Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Electronic Supplementary Information for:

Evaluation, Optimization, and Application of Three Independent Suspect Screening Workflows for the Characterization of PFASs in Water

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Table S1: List of 33 target PFASs included in suspect screening optimization.

Name	Acronym	Molecular Formula	Supplier	Concen-tration	Solvent
Perfluoro-2-methyl-3-oxahexanoic acid	GenX	C6HF11O3	SynQuest Laboratories	1 g/L	100% Methanol
Perfluorobutanoic acid	PFBA	C4HF7O2	Sigma-Aldrich	1 g/L	100% Methanol
Perfluoropentanoic acid	PFPeA	C5HF9O2	Alfa Aesar	1 g/L	100% Methanol
Perfluorohexanoic acid	PFHxA	C6HF11O2	TCI	1 g/L	96% MeOH + 4%H_2O
Perfluoroheptanoic acid	PFHpA	C7HF13O2	Sigma-Aldrich	1 g/L	96% MeOH + 4%H_2O
Perfluorooctanoic acid	PFOA	C8HF15O2	Sigma-Aldrich	1 g/L	100% Methanol
Perfluorononanoic acid	PFNA	C9HF17O2	Sigma-Aldrich	1 g/L	96% MeOH + 4%H_2O
Perfluorodecanoic acid	PFDA	C10HF19O2	Alfa Aesar	1 g/L	96% MeOH + 4%H_2O
Perfluoroundecanoic acid	PFUnA	C11HF21O2	Chem Cruz	1 g/L	96% MeOH + 4%H_2O
Perfluorododecanoic acid	PFDoA	C12HF23O2	Alfa Aesar	1 g/L	96% MeOH + 4%H_2O
Perfluorotridecanoic acid	PFTrDA	C13HF25O2	Chem Cruz	1 g/L	100% Ethyl Acetate
Perfluorotetradecanoic acid	PFTA	C14HF27O2	Chem Cruz	1 g/L	100% Ethyl Acetate
Perfluorobutanesulfonic acid	PFBS	C4HF9O3S	TCI	1 g/L	100% Methanol
Perfluoropentanesulfonic acid	PFPeS	C5HF11SO3	Wellington Laboratories	50 mg/L	100% Methanol
Perfluorohexanesulfonic acid	PFHxS	C6HF13O3S	Chem Cruz	1 g/L	100% Methanol
Perfluoroheptanesulfonic acid	PFHpS	C7HF15SO3	Wellington Laboratories	50 mg/L	100% Methanol

