



# Tools for the analysis of Per- and Polyfluoroalkyl Substances (PFAS)

Jessica L. Reiner, Chemical Sciences Division

# Acknowledgements



**Material Measurement Laboratory,  
Chemical Sciences Division**  
*Biochemical and Exposure Science  
Group*

John Kucklick  
Jennifer Lynch

*Organic Chemical Metrology Group*

Melissa Phillips  
Benjamin Place  
Kate Rimmer  
Alix Rodowa

*Chemical Informatics Group*  
Jared Ragland

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# National Institute of Standards and Technology



NIST is a non-regulatory Bureau within the Department of Commerce

NIST mission ... promote US innovation and industrial competitiveness by advancing measurement science, standards, and technology

The Chemical Sciences Division at NIST provides measurement services for a wide range of industries.

Provide traceability infrastructure for the nation for the measurement of chemical species in clinical, environmental, and food matrices

Develop new technologies based on separation and mass spectrometric techniques for measurement of chemical species

Assist industry to meet measurement goals



# NIST Laboratory Programs



Material  
Measurement  
Laboratory



Physical  
Measurement  
Laboratory



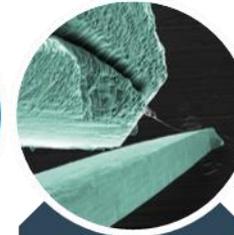
Engineering  
Laboratory



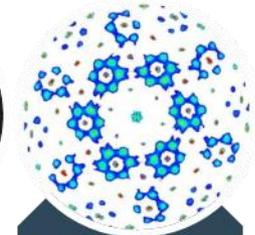
Information  
Technology  
Laboratory



Communication  
Technology  
Laboratory



Center for  
Nanoscale  
Science and  
Technology



NIST Center for  
Neutron  
Research

## Metrology Laboratories

Driving innovation through  
Measurement Science and Standards

## Technology Laboratories

Accelerating the adoption and deployment  
of advanced technology solutions

## National User Facilities

Providing world class, unique, cutting-  
edge research facilities

# Measurement Services



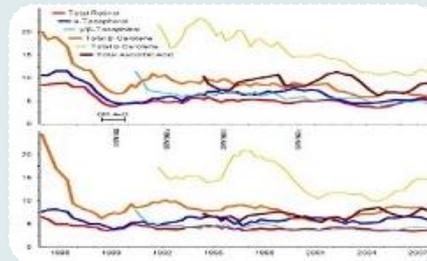
## Reference materials and data

- 1,200 Standard Reference Material products
- 100 Standard Reference Data products



## User facilities

- National Synchrotron Light Source II at Brookhaven National Laboratory
- nSoft consortium at NIST Center for Neutron Research



## Measurement quality assurance programs

- Cannabis
- Clinical measurements
- Dietary supplements
- Food nutrition and safety
- Marine environment

# Reference Materials (RMs)



**Standard Reference Materials (SRMs)** are Certified Reference Materials (CRMs) issued by the National Institute of Standards and Technology (NIST)

Homogeneous, well-characterized materials used to validate measurements and improve the quality of analytical data

Support accurate and comparable measurements

SRMs are provided to support clinical, food, environmental, fuel, metal alloy, cement, and advanced materials analyses

# SRMs for chemical measurements



- High Purity Neat Chemicals
  - Organic Calibration Solutions
  - Inorganic Calibration Solutions
  - Gas Mixture Standards
- Complex Matrix Standards
  - Advanced Materials
  - Biological Fluids/Tissues
  - Foods/Botanicals
  - Sediments/Soils/Particulates
  - House Dust



# SRMs



NIST has a variety of human SRMs including

- Serum, Plasma, Milk, Urine, Hair

NIST has a variety of natural matrix SRMs including

- Fish tissue, Mussel tissue, House dust

Measurements on these materials include chemical contaminants, elements, vitamins, metabolites, etc...



