Supporting Information:

Quantitation of Total PFAS including Trifluoroacetic Acid with Fluorine Nuclear Magnetic Resonance (^{19}F-NMR)

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Supporting Information includes: 13 pages, 5 tables, 1 figure, and 1 equation

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Materials and Methods

Experimental materials

Perfluoropropionic acid (PFPrA) reference standard and chromium (III) acetylacetonate ($Cracac_4$) were purchased from Acros Organics (Pittsburgh, PA). Perfluoro(2-ethoxyethane)sulfonic acid (PFEESA) and Perfluoro-3-methoxypropanoic acid (PFMPA) were purchased from Synquest Laboratories (Alachua, FL). Nonafluoro-3,6-dioxaheptanoic acid (NFDHA) was purchased from Apollo Scientific (Stockport, UK). Reference standards for Trifluoroacetic acid (TFA) and hexafluorobenzene (HFB) were purchased from Sigma Aldrich (Milwaukee, WI) and Trifluoro sulfonic acid (TFMS) was purchased from Alfa Aesar (Tewksbury, MA). The remaining 29 PFAS standards, ^{13}C-labelled PFOA (MPFOA) standard, and a mixture of isotopically labelled PFAS standards (MPFAC – 24 ES) (**Table S1**) were purchased from Wellington Laboratories, LLC (Overland Park, KS). Deuterated methanol was purchased from Cambridge Isotope Laboratories (Andover, MA). Liquid chromatography-mass spectrometry (LC-MS) grade acetonitrile and methanol for instrumental analysis was obtained from Omnisolv® through Millipore Sigma (Saint Louis, MO). American Chemical Society (ACS) grade glacial acetic acid and ammonium acetate were obtained from J. T. Baker (Philipsburg, NJ). Waters BEH

X-Bridge™ (3.5 μm particle size, 2.1 mm internal diameter, 150 mm length) analytical column was obtained from Waters (Milford, MA).

Sample Preparation and Analysis

Two wastewater samples were analyzed for the comparison of TOP and LC-HRMS to ^{19}F-NMR. The TOP wastewater *(WWa*) (10.0 mL) was prepared using a TOP assay and analyzed as discussed in Martin *et al.* 2019. After this, *WWa* was lyophilized (-40ºC) and reconstituted in D_4-MeOH (500 μL) with $Cracac_4$ (4 mg/mL) and analyzed with ^{19}F-NMR analysis. A second wastewater (*WWb*) sample (2.0L) was lyophilized, diluted in D_4-MeOH (1.4 mL), and split for individual ^{19}F-NMR and LC-HRMS analysis. The ^{19}F-NMR *WWb* aliquot was reduced 20%, followed by the addition of $Cracac_4$ (4 mg/mL) and hexafluorobenzene, an internal standard for quantification in all ^{19}F-NMR analyses. The LC-HRMS *WWb* aliquot (200 μL) was dried under N_2 and resuspended in 25:75 (v/v) 5mM ammonium acetate (pH 3.8): MeOH (700 μL) then analyzed by LC-HRMS analysis for targeted quantitation (n= 27) and PFAS suspect screening. Fluoromatch Flow™ 2.2 was used for suspect screening, matching theoretical exact masses to the EPA PFAS master list and known standards. Data dependent acquisition was performed for LC-HRMS samples, with any detections being manually inspected to ensure validity according to peak shape and fragmentation spectra. Detections were then graded according to the Schymanski[1] scale. Reference standards were used to confirm level 1 detections, 2a confirmed using reference spectra from Massbank of North America to compare to sample spectra, and 2b confirmed using a precursor match to the EPA PFAS Master list, -0.25 to 0.1 amu mass defect, and at least one common PFAS fragment. If an unknown PFAS had multiple potential isomers and fragmentation

is not sufficient to assist in the identification of the specific isomer, the identification as labeled as level 3.

The WW samples both underwent sample preconcentration through lyophilization to improve the method LOD. Lyophilization removes water from frozen samples at the sublimation point, so unlike SPE, lyophilization is inherently less biased because the highly polar low molecular weight PFAS are not lost during pre-concentration.