Name	Acronym	Molecular Formula	Supplier	Concen-tration	Solvent
Perfluorooctanesulfonic acid	PFOS	C8HF17O3S	Chem Cruz	1 g/L	100% Methanol
Perfluorononanesulfonic acid	PFNS	C9HF19SO3	Wellington Laboratories	50 mg/L	100% Methanol
Perfluorodecanesulfonic acid	PFDS	C10HF21SO3	Wellington Laboratories	50 mg/L	100% Methanol
Perfluoro-1-butanesulfonamide	FBSA	C4H2F9NO2S	Wellington Laboratories	50 mg/L	100% Methanol
Perfluoro-1-octanesulfonamide	FOSA	C8H2F17NO2S	Astatech	1 g/L	100% Methanol
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	C11H6F17NO4S	Wellington Laboratories	50 mg/L	100% Methanol
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	C12H8F17NO4S	Wellington Laboratories	50 mg/L	100% Methanol
1H,1H,2H,2H-Perfluorohexanesulphonic acid	4:2 FTS	C6H5F9O3S	Wellington Laboratories	50 mg/L	100% Methanol
3,3,4,4,5,5,6,6,7,7,8,8,8,-Tridecafluorooctane-1-sulphonic acid	6:2 FTS	C8H5F13O3S	Santa Cruz Biotechnology	1 g/L	100% Methanol
1h,1h,2h,2h-Perfluorooctanesulfonic acid	8:2 FTS	C10H5F17O3S	Wellington Laboratories	50 mg/L	100% Methanol
N-(3-dimethylaminopropan-1-yl)perfluoro-1-hexane-sulfonamide	AP-FHxSA	C11H13F13N2O2S	Wellington Laboratories	50 mg/L	100% Methanol
N-[3-perfluoro-1-hexanesulfonamido)propan-1-yl]-N,N,N-trimethylammonium	TAmP-FHxSA	C12H15F13N2O2S	Wellington Laboratories	50 mg/L	100% Methanol
6:2 fluorotelomer sulfonamide alkylbetanine	6:2 FTAB	C15H19F13N2O4S	Wellington Laboratories	50 mg/L	100% Methanol
2-[(4,4,5,5,6,6,7,7,8,8,8-Undecafluorooctyl)dimethylammonio]acetate	5:3 FTB	C12H14F11NO2	Wellington Laboratories	50 mg/L	100% Methanol
2-[(3,4,4,5,5,6,6,7,7,8,8,8-Dodecafluorooctyl)dimethylammonio]acetate	5:1:2 FTB	C12H13F12NO2	Wellington Laboratories	50 mg/L	100% Methanol
2H-Perfluoro-2-octenoic acid (6:2)	FHUEA	C8H2O2F12	Wellington Laboratories	50 mg/L	100% Isopropanol
2H-Perfluoro-2-decenoic acid (8:2)	FOUEA	C10H2O2F16	Wellington Laboratories	50 mg/L	100% Isopropanol

Sample analysis.

Table S2: Loading and elution pump gradients for samples prepared for large-volume injection (LVI).

Retention time (min)	**Flow** ($mL \cdot min^{-1}$)	**%A** (Water)	**%B*** (Methanol)
Loading pump			
0.0	1.000	99	1
5.1	1.000	99	1
5.11	0.000	99	1
37.2	0.000	99	1
37.3	1.000	2	98
41.3	1.000	2	98
41.4	1.000	99	1
42.1	1.000	99	1
Elution pump			
0.0	0.300	60	40
6.1	0.300	60	40
30.1	0.300	10	90
37.1	0.300	10	90
37.2	0.300	60	40
42.1	0.300	60	10

* A = LC-MS-grade water with 20 mM ammonium acetate. B = LC-MS-grade methanol.

Table S3: Elution pump gradient for samples prepared for small-volume injection (SVI).

Retention time (min)	**Flow** ($mL \cdot min^{-1}$)	**%A** (Water)	**%B*** (Methanol)
Elution pump			
0.0	0.300	60	40
1.0	0.300	60	40
25.0	0.300	10	90
32.0	0.300	10	90
32.1	0.300	60	40
37.0	0.300	60	40

* A = LC-MS-grade water with 20 mM ammonium acetate acid. B = LC-MS-grade methanol.

Table S4: Source, full-scan MS, and data-dependent MS2 (dd-MS^2) parameters.

Source parameters	
Voltage	+3.5 kV / -2.5 kV
Sheath gas flow	48 AU
Auxiliary gas flow	11 AU
Sweep gas flow	2 AU
Capillary temperature	320 ºC
S-lens RF level	50 AU
Auxiliary gas heater temperature	413 ºC
Full-scan MS	
m/z range	100-1500
Resolution	140,000 at 200 *m/z*
automatic gain control target	500,000
Maximum injection time	200 ms
dd-MS^2	
Resolution	17,500 at 200 *m/z*
Automatic gain control target	200,000
Maximum injection time	100 ms
Loop count	3
Isolation window	1.0 *m/z*
(N)CE/stepped NCE	20, 40, 60
Dynamic exclusion	6 s
When idle	Pick others

Table S5: Analytical details for each of the 33 PFASs included in the test samples.

