 | | Perfluoro ether undecane sulfonic acid | 1 |

## DIMSpec Database Schema Description

The schema for the underlying database is defined by a series of SQL scripts in the project's "config" directory. Data are structured in a series of "nodes" and are detailed in this section. Each node contains a series of conceptually-interrelated tables and views. Complete descriptions of each node, and the tables and views therein, are provided in the DIMSpec User Guide (https://pages.nist.gov/dimspec/docs/technical-details.html). Graphical examples of each node are provided there, and a lower resolution copy of the entity relationship diagram is provided, along with links to each, in the caption of **Figure 1210**.

### *The Analyte Node*

This node contains information relevant to analytical targets. This node does not contain analytical data, but rather identifying information and views to compare that identifying information with measurements held in the "data" node and is also linked to the "contributors" node.

### *The Contributors Node*

This node contains information relevant to identifying data contributors, similar to a "users" table. It is used primarily to provide contribution statistics and tie data to data producers in the samples and analytes node, both of which are connected to the peaks node. When the database is built, a "sys" username with the affiliation "system" is automatically added as a default user.

### *The Data Node*

This node contains mass spectral data, metadata about samples and the software used to generate it, identification confidence, and quality control measures, as well as views to consume it directly. It is linked to the analyte node through the "compound_fragments" table and to the contributors and methods nodes through the samples table. This node contains two sub-nodes and is the main location of analytical data. One describes samples and one describes peaks generated from those samples.

### *The Logging Node*

This node is included for automatic logging within the database itself (a future development opportunity), with tables to store and normalize logs and store a database version history. It is not used by default, but rather serves as a placeholder in case logging should be enabled via triggers if required by the data management and quality control systems for a given project. As it is not in use and has not been tested, details are not included but can be queried like any other tables.

### *The Methods Node*

This node contains data describing experimental settings, both for the chromatographic separation and the mass spectrometer. It is the largest node, composed of four subnodes. The mass spectrometer ("mass spec") subnode contains information about the mass spectrometer settings used to collect data for an experiment and is closely related to the "descriptions" node which contains vendor descriptions for all instrumentation used in the experiment, allowing a single mass spectrometric method to describe multiple detectors and chromatographic separators. The quality control subnode describes the quality control procedures that were applied. Finally, the “mobile phase” subnode describe chromatographic conditions, allowing for multiple chromatographic components to be described, and multiple stages of mobile phase conditions.

### *The Reference Node*

This node contains universally applicable reference information for chemical metrology. Data for elements, their exact masses, and their natural isotopic abundances are automatically added as part of the database build process. This node does not directly connect to any others but serves only for computational convenience.

Figure 12. Entity Relationship Diagram (ERD) of the DIMSpec relational database (SQLite) schema demonstrating modularity by collecting tables and view into conceptually related nodes. Individual nodes share a color and visual proximity. Tables are colored, and related views are in white. Details may be difficult to see in this medium, and interested parties are directed to descriptions and higher resolution images of the individual nodes in the User Guide (https://pages.nist.gov/dimspec/docs/technical-details.html#database-schema), or the full resolution version hosted in GitHub at (https://raw.githubusercontent.com/usnistgov/dimspec/refs/heads/main/dimspec_user_guide/assets/ERD.png).

**Table 18. Number of laboratories that reported of PFAS included in Sample A.**

| PFAS Preferred Name | Acronym | NIST ID | Number of Reporting Laboratories |
|---|---|---|---|
| 5:3 fluorotelomer betaine | 5:3 FTB | NISTPFAS003794 | 7 |
| Chloro-perfluorooctane sulfonate | 8Cl-PFOS | NISTPFAS003238 | 22 |
| Perfluorohexane sulfonamido amine | N-AP-FHxSA | NISTPFAS000878 | 23 |
| Perfluorobutanoic acid | PFBA | NISTPFAS002649 | 20 |
| Perfluoropentanoic acid | PFPeA | NISTPFAS002646 | 21 |
| Perfluorohexanoic acid | PFHxA | NISTPFAS002643 | 25 |
| Perfluoroheptanoic acid | PFHpA | NISTPFAS002640 | 26 |
| Perfluorooctanoic acid | PFOA | NISTPFAS002637 | 24 |
| Perfluorononanoic acid | PFNA | NISTPFAS002635 | 25 |
| Perfluorodecanoic acid | PFDA | NISTPFAS002632 | 23 |
| Perfluorobutanesulfonic acid | PFBS | NISTPFAS003045 | 25 |
| Perfluorohexanesulfonic acid | PFHxS | NISTPFAS003043 | 26 |
| Perfluorooctanesulfonic acid | PFOS | NISTPFAS003041 | 24 |