# Use of Complex or Natural-Matrix SRMs



## Method Development

You can analyze the SRM using a new analytical method as you develop it. If your result agrees with the assigned value, then your method will probably give good values for other similar samples

## Method Validation

You can analyze the SRM and other samples typical of those that you will analyze (matrix, concentration) to demonstrate your method's scope of applicability and that your method has acceptable accuracy, repeatability, and selectivity. (AOAC recommends analysis of at least three materials, at least in duplicate, on at least two days.)

## Quality Control (QC)

You can prepare and analyze the SRM the same way as you would an unknown sample. If your result for the SRM agrees with the assigned value, then your results for your own sample are probably right.

## Traceability

You can prepare and analyze the SRM along with an in-house QC material over a set timeframe to establish values for your in-house QC, and then use that material for routine quality assurance.

# NIST's Role in PFAS Measurement



NIST (2004-present)

NIST developed measurement methods for PFAS using LC-MS/MS

Added PFAS values to existing SRM

15 materials with PFAS measurements

Four **New AFFF Reference Materials** (7/2023)

Working on contaminated soil, contaminated food, low level drinking water

Sediment	Tissue	Human Fluids	Calibration Standards	Other
2586 – Soil containing lead from paint	1946 – Lake Superior Fish Tissue	1957/1958 - Human Serum	RM 8446	2781 – Domestic Sludge
1936 – Great Lakes Sediment	1947 – Lake Michigan Fish Tissue	1950 - Human Plasma	RM 8447	2585 – House Dust



# PFAS in Environmental Matrices



# PFAS in Aqueous Film-Forming Foams (AFFF)



- Four individual formulations of AFFF (diluted and modified)
- Wide range of concentrations for up to 16 PFAS
- Designed for the identification and quantification of PFAS in AFFF



# PFAS in Food



- Fish tissue (in production)
- Frozen meat (in progress)
  - Bull
  - Dairy cow
  - Porcine
- Plant materials (in progress)
  - Corn silage
  - Spinach
- Quality Assurance Programs
  - *Freeze-dried milk (cow)*
  - *Freeze-dried egg (chicken)*

# Interlaboratory Studies for PFAS



NIST routinely coordinates interlaboratory studies where participating laboratories are given select materials and laboratories provide data for the concentration of PFAS (or other chemicals) in the materials.

Quality Assurance Programs (QAPs) are interlaboratory studies aimed at providing results and feedback to laboratories, and sometimes subsequent interlaboratory studies, aimed at improving measurement capabilities of participating laboratories.

# Interlaboratory Study for PFAS in AFFF

- Participating laboratories were sent four AFFF commercial mixtures and asked to provide quantitative data for specific PFAS.
- Project was supported in part by DOD SERDP Project ER18-1664.



National Institute of Standards and  
Technology Internal Report (NISTIR 8399)  
<https://doi.org/10.6028/NIST.IR.8399>

# Quality Assurance Program for PFAS in Food

- In 2023, as part of the Food Nutrition and Safety Measurements Quality Assurance Program (FNSQAP), three materials were sent to participating laboratories to measure select PFAS:
  - Frozen meat (beef)
  - Freeze-dried milk (cow)
  - Freeze-dried egg (chicken)
- Data is still being analyzed and a final report is expected soon.



# NIST Data Tools for the Non-Targeted Analysis of PFAS



- NIST List of Possible Per- and Polyfluoroalkyl Substances
- PFAS in the NIST Mass Spectral Libraries
- Database Infrastructure for Mass Spectrometry (DIMSpec)