Table S1. PFAS analytes (n=32) and their respective abbreviations, target surrogate, chemical shift, and supplier for the analytes targeted in the F-NMR and LC-MS analysis, organized by class. The isotopically labelled surrogates identified are used for isotope dilution quantification in LC-MS analysis. NA denotes not applicable for analytes that are not amenable to the analysis by the LC-MS method used in this study. The reported chemical shift is the observed shift for the terminal -CF_3 group of each PFAS.

Analyte	Abbreviation	Isotopically labelled surrogate	F-NMR -CF_3 chemical shift (ppm)	Supplier
Carboxylates				
Trifluoroacetic acid[a]	TFA	NA	-77.0	Sigma Aldrich
Perfluoropropionic acid	PFPrA	NA	-84.9	Acros Organics
Perfluorobutanoic acid	PFBA	$^{13}C_4$-PFBA	-82.5	Wellington Laboratories
Perfluoropentanoic acid	PFPeA	$^{13}C_5$-PFPeA	-82.6	Wellington Laboratories
Perfluorohexanoic acid	PFHxA	$^{13}C_5$-PFHxA	-82.5	Wellington Laboratories
Perfluoroheptanoic acid	PFHpA	$^{13}C_4$-PFHpA	-82.4	Wellington Laboratories
Perfluorooctanoic acid	PFOA	$^{13}C_8$-PFOA	-82.4	Wellington Laboratories
Perfluorononanoic acid	PFNA	$^{13}C_9$-PFNA	-82.4	Wellington Laboratories
Perfluorodecanoic acid	PFDA	$^{13}C_6$-PFDA	-82.4	Wellington Laboratories
Perfluoroundecanoic acid	PFUdA	$^{13}C_7$-PFUDA	-82.4	Wellington Laboratories
Perfluorododecanoic acid	PFDoA	$^{13}C_2$-PFDoA	-82.4	Wellington Laboratories
Perfluorotridecanoic acid	PFTrDA	$^{13}C_2$-PFDoA	-82.4	Wellington Laboratories
Perfluorotetradecanoic acid	PFTeDA	$^{13}C_2$-PFTeDA	-82.4	Wellington Laboratories
Sulfonic acid				
Trifluoromethane sulfonic acid	TFMS	NA	-80.2	Alfa Aesar
Perfluorobutanesulfonic acid	PFBS	$^{13}C_3$-PFBS	-82.3	Wellington Laboratories
Perfluoropentanesulfonic acid	PFPeS	$^{13}C_3$-PFHxS	-82.5	Wellington Laboratories
Perfluorohexanesulfonic acid	PFHxS	$^{13}C_3$-PFHxS	-82.4	Wellington Laboratories

Perfluoroheptanesulfonic acid	PFHpS	$^{13}C_8$-PFOS	-82.4	Wellington Laboratories
Perfluorooctanesulfonic acid	PFOS	$^{13}C_8$-PFOS	-82.4	Wellington Laboratories
Perfluorononanesulfonic acid	PFNS	$^{13}C_8$-PFOS	-82.4	Wellington Laboratories
Perfluorodecanesulfonic acid	PFDS	$^{13}C_8$-PFOS	-82.4	Wellington Laboratories
Aminated				
Perfluorooctanesulfonamide	FOSA	$^{13}C_8$-FOSA	-82.4	Wellington Laboratories
2-(N-Methylperfluorooctanesulfonamido) acetic acid	NMeFOSAA	d3-N-MEFOSAA	-82.4	Wellington Laboratories
2-(N-Ethylperfluorooctanesulfonamido) acetic acid	NEtFOSAA	d5-EtFOSAA	-82.4	Wellington Laboratories
Fluorotelomers				
Fluorotelomer sulfonic acid 4:2	4:2 FTS	$^{13}C_2$-4:2 FTS	-82.7	Wellington Laboratories
Fluorotelomer sulfonic acid 6:2	6:2 FTS	$^{13}C_2$-6:2 FTS	-82.4	Wellington Laboratories
Fluorotelomer sulfonic acid 8:2	8:2 FTS	$^{13}C_2$-8:2 FTS	-82.4	Wellington Laboratories
Carboxylate Ethers				
Hexafluoropropylene oxide dimer acid	HFPO-DA	NA	-82.9	Wellington Laboratories
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	NA	-88.3	Synquest
Perfluoro-4-methoxybutanoic acid	PFMBA	NA	-56.8	Sigma Aldrich
Perfluoro-3-methoxypropanoic acid	PFMPA	NA	-56.9	Synquest
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	NA	-57.0	Apollo Scientific

[a]TFA was not included in the initial training set for method development, but was added later for detection confirmation

Table S2.

