Environmental Science & Technology Letters

pubs.acs.org/journal/estlcu

Global Perspective

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

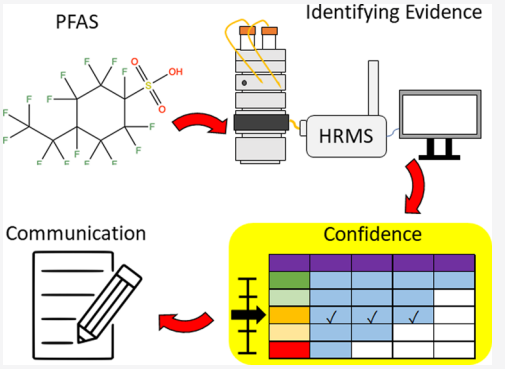
Joseph A. Charbonnet, Carrie A. McDonough, Feng Xiao, Trever Schwichtenberg, Dunping Cao, Sarit Kaserzon, Kevin V. Thomas, Pradeep Dewapriya, Benjamin J. Place, Emma L. Schymanski, Jennifer A. Field, Damian E. Helbling, and Christopher P. Higgins*

Cite This: https://doi.org/10.1021/acs.estlett.2c00206

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Per- and polyfluoroalkyl substances (PFASs) are important environmental contaminants, yet relatively few analytical reference standards exist for this class. Nontarget analyses performed by means of high-resolution mass spectrometry (HRMS) are increasingly common for the discovery and identification of PFASs in environmental and biological samples. The certainty of PFAS identifications made via HRMS must be communicated through a reliable and harmonized approach. Here, we present a confidence scale along with identification criteria specific to suspect or nontarget analysis of PFASs by means of nontarget HRMS. Confidence levels range from level 1a—"Confirmed by Reference Standard," and level 1b—"Indistinguishable from Reference Standard," to level 5—"Exact Masses of Interest," which are identified by suspect screening or data filtering, two common forms of feature prioritization. This confidence scale is consistent with general criteria for communicating confidence in the identification of small organic molecules by HRMS (e.g., through a match to analytical reference standards, library MS/MS, and/or retention times) but incorporates the specific conventions and tools used in PFAS classification and analysis (e.g., detection of homologous series and specific ranges of mass defects). Our scale clarifies the level of certainty in PFAS identification and, in doing so, facilitates more efficient identification.



KEYWORDS: *per- and polyfluoroalkyl substances (PFASs), high-resolution mass spectrometry (HRMS), confidence scale, nontarget analysis (NTA), suspect screening, isomers*

INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are a large class of environmental contaminants, many of which are ubiquitous and persistent in the environment.[1−3] Since PFAS structures are often proprietary and analytical reference standards exist for relatively few PFASs, nontarget analyses are essential for the discovery and identification of PFASs in environmental and biological samples. High-resolution mass spectrometry (HRMS) is a powerful technique that can be used for nontarget analysis of environmental organic contaminants and is an increasingly common analytical choice for PFAS investigations.

Guidance[4] and tools[5−7] have been developed to facilitate nontarget HRMS PFAS analysis. However, no PFAS-specific guidance exists for communicating confidence in the certainty of HRMS identifications. Schymanski et al. provided a broad guidance for communicating confidence in nontarget HRMS identifications with varying levels of structural certainty and invited researchers to define sublevels "on a per-study basis where evidence supporting different proposed structures is clearly presented."[8] In this Global Perspective, we contextualize those 2014 guidelines and define sublevels and identification criteria specific to the study of PFASs which contain a C_nF_{2n+1} moiety with $n \geq 2$ (i.e., typically PFASs which occur in homologous series). This clarification and contextualization is necessary due to the characteristics of PFASs. For example, the structures of PFASs found in a homologous series differ only by the number of perfluorinated carbons in their tail. Consequently, confident identification of a single homologue can provide evidence to support the identification of other homologues in the series. However, studies have varied in their interpretations of the certainty provided by homologue detections,[4,9,10] suggesting the need for more harmonized communication of confidence.

Greater clarity is also needed in communicating confidence in isomeric PFAS structures. For example, electrochemical fluorination produces both branched and linear PFAS isomers,

Received: March 25, 2022
Revised: May 19, 2022
Accepted: May 19, 2022

and the relevant branching occurs only in the perfluoroalkyl tail.[11] Branched and linear isomers are conventionally considered the same PFASs for regulatory purposes, and data are often provided for summed isomer concentrations. The position of the branching is not routinely distinguished by liquid chromatography (LC)-HRMS.[12] However, when the isomerization occurs in the headgroup, the structures are conventionally considered distinct PFASs. Similarly, constitutional isomers (i.e., structural isomers with distinct functional groups, rather than different positions of the same functional group) are considered distinct PFASs, even when the isomerization occurs only in the fluorinated tails of their structures (e.g., unsaturated perfluorooctanesulfonate [UPFOS] and perfluoro-4-ethylcyclohexane [PFEtCHxS]; Figure 1).