# Per- and polyfluoroalkyl substances

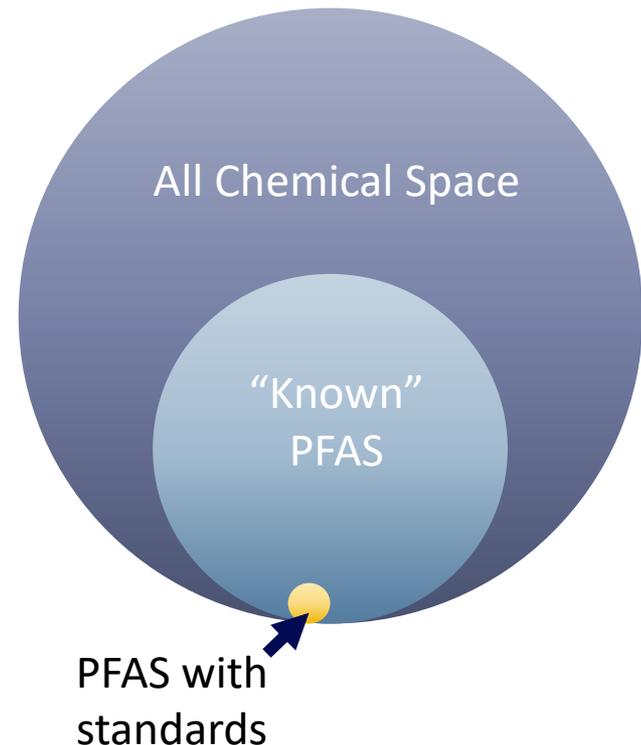


Estimates for the total number of different PFAS in the environment and/or used in varying industries can be over 4,000.

There are a limited number of analytical standards available for these compounds.

- Estimated ~100 standards commercially available.
- This makes traditional targeted approaches (LC-MS/MS) more difficult

**Non-Targeted Analysis** and **Suspect Screening Analysis** are two approaches towards the detection of known and unknown PFAS without the availability of authentic analytical standards.

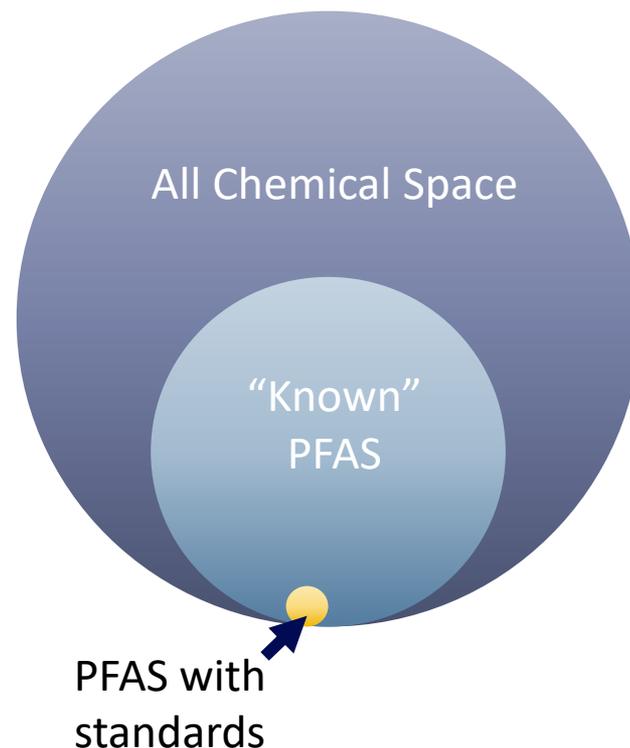


# Non-Targeted Analysis



**Non-targeted analysis (NTA)** is a theoretical concept that can be broadly defined as the characterization of the chemical composition of any given sample without the use of *a priori* knowledge regarding the sample's chemical content.

**Suspect screening analysis (SSA)** is the identification of chemicals and/or chemical classes detected by an instrument, typically a mass spectrometer, by comparison to a predefined user list or library containing known chemicals of interest.



Benchmarking and Publications for Non-Targeted Analysis Working Group. <https://nontargetedanalysis.org/>

# Standardized and Consistent Reporting of PFAS Identities



Naming protocols for PFAS have varied over the years.

- We recommend the use of compound identifiers and/or structural notation for reporting the discovery and identification of novel PFAS.

Use of standard protocols for stating the identification confidence of a novel PFAS.

- Charbonnet et al. provides a new scale for communicating the confidence for PFAS identification.