Name	Acronym	Chemical Formula	Adduct	Extract Mass (*m/z*)	LVI RT (min)	SVI RT (min)	Diagnostic Fragment (*m/z*)
Perfluoro-2-methyl-3-oxahexanoic acid	GenX	C6HF11O3	[M-H]-	328.9672	17.47	11.22	118.99
Perfluorobutanoic acid	PFBA	C4HF7O2	[M-H]-	212.9786	9.63	3.76	168.99
Perfluoropentanoic acid	PFPeA	C5HF9O2	[M-H]-	262.9760	12.76	6.88	218.99
Perfluorohexanoic acid	PFHxA	C6HF11O2	[M-H]-	312.9722	16.53	10.32	268.98
Perfluoroheptanoic acid	PFHpA	C7HF13O2	[M-H]-	362.9690	19.48	13.32	318.98
Perfluorooctanoic acid	PFOA	C8HF15O2	[M-H]-	412.9659	21.89	15.77	368.98
Perfluorononanoic acid	PFNA	C9HF17O2	[M-H]-	462.9627	23.91	17.87	418.97
Perfluorodecanoic acid	PFDA	C10HF19O2	[M-H]-	512.9600	25.64	19.60	468.97
Perfluoroundecanoic acid	PFUnA	C11HF21O2	[M-H]-	562.9563	27.10	21.08	518.97
Perfluorododecanoic acid	PFDoA	C12HF23O2	[M-H]-	612.9531	28.37	22.38	568.96
Perfluorotridecanoic acid	PFTrDA	C13HF25O2	[M-H]-	662.9499	29.48	23.49	618.96
Perfluorotetradecanoic acid	PFTA	C14HF27O2	[M-H]-	712.9467	30.48	24.47	668.96
Perfluorobutanesulfonic acid	PFBS	C4HF9O3S	[M-H]-	298.9424	13.68	7.53	79.96
Perfluoropentanesulfonic acid	PFPeS	C5HF11SO3	[M-H]-	348.9398	16.65	10.78	79.96
Perfluorohexanesulfonic acid	PFHxS	C6HF13O3S	[M-H]-	398.9360	19.67	13.54	79.96
Perfluoroheptanesulfonic acid	PFHpS	C7HF15SO3	[M-H]-	448.9334	21.68	15.84	79.96
Perfluorooctanesulfonic acid	PFOS	C8HF17O3S	[M-H]-	498.9296	23.86	17.86	79.96
Perfluorononanesulfonic acid	PFNS	C9HF19SO3	[M-H]-	548.9270	25.28	19.53	79.96

Name	Acronym	Chemical Formula	Adduct	Extract Mass (*m/z*)	LVI RT (min)	SVI RT (min)	Diagnostic Fragment (*m/z*)
Perfluorodecanesulfonic acid	PFDS	C10HF21SO3	[M-H]-	598.9238	26.73	20.98	79.96
Perfluoro-1-butanesulfonamide	FBSA	C4H2F9NO2S	[M-H]-	297.9590	15.77	10.3	77.96
Perfluoro-1-octanesulfonamide	FOSA	C8H2F17NO2S	[M-H]-	497.9462	25.86	20.55	77.96
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	C11H6F17NO4S	[M-H]-	569.9673	26.16	20.42	82.96
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	C12H8F17NO4S	[M-H]-	583.9830	26.89	21.71	82.96
1H,1H,2H,2H-Perfluorobutanesulfonic acid	4:2 FTS	C6H5F9O3S	[M-H]-	326.9743	15.85	9.99	80.96
1H,1H,2H,2H-Perfluorohexanesulfonic acid	6:2 FTS	C8H5F13O3S	[M-H]-	426.9679	21.46	15.64	80.96
1H,1H,2H,2H-Perfluorooctanesulfonic acid	8:2 FTS	C10H5F17O3S	[M-H]-	526.9615	25.31	19.56	80.96
N-(3-dimethylaminopropan-1-yl)perfluoro-1-hexane-sulfonamide	AP-FHxSA	C11H13F13N2O2S	[M+H]+ [M-H]-	485.0563 483.0428	23.43	18.2	85.09 118.99
N-[3-perfluoro-1-hexanesulfonamido)propan-1-yl]-N,N,N-trimethylammonium	TAmP-FHxSA	C12H15F13N2O2S	[M+H]+	499.0719	21.66	16.40	60.08
6:2 fluorotelomer sulfonamide alkylbetanine	6:2 FTAB	C15H19F13N2O4S	[M+H]+	571.0931	22.38	17.1	58.07
2-[(4,4,5,5,6,6,7,7,8,8,8-Undecafluorooctyl)dimethylammonio]acetate	5:3 FTB	C12H14F11NO2	[M+H]+	414.0922	18.92	13.64	58.07
2-[(3,4,4,5,5,6,6,7,7,8,8,8-Dodecafluorooctyl)dimethylammonio]acetate	5:1:2 FTB	C12H13F12NO2	[M+H]+	432.0827	18.55	13.27	58.07
2H-Perfluoro-2-octenoic acid (6:2)	FHUEA	C8H2O2F12	[M-H]-	356.9790	19.40	15.90	92.99
2H-Perfluoro-2-decenoic acid (8:2)	FOUEA	C10H2O2F16	[M-H]-	456.9727	23.90	19.70	118.99