Extrapolation of limit of detection (LOD) based on scans performed on PFOS solutions in D_4-MeOH with $Cracac_4$. The S/N ratios for the -CF_3 shift was used to extrapolate the methods LOD, which was concluded to be 50 µg/L.

Concentration (µg/L):	Transients (nt):	S/N:
2,000	100	12
2,000	1800	48
500	1800	12
500	16000	36
50	16000	3.6

Equation S1. Quantification of PFAS analytes with internal standard, hexafluorobenzene (HFB).

$$C_{PFAS} = \frac{Integral\ of\ Target\ Peak}{Integral\ of\ HFB} \times \frac{\#\ of\ Nucleii\ (HFB)}{\#\ of\ Nucleii\ (-CF_3)} \times C_{HFB}$$

Where:

C_{PFAS}:$Concentration\ of\ total\ PFAS\ (-CF_3)$

C_{HFB}:$Concentration\ of\ HFB\ in\ NMR\ tube\ (\mu M)$

Ex. TOP total PFAS

$$C_{PFAS} = \frac{11.18}{20.79} \times \frac{6}{3} \times 4.53 \times 10^{-5}$$

$$C_{PFAS}: 195.04\ \mu M$$

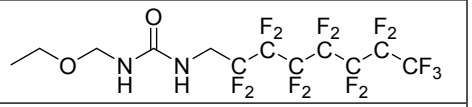
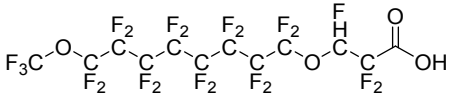
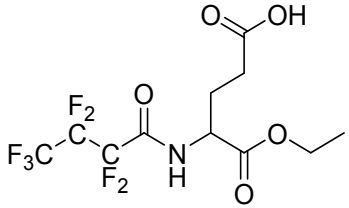
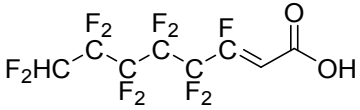
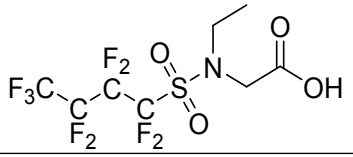
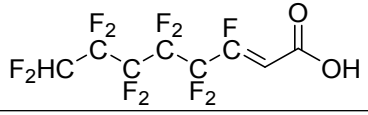
Table S3. Total oxidizable precursor quantitation using liquid chromatography with mass spectrometry. Results are expressed as µM for each carboxylate and sulfonate end product of the oxidation reaction. A total of 6.66 µM of PFAS were detected in the *WWa* sample.

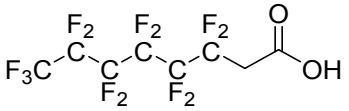
Analyte	Concentration (µMole / L)	Concentration (µg/L)
PFBS	1.57E-05	4.71E-03
PFPeS	4.00E-06	1.40E-03
PFHxS	3.81E-06	1.52E-03
PFBA	1.92E+00	4.11E+02
PFPeA	3.45E+00	9.11E+02
PFHxA	1.14E+00	3.58E+02
PFHpA	1.59E-01	5.79E+01
PFOA	7.26E-05	3.01E-02
PFNA	2.85E-05	1.32E-02
PFDA	3.89E-06	2.00E-03
Total PFAS	6.66E+00	1.74E+03

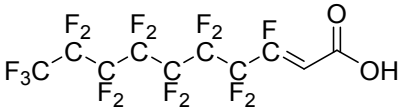
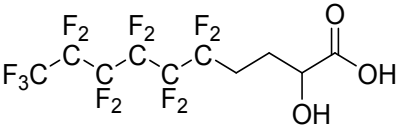
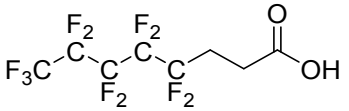
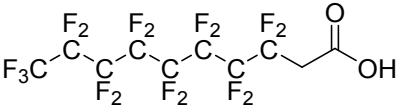
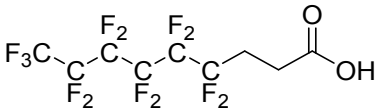
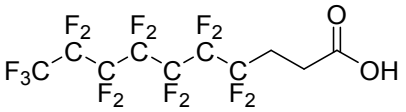
Table S4. LC-HRMS quantitative targeted analysis results for individual PFAS detected in WWb (μM) and the calculated total PFAS within the sample. The total PFAS detected was 6.926 μM.