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Science & Technology

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Viewpoint

## Speaking the Same Language: The Need for Accurate and Consistent Reporting of Novel Per- and Polyfluoroalkyl Substances

Benjamin J. Place\* and Jared M. Ragland

Cite This: <https://doi.org/10.1021/acs.est.2c04273>

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Science & Technology LETTERS

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Global Perspective

## Communicating Confidence of Per- and Polyfluoroalkyl Substance Identification via High-Resolution Mass Spectrometry

Joseph A. Charbonnet, Carrie A. McDonough, Feng Xiao, Trever Schwichtenberg, Dunning Cao, Sarit Kaserzon, Kevin V. Thomas, Pradeep Dewapriya, Benjamin J. Place, Emma L. Schymanski, Jennifer A. Field, Damian E. Helbling, and Christopher P. Higgins\*

Cite This: *Environ. Sci. Technol. Lett.* 2022, 9, 473–481

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# NIST Suspect List of Possible PFAS



Purpose: To create a central location for PFAS identified by peer-reviewed manuscripts, other documentations, or computational tools.

- Identities are attributed to discovery source.
- Structures (as InChI) are the key value (not name, acronym, elemental formula, etc.).
- Index values are constant
- Additional information (acronym, chemical class, etc.) available.

Built upon efforts by Colorado School of Mines, Oregon State University, and others.

Public contributions are allowed, does not require chemical registration or other official entry protocol.

## NIST Suspect List of Possible Per- and Polyfluoroalkyl Substances

ID	CHEMICAL_NAME	INCHI	SMILES	INCHIKE'	FORMUL	FIXEDMASS
1	1H-Perfluoro-3,3-bis(trifluoromethyl)propane	InChI=1S/C8HF13/c1-2-3(6(13,14)15,7(16,17)18)4(9,10)5(11,12)8(19,20)21/h1H	C#CC(C(C(F)F)F)F	C8HF13		343.9870669
2	Bis(heneicosafluorodecyl)phosphine oxide	InChI=1S/C20H9F34O2P/c21-5(22,7(25,26)9(29,30)11(33,34)13(37,38)15(41,42)17(CP(=O)(C)C)20H9F34	(F)C(C(F)F)OP(=O)(F)F	C20H9F34O2P		957.9797256
3	Tris(2-(perfluorododecyl)ethyl)phosphine oxide	InChI=1S/C42H12F50O4P/c43-7(44,10(49,50)13(55,56)16(61,62)19(67,68)22(73,74)COP(=O)JKDZBIHM	C(C(F)F)OP(=O)(C(F)F)C(F)F	C42H12F50O4P		2035.927562
4	2-Chloro-2-propenoic acid	InChI=1S/C9H6ClF9O2/c1-4(10)5(20)21-3-2-6(11,12)7(13,14)8(15,16)9(17,18)19/h	C=C(C(=O)O)C(F)F	C9H6ClF9O2		351.9912611
5	Bicyclo[2.2.1]hept-2-ene, 5,5,6-trifluoro-	InChI=1S/C10H6F10O/c11-6(12)4-1-2-5(3-4)7(6,13)21-10(19,20)8(14,15)9(16,17)1:	C1=CC2CC.JZEKVGWIW	C10H6F10O		332.025897
6	3,3,4-Trifluoro-4-(heptafluoropropyl)butanoic acid	InChI=1S/C12H8F10O/c13-8(14)6-4-1-2-5(3-4)7(6)9(8,15)23-12(21,22)10(16,17)11	C1=CC2CC.NGEYGRCC	C12H8F10O		358.0415471
7	5-(Nonafluorobutyl)bicyclo[2.2.1]hept-2-ene	InChI=1S/C11H9F9/c12-8(13,7-4-5-1-2-6(7)3-5)9(14,15)10(16,17)11(18,19)20/h1-2	C1=CC2CC.QAIOQFZL	C11H9F9		312.0560543
8	3-(Heptafluorobutyl)camphor	InChI=1S/C14H15F7O2/c1-10(2)6-4-5-11(10,3)8(22)7(6)9(23)12(15,16)13(17,18)14	CC1(C)C2C.PEWoesyE	C14H15F7O2		348.0960273
9	Perfluorobutylsulfonamide	InChI=1S/C4H2F9NO2S/c5-1(6,3(9,10)11)2(7,8)4(12,13)17(14,15)16/h(H)2,14,15,16	C(C(C(F)F)F)FUVKFLJW	C4H2F9NO2S		298.9662533
10	N-(3,4-Dichlorophenyl)-2,2,3,3-tetrafluorobutanoic acid	InChI=1S/C10H4Cl2F7NO/c11-5-2-1-4(3-6(5)12)20-7(21)8(13,14)9(15,16)10(17,18)	c1cc(c(cc1)N)NMDAGXI	C10H4Cl2F7NO		356.9558166
11	N-(3,5-Dichlorophenyl)-2,2,3,3-tetrafluorobutanoic acid	InChI=1S/C10H4Cl2F7NO/c11-4-1-5(12)3-6(2-4)20-7(21)8(13,14)9(15,16)10(17,18)	c1c(cc(cc1)N)WGNKYUV	C10H4Cl2F7NO		356.9558166
12	2-Propenoic acid, 2-methyl-, 2-(perfluorooctyl)ethyl ester	InChI=1S/C28H28F17N3O8S/c1-6-48(9-10-54-19(50)46-16-8-7-14(4)17(11-16)47-2	CCN(CCO)WUGSPVX	C28H28F17N3O8S		889.1325656