Post-acquisition data processing.

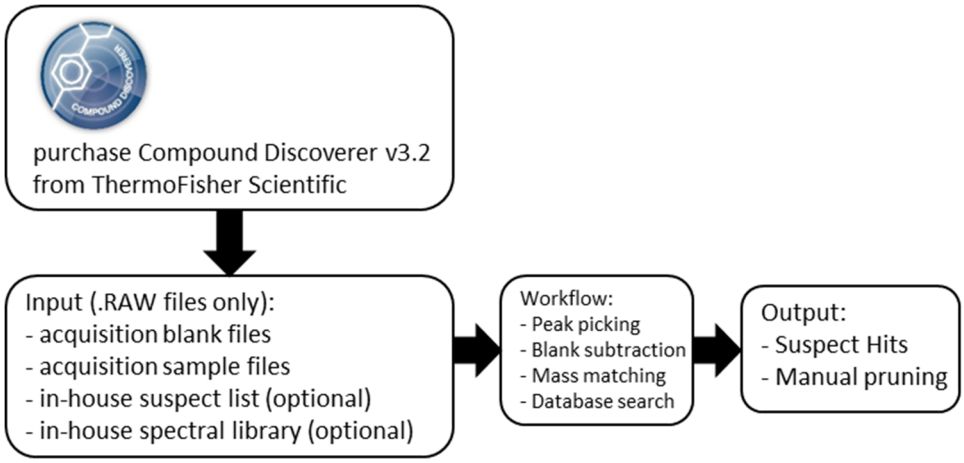

Figure S1: Requirements for implementation of the Compound Discoverer PFAS suspect screening workflow. The analyst must import .RAW files from a Thermo mass spectrometer to initiate a workflow. We imported .RAW files representing "blank" and "sample" acquisitions. We also imported our suspect list (see Methods section of main manuscript) as a Local Database and used matches to this list as one of our final criteria for identifying suspect PFASs. The result of the workflow is exported as an .xlsx and manually curated (or pruned) as described in the main manuscript to derive a final list of suspect PFAS detections.

Table S6: Optimized parameter settings for PFAS suspect screening with *Compound Discoverer v3.2*.