Analyte	Concentration (μMole / L)	Concentration (μg/L)
6:2 FTS	0.738	316
8:2 FTS	0.009	4.75
N-Me-FOSAA	0.124	70.8
N-Et-FOSAA	0.033	19.3
PFHxS	0.065	26.0
PFOA	1.365	565
PFOS	0.096	48.0
PFBA	0.738	158
PFBS	0.034	10.2
PFDA	0.136	69.9
PFHpA	0.625	228
PFHxA	2.065	649
PFNA	0.047	21.8
PFPeA	0.844	223
PFPeS	0.006	2.10
PFUdA	0.002	1.13
Total PFAS	6.926	2412

Table S5. PFAS detected with high resolution mass spectrometry suspect screening. Detections were supported by an exact mass match (±5 ppm mass error), at least 2 structurally relevant fragments, and isotopic pattern matches. Homologous series are considered detections separated by a single -CF_2 unit and are denoted by *,‡,†.

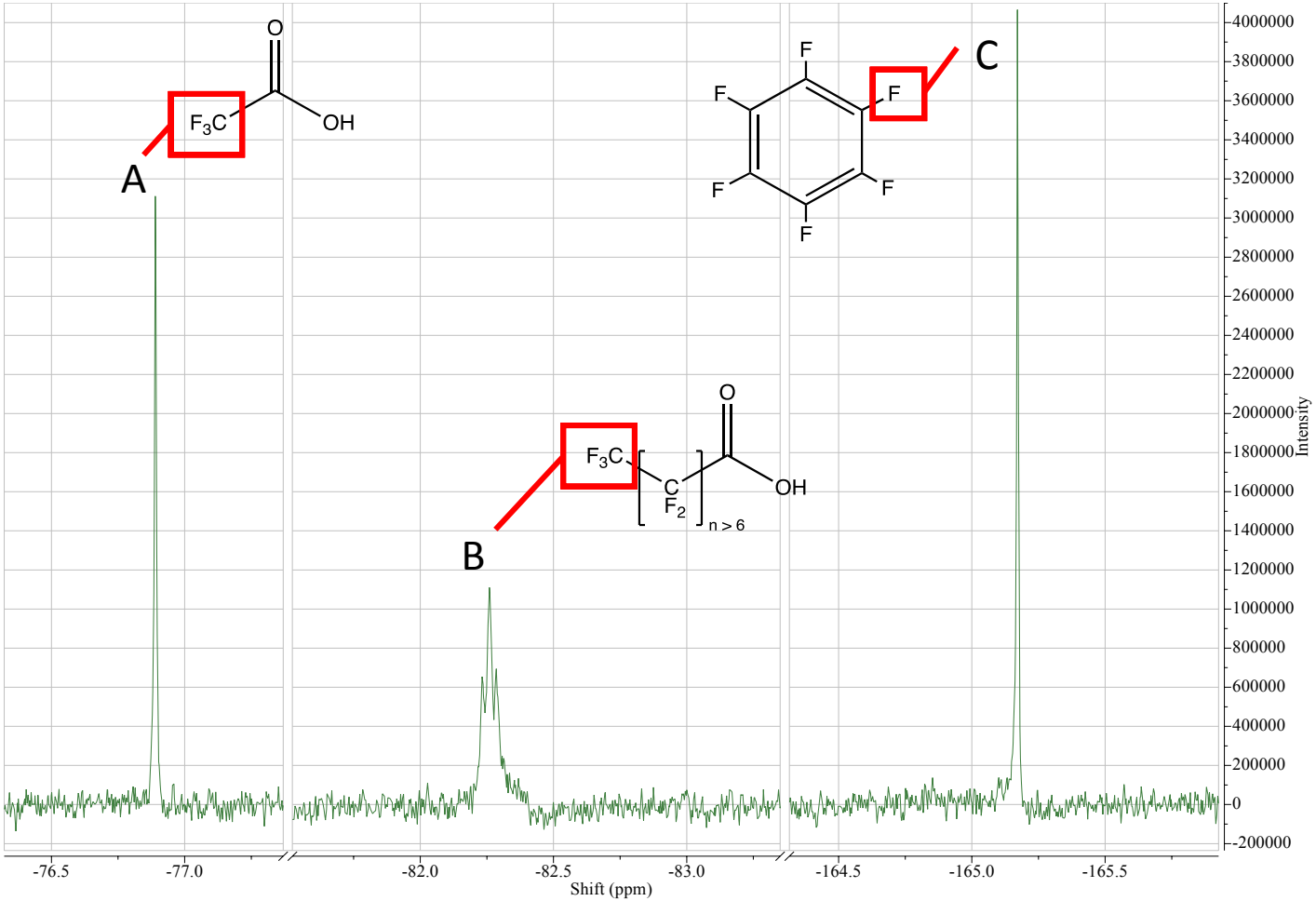
Name	m/z	Retention time (min)	Chemical Formula	Fragments	Detection Confidence	Structure
1-(ethoxymethyl)-3-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctyl)urea	499.0533	11.95	$C_{12}H_{11}F_{15}N_2O_2$	C_4F_9	3c	
2,2,3-trifluoro-3-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-hexadecafluoro-8-(trifluoromethoxy) octyl)oxy) propanoic acid	626.9533	14.09	$C_{12}H_2F_{22}O_4$	C_2F_5, C_5F_{11}	3c	
N-(Perfluorobutanoyl)glutamic acid 1-ethyl ester	370.0542	14.86	$C_{11}H_{12}F_7NO_5$	$C_9H_7F_6NO_3$, $C_{10}H_{11}F_7NO_3$	3a	
3,4,4,5,5,6,6,7,7,8,8,8-dodecafluorooct-2-enoic acid*	356.9794	16.46	$C_8H_2F_{12}O_2$	C_7F_{11}	1b	
N-Ethyl-N-[(nonafluorobutyl)sulfonyl]glycine	383.9960	17.87	$C_8H_8F_9NO_4S$	C_4F_9, $C_4F_9SO_2$, $C_6H_7F_9NSO_2$	2b	
3,4,4,5,5,6,6,7,7,8,8-Undecafluorooct-2-enoic acid	338.9885	17.89	$C_8H_3F_{11}O_2$	C_2F_5, C_5F_{11}	3c	