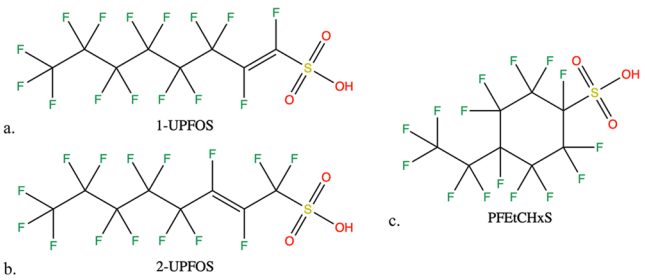


Figure 1. (a) 1-UPFOS (CID 162420437) and (b) 2-UPFOS (CID 162420438) are (tail) positional isomers which differ by the position of the same functional group (i.e., unsaturated bond), whereas UPFOS and (c) PFEtCHxS (CID 101650) are constitutional isomers with distinct functional groups.

To account for these and other nuances, our scale provides detailed guidance for communicating the certainty of PFAS identification from HRMS-derived data. Nonetheless, other evidence can (and typically should) be used to confidently ascertain the identities of PFASs found in the environment. We first present confidence levels and identification criteria tailored to nontarget HRMS studies of PFASs which contain a C_nF_{2n+1} moiety with $n \geq 2$. We then provide guidance on specific identification criteria used to establish confidence. Our confidence scale arises from a need for a more reliable and harmonized identification and reporting approach for PFASs.

IDENTIFICATION CONFIDENCE

Table 1 lists the proposed confidence levels for PFAS identification. Criteria highlighted in blue are required for PFAS identification at the given confidence level. At least one of the criteria highlighted in gray must be met at the given confidence level, but not all are required. Because matching to an analytical reference standard or MS/MS library spectrum provides a high degree of certainty, high-confidence identifications meeting those rigorous criteria need not additionally present a prescribed number of matching MS/MS fragments in experimental results. However, MS/MS fragmentation is a crucial component of all library matching algorithms, and presenting spectra for level 2a and greater identifications may be nonetheless useful. An example decision tree for establishing confidence levels is provided in the Supporting Information (Figure S1).

Level 1a: Confirmed by Reference Standard. Level 1a identification requires confirmation by matching a feature's exact mass, isotope pattern, retention time, and MS/MS spectrum to those of an analytical reference standard in a matrix-matched sample analyzed on the same instrument and run as the feature. Due to the varied and proprietary methods through which HRMS platforms compare experimental and

Table 1. Criteria for PFAS Identification at Various Confidence Levels[a]

Level	Identification Confidence	Accurate Mass	Mass Defect	Isotopic Pattern Match	Consistent RT	Homologue (number; level)	MS^2 Fragments (number; type)	Library MS^2	Reference Standard
Level 1a	Confirmed by reference standard	✓	✓	✓	✓			✓	✓
Level 1b	Indistinguishable from reference standard	✓	✓	✓	✓			✓	✓
Level 2a	Probable by library spec. match	✓	✓	✓	✓			✓	
Level 2b	Probable by diagnostic fragmentation evidence	✓	✓	✓	✓	*≥ 1; ≥ level 3*	**≥ 3; diagnostic**		
Level 2c	Probable by diagnostic homologue evidence	✓	✓	✓	✓	**≥ 2; ≥ level 2a**	**≥ 2; diagnostic**		
Level 3a	Positional isomer candidates	✓	✓	✓	✓	*≥ 1; ≥ level 3*	**≥ 1; subclass-aligned**		
Level 3b	Fragmentation-based candidate	✓	✓	✓	✓	*≥ 1; ≥ level 3*	**≥ 1; subclass-aligned**		
Level 3c	Circumstantial candidate with fragmentation evidence	✓	✓	✓	✓	*≥ 1; ≥ level 3*	**≥ 1; subclass-aligned (in silico)**		
Level 3d	Circumstantial candidate with homologue evidence	✓	✓	✓	✓	**≥ 2; ≥ level 2a**			
Level 4	Unequivocal molecular formula	✓	✓	✓					
Level 5a	PFAS suspect screening exact mass match	**✓ (suspect list match)**							
Level 5b	Nontarget PFAS exact mass of interest	✓	✓			*≥ 3*	*≥ 2; subclass-aligned (in silico)*		

[a]Blue highlights with bold typeface indicate required criteria for PFAS identification at a certain confidence level. Gray highlights and italic typeface indicate where any of several criteria may be used in identification.