**Table 19. Number of laboratories that reported PFAS known to be present in Sample B.**

| PFAS Preferred Name | Acronym | NIST ID | Number of Reporting Laboratories |
|---|---|---|---|
| Perfluorohexane sulfonamido amine | N-AP-FHxSA | NISTPFAS000878 | 22 |
| Perfluorobutanoic acid | PFBA | NISTPFAS002649 | 11 |
| Perfluorohexanoic acid | PFHxA | NISTPFAS002643 | 19 |
| Perfluoroheptanoic acid | PFHpA | NISTPFAS002640 | 12 |
| Perfluorooctanoic acid | PFOA | NISTPFAS002637 | 18 |
| Perfluoropropanesulfonic acid | PFPrS | NISTPFAS003492 | 18 |
| Perfluorobutanesulfonic acid | PFBS | NISTPFAS003045 | 22 |
| Perfluoropentanesulfonic acid | PFPeS | NISTPFAS003044 | 23 |
| Perfluorohexanesulfonic acid | PFHxS | NISTPFAS003043 | 26 |
| Perfluoroheptanesulfonic acid | PFHpS | NISTPFAS003042 | 18 |
| Perfluorooctanesulfonic acid | PFOS | NISTPFAS003041 | 24 |
| 6:2 fluorotelomer sulfonic acid | 6:2 FTS | NISTPFAS003052 | 23 |
| Perfluorobutanesulfonamide | FBSA | NISTPFAS000009 | 4 |
| Perfluorohexanesulfonamide | FHxSA | NISTPFAS002560 | 7 |
| N,N,N-trimethyl-3-[[(1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluorohexyl)sulfonyl]amino]-1-Propanaminium | N-TAmP-FHxSA | NISTPFAS003633 | 3 |
| 6:2 Fluorotelomer sulfonamide betaine | 6:2 FTAB | NISTPFAS003736 | 10 |

**Table 20. Number of laboratories that reported PFAS known to be present in Sample C.**

| PFAS Preferred Name | Acronym | NIST ID | Number of Reporting Laboratories |
|---|---|---|---|
| Perfluorohexane sulfonamido amine | N-AP-FHxSA | NISTPFAS000878 | 23 |
| Perfluorohexanoic acid | PFHxA | NISTPFAS002643 | 23 |
| Perfluoroheptanoic acid | PFHpA | NISTPFAS002640 | 18 |
| Perfluorooctanoic acid | PFOA | NISTPFAS002637 | 21 |
| Perfluoropentanesulfonic acid | PFPeS | NISTPFAS003044 | 24 |
| Perfluorohexanesulfonic acid | PFHxS | NISTPFAS003043 | 26 |
| Perfluoroheptanesulfonic acid | PFHpS | NISTPFAS003042 | 24 |
| Perfluorooctanesulfonic acid | PFOS | NISTPFAS003041 | 25 |
| Perfluorononanesulfonic acid | PFNS | NISTPFAS003040 | 23 |
| Perfluorodecanesulfonic acid | PFDS | NISTPFAS003039 | 23 |
| Perfluorohexanesulfonamide | FHxSA | NISTPFAS002560 | 25 |
| Perfluorooctanesulfonamide | FOSA | NISTPFAS002559 | 24 |

# APPENDIX B: LIST OF SCIENTIFIC/TECHNICAL PUBLICATIONS

## Peer-reviewed journal articles

Place, Benjamin J. and Ragland, Jared M. “Speaking the Same Language: The Need for Accurate and Consistent Reporting of Novel Per- and Polyfluoroalkyl Substances.” *Environmental Science & Technology*, 2022, 56, 15, 10546-10566. https://doi.org/10.1021/acs.est.2c04273

Ragland, Jared M. and Place, Benjamin J. “A Portable and Reusable Database Infrastructure for Mass Spectrometry, and Its Associated Toolkit (The DIMSpec Project).” *Journal of the American Society for Mass Spectrometry*, 2024, 35, 6, 1282-1291. https://doi.org/10.1021/jasms.4c00073

## Public data repositories

Place, Benjamin J. “Suspect List of Possible Per- and Polyfluoroalkyl Substances (PFAS).” National Institute of Standards and Technology, 2021. https://doi.org/10.18434/mds2-2387

Ragland, Jared M. and Place, Benjamin J. “Database Infrastructure for Mass Spectrometry - Per- and Polyfluoroalkyl Substances.” National Institute of Standards and Technology, 2023. https://doi.org/10.18434/mds2-2905

## Technical Reports

Place, Benjamin J., Reiner, Jessica L., Ragland, Jared M., Rodowa, Alix E., Burdette, Carolyn Q., Cuthbertson, Amy A., Rimmer, Catherine A., Kucklick, John R., Mahynski, Nathan. “Per- and Polyfluoroalkyl Substances – Non-Targeted Analysis Interlaboratory Study Final Report.” National Institute of Standards and Technology – Interagency Report (NIST-IR). *In review*.

## Conference/Symposium Abstracts

Ragland, Jared M. and Place, Benjamin J. 2023, August 13-17. “Creating a portable database infrastructure for non-targeted analysis in mass spectrometry.” ACS Fall 2023, San Francisco, CA, USA.

Ragland, Jared M. and Place, Benjamin J. 2023, November 12-16. "A portable database infrastructure for per- and polyfluorinated alkyl substances high resolution accurate mass data." SETAC North America 44th Annual Meeting, Louisville, KY, USA.

Ragland, Jared M., Place, Benjamin J., and Reiner, Jessica L. 2023, December 13. "Improving Access and Utility of Analytical Data for the Confident Discovery, Identification, and Source Attribution of PFAS in Environmental Matrices." SERDP & ESTCP Webinar Series, Virtual.

# APPENDIX C: OTHER SUPPORTING MATERIALS

**Awards:**

- 2023 National Institute of Standards & Technology Material Measurement Laboratory – Technical Excellence Award, “For development of a Web-based user interface for the collection and analysis of PFAS mass spectra and the associated metadata.”
- 2024 National Institute of Standards & Technology Material Measurement Laboratory – Technology Transfer Award, “For developing a series of video tutorials for the Database Infrastructure of the Mass Spectrometry project.”
- 2024 National Institute of Standards & Technology Allen V. Astin Measurement Science Award for “Establishing a measurement infrastructure for the global assessment of per- and polyfluorinated alkyl substances (PFAS).”