Select Spectra	
Lower RT Limit (min)	0
Upper RT Limit (min)	0
First Scan	0
Last Scan	0
Ignore Specified Scans	
Lowest Charge State	0
Highest Charge State	0
Min. Precursor Mass	100 Da
Max. Precursor Mass	1500 Da
Total Intensity Threshold	0
Minimum Peak Count	1
Mass Analyzer	Any
MS Order	Any
Activation Type	Any
Min. Collision Energy	0
Max. Collision Energy	1000
Scan Type	Any
Polarity Mode	Any
S/N Threshold (FT-only)	3
Unrecognized Charge Replacements	1
Unrecognized Mass Analyzer Replacements	ITMS
Unrecognized MS Order Replacements	MS2
Unrecognized Activation Type Replacements	CID
Unrecognized Polarity Replacements	+
Unrecognized MS Resolution@200 Replacements	60000
Unrecognized MSn Resolution@200 Replacements	30000
Precursor Selection	Use MS(n-1) Precursor
Use Isotope Pattern in Precursor Reevaluation	True
Provide Profile Spectra	Automatic
Store Chromatograms	False
Align retention times	
Alignment Model	Adaptive Curve
Alignment Fallback	Use Linear Model
Maximum Shift (min)	2
Shift Reference File	True
Mass Tolerance	5 ppm
Remove Outlier	True
Detect Compounds	
Mass Tolerance (ppm)	5 ppm
Intensity Tolerance (%)	30

S/N Tolerance	3
Min. Peak Intensity	10000
Ions	[2M-H]-1; [2M-H+HAc]-1; [M+Cl]-1; [M+FA-H]-1; [M+H]+1; [M-2H]-2; [M-2H+K]-1; [M-H]-1; [M-H+HAc]-1; [M-H+TFA]-1; [M-H-H2O]-1
Base Ions	[M+H]+1; [M-H]-1
Min. Element Counts	CHF
Max. Element Counts	C90 H190 Br3 Cl4 F40 K2 N10 Na2 O18 P3 S5
Filter Peaks	True
Max. Peak Width (min)	0.8
Remove Singlets	True
Min. # Scans per Peak	10
Min. # Isotopes	3
Min. Spectral Distance Score	50
Remove Potentially False Positive Isotopes	True
Merge Features	
Mass Tolerance	5 ppm
RT Tolerance (min)	0.2
Group Compounds	
Mass Tolerance	5 ppm
RT Tolerance (min)	0.2
Preferred Ions	[M+H]+1; [M-H]-1
Fill Gaps	
Mass Tolerance	5 ppm
S/N Threshold	1.5
Use Real Peak Detection	True
Mark Background Compounds	
Max. Sample/Blank	5
Max. Blank/Sample	5
Hide Background	True
Predict Compositions	
Mass Tolerance	5 ppm
Min. Element Counts	CHF
Max. Element Counts	C90 H190 Br3 Cl8 F40 N10 O18 P3 S5
Min. RDBE	0
Max. RDBE	40
Min. H/C	0
Max. H/C	3.5
Max. # Candidates	10
Max. # Internal Candidates	500
Intensity Tolerance (%)	30
Intensity Threshold (%)	0.1
S/N Threshold	3

Min. Spectral Fit (%)	50
Min. Pattern Cov. (%)	80
Use Dynamic Recalibration	True
Use Fragments Matching	True
Mass Tolerance	40 ppm
S/N Threshold	3
Search Mass Lists	
Mass lists	PFAS Suspect List (defined in Methods section on manuscript)
Use Retention Time	True
RT Tolerance (min)	4
Mass Tolerance	5 ppm
Assign Compound Annotations	
Mass Tolerance	5 ppm
Data Source #1	MassList Search
Data Source #2	mzCloud Search
Data Source #3	ChemSpider Search
Data Source #4	Predicted Compositions
Use mzLogic	True
Use Spectral Distance	True
SFit Threshold	20
SFit Range	20
Compound Class Scoring	
Compound Classes[a]	\PFAS General .cLib|\PFSAs.cLib
S/N Threshold	5
High Acc. Mass Tolerance	50 ppm
Low Acc. Mass Tolerance	0.5 Da
Use Full MS Tree	False
Allow DIA Scoring	True

[a] The Compound Classes manually inputted into the "Compound Classes Library" were the "PFAS General Class" and the "PFSAs" (adapted from Nason et al.[1]). The "PFAS General" compound class included the fragments: $C_2F_5^-$ (118.9926), $C_3F_7^-$ (168.9894), $C_4F_9^-$ (218.9862), $C_5F_{11}^-$ (268.9830), FSO_3^- (98.9556), FSO_2^- (82.9609), CF_3O^- (84.9907), and CF_3^- (68.9958). The "PFSAs" compound class included the fragments: SO_3^- (79.9574) and FSO_3^- (98.9558).