6:2 Fluorotelomer carboxylic acid‡	376.9855	18.11	$C_8H_3F_{13}O_2$	CO_2F, C_7F_{11}	1b	

Name	m/z	Retention time (min)	Chemical Formula	Fragments	Detection Confidence	Structure
3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-hexadecafluorodec-2-enoic acid*	456.9728	19.60	$C_{10}H_2F_{16}O_2$	C_9F_{15}	2c	
5,5,6,6,7,7,8,8,9,9,10,10,10-Tridecafluoro-2-hydroxydecanoic acid	421.0118	20.32	$C_{10}H_7F_{13}O_3$	C_7F_9, C_7F_7	3c	
5:3 Fluorotelomer carboxylic acid†	341.0042	20.70	$C_8H_5F_{11}O_2$	C_7F_7, C_7HF_8	1b	
7:2 Fluorotelomer carboxylic acid‡	476.9791	21.31	$C_8H_2F_{12}O_2$	CO_2F, C_9F_{15}	2c	
6:3 Fluorotelomer carboxylic acid†	391.0012	22.43	$C_9H_5F_{13}O_2$	C_7F_7, C_8F_9, C_8HF_{10}	2c	
7:3 Fluorotelomer carboxylic acid†	440.9979	24.06	$C_{10}H_5F_{15}O_2$	C_8F_9, C_9F_{11}, C_9HF_{12}	1b	

* unsaturated polyfluorocarboxylic acid homologous series
‡ n:2 Fluorotelomer carboxylic acid homologous series
† n:3 Fluorotelomer carboxylic acid homologous series

Figure S1. F-NMR spectra of the landfill leachate extract, with a 100 µg/L PFOS standard addition to elucidate the triplet character of the -CF_3 peak. Peaks are labeled at (A) trifluoroacetic acid (TFA), (B) -CF_3 of per- and polyfluoroalkyl substances (PFAS), and (C) hexafluorobenezene. In this example, (B) shows the clear triplet peak of the -CF_3 shift from the PFOS standard addition. This supports the environmental detection of -CF_3 proximal to the LOD.



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