<https://data.nist.gov/od/id/mds2-2387>

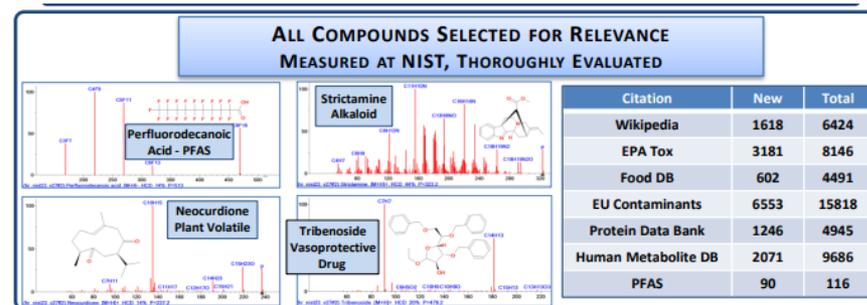
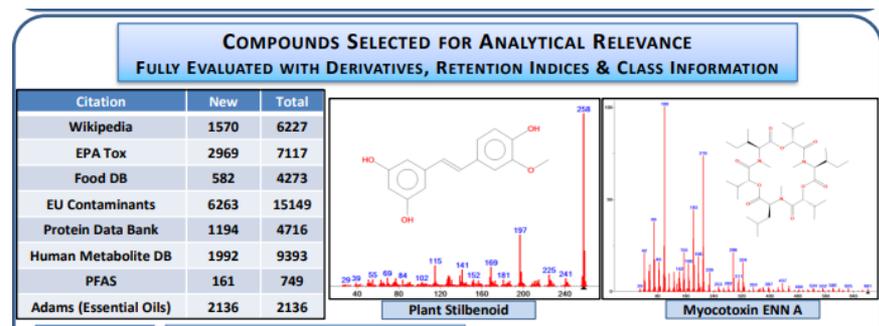


# NIST Mass Spectral Libraries: 2023 Release



The NIST Mass Spectrometry Data Center have a total of 865 PFAS to their mass spectral libraries, with 251 PFAS added in the 2023 release.

- 161 empirical mass spectra added to the EI-MS library
- 90 empirical mass spectra added to the tandem MS library