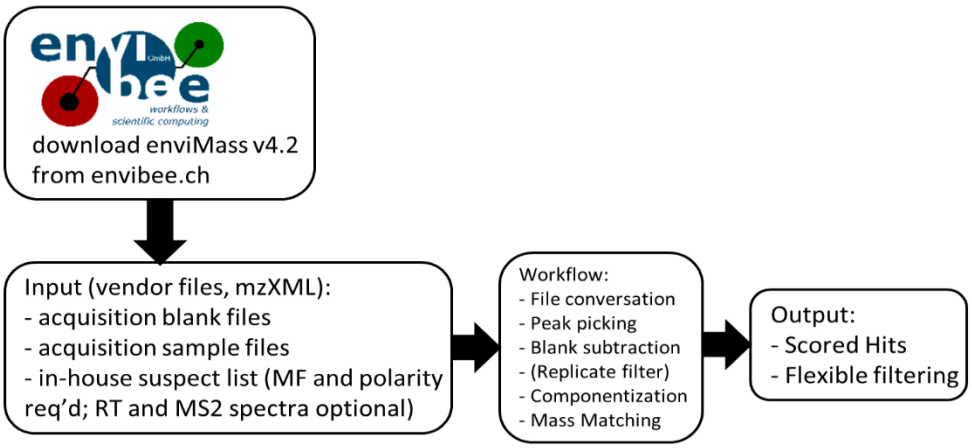

Figure S2: Requirements for implementation of the enviMass PFAS suspect screening workflow. MF = neutral molecular formula; RT = retention time; MS2 = MS2 fragment masses. The analyst must import acquisition files or .mzXML files derived from the acquisition files to initiate a workflow. We imported .mzXML files representing "blank" and "sample" acquisitions. We also imported our suspect list (see Methods section of main manuscript) as a Target List and used matches to this list as one of our final criteria for identifying suspect PFASs. We note that we did include "characteristic" MS2 spectra into the *enviMass* workflow, although the use of MS2 fragments did not improve the performance of the suspect screening workflow and they were not used in interpreting the final optimized results.

Table S7: Optimized parameter settings for PFAS suspect screening with *enviMass v4.2*.

Instrument/Resolution	
Instrument resolution	Q-Exactive,ExactivePlus_R140000@200
Method Setup	
Define new method	Type in File ID → Select above scan types to include → [1] and [2] → Save
Peak picking	
Include estimation?	No
Maximum RT gap in an EIC	300 s
Maximum *m/z* deviation of an EIC centroid	3.5 ppm
Minimum number of points per peak	4 within 8 s
Maximum RT gap to be interpolated	10 s
Maximum RT width of a peak	120 s
Minimum intensity threshold	10^3
Minimum signal/noise	4
Minimum signal/base	2
Maximum possible number of peaks with EIC	3
Tolerances	
± m/z tolerance	5 ppm
Maximum RT deviation	2 s
Replicates	
m/z tolerance	5 ppm
RT tolerance	20 s
Screening	
RT tolerance from expected	240 s
Screen for MS2 fragments?	TRUE
± m/z tolerance	100 ppm
Only screen MS2 if MS1…	TRUE
Cutoff score	0.9
Adducts	$[M+H]^+$, [M+], $[M-H]^-$
Profiles (Fast)	
m/z tolerance	5 ppm
RT tolerance	20 s
Blind	
Ratio by which the sample intensity…	100
m/z tolerance	12 ppm
RT tolerance	30 s
Componentization (isotopologue/adduct)	
m/z tolerance	2.5 ppm
RT tolerance	5 s
Adducts (Positive)	$[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, $[M]^+$
Adducts (Negative)	$[M-H]^-$, $[M+FA-H]^-$, $[M+Cl]^-$, $[M]^-$

Table S8: Optimized workflow for PFAS suspect screening with *enviMass v4.2*.