# Database Infrastructure for Mass Spectrometry (DIMSpec)

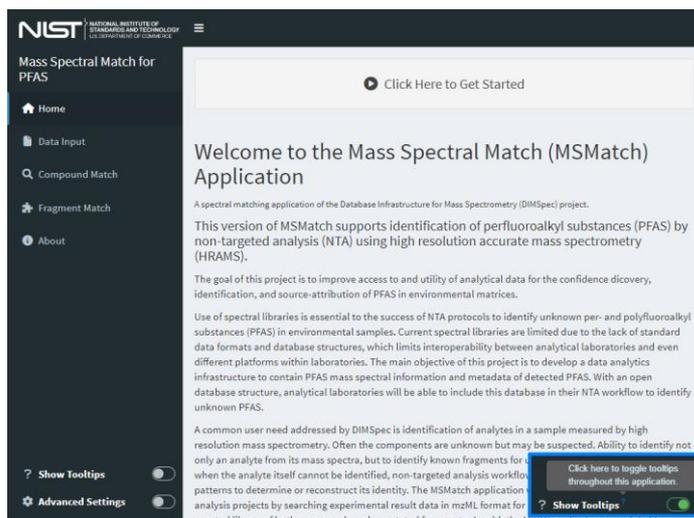


## Project funded by the DOD Strategic Environmental Research and Development Program (SERDP)

Objective: develop a data analytics 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.



# Database Infrastructure for Mass Spectrometry (DIMSpec)



## Mass Spectral Match (MSMatch) App

# Database Infrastructure for Mass Spectrometry (DIMSpec)



The screenshot displays the NIST Mass Spectral Match (MSMatch) web interface. The top navigation bar includes the NIST logo and the text 'NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY U.S. DEPARTMENT OF COMMERCE'. Below the navigation bar, the main content area is divided into two primary sections: 'Load Data' and 'Feature Identification'. The 'Load Data' section is currently active and contains the following elements:

- Choose a data file (.mzML):** A 'Load' button with the text 'Select a file to begin'.
- Set Instrument Parameters:** A section with several input fields:
  - MS Experiment Type:** A dropdown menu currently set to 'DDA (data-dependent acquisition)'.
  - Relative Error (ppm):** A text input field containing the value '5'.
  - Minimum Error (Da):** A text input field containing the value '0.002'.
  - Isolation Width (Da):** A text input field containing the value '0.7'.

The 'Feature Identification' section is partially visible on the right and includes:

- Select parameters identifying peaks to examine:** A '+ Add' button.
- Import:** A button with the text 'Select a file to import parameters'.

A dark sidebar on the left side of the interface contains navigation links: Home, Data Help, Compound, Fragmentation, and About. At the bottom of the sidebar, there are links for '? Show Tool' and '⚙️ Advance'.

MSMatch – Data Load

# Database Infrastructure for Mass Spectrometry (DIMSpec)



Mass Spectral Match for PFAS

Load Data

Choose a data file (.mzML)

Feature Identification

Select parameters identifying peaks to examine.

Top match is **Perfluorohexanoic acid** from an analytical standard

Set Instrument: DDA (data-d)

MS Experiment: Comparison Mass Spectrum

Relative Error: 5

Minimum Error: 0.002

Isolation Width: 0.7

Your measurement is in black. Comparison spectrum is in red.

Relative Intensity vs m/z

Compound	MS1 Score	MS1 Score (Rev)	MS2 Score	MS2 Score (Rev)	# Annotated Fragments	# Annotated Structures	# Annotated Citations
Perfluorohexanoic acid	0.9989	0.9972	0.9939	0.9999	2	2	2
Perfluorohexanoic acid	0.9883	0.9843	0.7424	0.6647	1	1	1
Perfluorohexanoic acid	0.965	0.7849	0.3342	0.4294	1	0	0
Perfluorohexanoic acid	0.9633	0.7331	0.3342	0.3357	0	0	0
Perfluorohexanoic acid	0.9717	0.626	0.3342	0.4054	1	0	0
Perfluorohexanoic acid	0.9419	0.7254	0.3342	0.2375	1	0	0
Perfluorohexanoic acid	0.9695	0.7948			1	0	0
Perfluorohexanoic acid	0.9359	0.8033			1	0	0
Perfluorohexanoic acid	0.964	0.6962			1	0	0

This reference spectrum was measured by LC (ThermoFisher Scientific) quadrupole orbitrap MS (ThermoFisher Scientific), separated by octadecyl (C18) analytical column in negative ESI mode at 2500.0 volts and fixed fragmentation by HCD (higher energy collision-induced dissociation) at 30 volts.