Preprocessing	
Mass recalibration	No
RT Alignment	No
Replicate filter	Yes
Blank/Blind peak detection	Yes
Targets	
Screen internal standards	No
Screen target compounds	Yes
Intensity Normalization using ISTD-profiles	No
Non-targets	
Peak-shape correlation	Yes
Isotopologue grouping	Yes
Adduct grouping	Yes
Homologue series detection	No
Profile componentization	Yes
Watchlist screening	No
Concentrations	
Calibration	No
Quantification	No
Recovery	No
Profiling	
Profile extraction	Yes
Omit peaks from blind files	Yes
Omit peaks from spiked files	No
Trend detection	No
Comparisons	No

Table S9: Optimized filtering of results for PFAS suspect screening with *enviMass v4.2*.

Results → Cross-file profiling → Filtering	
Components	
Omit lower ranked profiles in each component	Yes (greedy)
... but omit profiles not having any isotopologues in their component prior to that?	No
... but omit profiles not being part of homologue series prior to that?	No
... and extend componentization over all homologue series relations (experimental)?	No
Filter	
Filter profiles by mean sample vs. blind intensity ratio ..	Yes
Mean sample vs. blind intensity ratio:	1.5
... or remove all profiles which contain any blind peaks at all?	No
Filter profiles by time-consecutive number of peaks (i.e., to omit noise profiles with only sporadic peak detections in times series):	Yes
Minimum number of consec. time points:	3
Include profiles of certain target compounds ONLY, by their tag1 label? Intersects with the above selection by compound name or ID, if any.	All
Filter by minimum number of sample peaks in a profile?	No
Filter by maximum number of sample peaks in a profile?	No

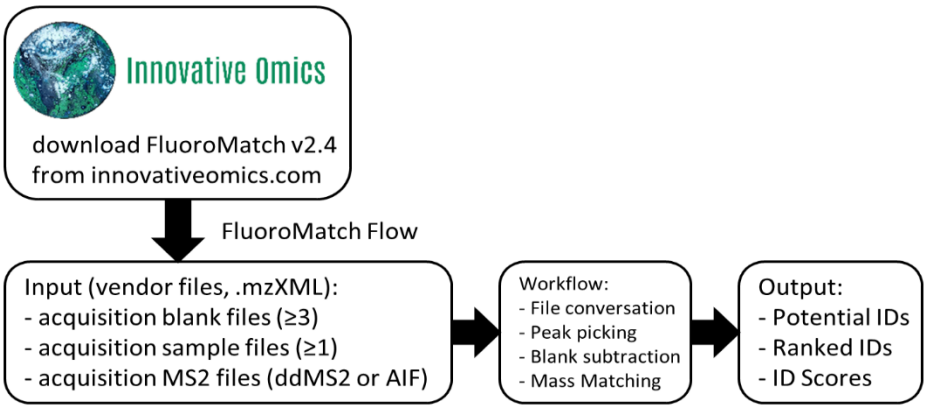

Figure S3: Requirements for implementation of the *FluoroMatch* PFAS suspect screening workflow. The analyst must import acquisition files to initiate a workflow. No suspect list is needed and no MS2 spectra are needed; *FluoroMatch* relies on internal libraries and external databases for exact mass matching and uses literature data or *in silico* techniques for MS2 fragment screening. The results are contained in a .csv file that can be interpreted in different ways to identify suspect PFASs in the samples (see discussion in the main manuscript).

References

1 S. L. Nason, J. Koelmel, N. Zuverza-Mena, C. Stanley, C. Tamez, J. A. Bowden and K. J. Godri Pollitt, Software Comparison for Nontargeted Analysis of PFAS in AFFF-Contaminated Soil, *J. Am. Soc. Mass Spectrom.*, 2021, **32**, 840–846.