Estimate Match Score Uncertainty

## MSMatch – Compound Search

# Database Infrastructure for Mass Spectrometry (DIMSpec)



The screenshot displays the NIST Mass Spectral Match (MSMatch) software interface. The main window shows a comparison mass spectrum for Perfluorohexanoic acid. The top match is identified as Perfluorohexanoic acid from an analytical standard. The MSMatch interface includes a sidebar with navigation options (Home, Data, Compound, Fragment, About) and a main panel with sections for 'Load Data', 'Feature Identification', and 'Uncertainty Evaluation'. The 'Uncertainty Evaluation' section displays a table of match scores and a box plot of match scores for 100 iterations.

Compound	MS1	MS1 (Rev)	MS2	MS2 (Rev)	# Annotated Fragments
Perfluorohexanoic acid	0.9983	0.9983	0.7428	0.6947	2

# Annotated Fragments	# Annotated Structures	# Annotated Citations
2	2	2
1	1	1
1	0	0
0	0	0
1	0	0
1	0	0
1	0	0
1	0	0
1	0	0

Range of Match Scores (100 Iterations)  
MS2 match for Perfluorohexanoic acid

Forward match scores ranged 0.632 - 0.833 with an IQR of 0.6517 and median of 0.7345.  
Reverse match scores ranged 0.5294 - 0.7546 with an IQR of 0.6055 and median of 0.6605.

## MSMatch – Uncertainty Analysis

# Database Infrastructure for Mass Spectrometry (DIMSpec)



**Mass Spectral Match for PFAS**

**Load Data**  
Choose a data file (.mzML)

**Feature Identification**  
Select parameters identifying peaks to examine.

**Top match is Perfluorohexanoic acid from an analytical standard**  
MS1 Score: 0.9999 (1) (score: 0.9999)

**Comparison**

Fragment	Measured at m/z	Exact Mass
C2F5	118.9913	118.9920
CSF11	268.9834	268.9824
CSF11	268.9834	268.9824
C2F5	118.9913	118.9920
CSF11	268.9834	268.9824

**Mass Error (ppm)**: -5.4847  
**SMILES**: F[C-](F)(F)(F)F  
**Radical**: Not Recorded  
**Net Charge**: -1  
**Found in n Compounds**: 1  
**Found in n Peaks**: 1

**Chemical Structure**: F[C-](F)(F)(F)F

**Uncertainty Evaluation**

Compound	MS1 (Rev)	MS2 (Rev)	# Annotated Fragments
Perfluorohexanoic acid	1	1	1

**Properties of Perfluorohexanoic acid**

- Formula: C6HF11O2
- Exact Mass: 313.9801
- Source Type: Curated
- Obtained From: <https://comptox.epa.gov/dashboard>
- Local (+): 0
- Local (-): 0
- Net Charge: 0

**Other Compounds**

Compound	Identification Confidence	n Points
Perfluoro-2-methyl-3-oxahexanoic acid	1	2
Perfluoroheptanoic acid	1	2
Perfluorohexanesulfonic acid	1	2
Perfluorooctanoic acid	1	2
Perfluorheptanesulfonic acid	1	2
perfluoro-n-propanoic acid	1	2
Perfluorononanoic acid	1	2
N-Methylperfluorooctanesulfonamide	1	2
Perfluorodecanoic acid	1	2

**Level 1a - Confirmed by reference standard**

Property	Value
Precursor m/z	312.973
Ion State	[M-H]
RT Centroid	30.8
RT Start	30.0
RT End	31.1
Peak ID	170
Sample ID	3

**A.** Copy CSV Excel

**B.** Copy CSV Excel Previous 1 2 Next

## MSMatch – Fragment Search

# Questions?



For more information on any of the ongoing PFAS projects, feel free to reach out at:  
[pfas@nist.gov](mailto:pfas@nist.gov)

To learn about NIST's PFAS Program go to:

<https://www.nist.gov/programs-projects/and-polyfluoroalkyl-substances-pfas>



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