https://doi.org/10.1021/acs.estlett.2c00206
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

library spectra, it remains challenging to define a threshold score for an acceptable library spectrum match. However, an identification with level 1a confidence should provide every indication that the feature identified matches the exact structure of the analytical reference standard. The analytical reference standard should be of high quality and fully characterized for isomer-specific identity and purity using several orthogonal analytical approaches. Such standards typically come from a recognized manufacturer but can also be synthesized in-house (so long as that synthesized standard has been conclusively characterized, e.g., to NIST Highest Confidence standard).[13]

Level 1b: Indistinguishable from Reference Standard. Certain PFASs have isomers with different positions of substitution, branching, or unsaturated bonds within their fluorinated tails that are virtually indistinguishable based on MS/MS fragmentation. Such PFASs may be identified at level 1b if they can be confirmed with an analytical reference standard (i.e., by accurately matching mass, isotopic pattern, MS/MS spectrum, and retention time), despite the existence of known structures with potentially indistinguishable fragments. For instance, the position of a substituted chlorine atom in a PFAS structure may be difficult to distinguish by HRMS, as the chlorine atom may be lost as chloride and leave a neutral chain fragment.[14]

Branched and linear isomers are often distinguishable from analytical reference standards by their distinct chromatographic signal. Consistent with the convention that branched isomers are grouped together, such isomers should be identified at the highest confidence level determined for any single isomer within a sample (see Level 3a: Positional Isomer Candidates). If specific branched isomers are not distinguishable, their match to a reference standard cannot achieve greater confidence than level 1b.

Level 2: Probable Structures. At level 2, the probable structure of an analyte is identified principally using a library MS/MS spectrum or diagnostic chemical characteristics (e.g., accurate mass, mass defect, and isotopic pattern). Frequently—though not always—these PFASs also match an exact mass on a suspect list.

If an MS/MS library spectrum is not available to support the identification with level 2a certainty, diagnostic evidence including the experimental conditions,[8] MS/MS fragmentation pattern, and co-occurrence of homologues or a retention time consistent with the proposed structure enables identification of a probable structure at confidence level 2b (see High-Resolution MS/MS Spectra). Such information can also be used to identify a probable PFAS structure with fewer diagnostic fragments at confidence level 2c. Since levels 2b and 2c describe different methods of determining a probable structure from diagnostic evidence, the two levels should generally be considered comparable to each other. However, consecutive letters have been chosen for the ease of presentation and reporting.

Level 2a: Probable by Library Spectrum Match. At level 2a, a probable structure is identified by its accurate mass, mass defect, and isotopic pattern and matching an MS/MS spectrum in a mass spectral library, because no analytical reference standard was used (or available) to establish level 1 confidence. The deconvoluted spectrum should contain a sufficient number of diagnostic fragments consistent with the library spectrum to rule out other structural isomers.

To ensure that the analyte and library MS/MS spectra originate from the same structure, a level 2a identification should match a spectrum found in a curated and widely accessible spectral library (i.e., the library spectrum and its match to the experimental data must be reproducible). Such libraries are available from HRMS platform manufacturers, government agencies, and the public domain (e.g., MassBank).[15,16] In-house amendments to these libraries are commonplace in many research laboratories; however, researchers should be mindful of the "environmental purity" of the library sample, with environmental samples often generating less pristine spectra than either technical grade standards or components in commercial mixtures (e.g., aqueous film-forming foam; AFFF).

Level 2b: Probable by Diagnostic Fragmentation Evidence. Many forms of evidence in addition to MS/MS fragmentation (e.g., parent compound identity, ionization behavior, synthesis pathway) may contribute to identifying an analyte with level 2b certainty. However, at level 2b confidence, the MS/MS spectrum of a PFAS should contain at least three fragments that qualify as diagnostic evidence (see High-Resolution MS/MS Spectra). For many novel PFASs, this amount of fragmentation is the minimum necessary criterion to distinguish a specific headgroup.[17]

Ideally, at least one candidate homologue can be detected to support fragmentation evidence at level 2b. However, for some novel PFASs, no candidate homologues may be detected. In such cases, the identification may retain level 2b confidence if the retention time is consistent with the proposed structure (e.g., the retention time fits the trend for a wide range of compounds with a similar m/z; see Retention Time).

Level 2c: Probable by Diagnostic Homologue Evidence. If spectral data are insufficient to determine the probable PFAS structure, an analyte may be identified with level 2c confidence if at least two homologues are identified with level 2a or greater certainty in the same sample. The retention time of the analyte should be consistent with the trend defined by the homologues (see Retention Time).

At level 2c, the MS/MS spectrum should also contain at least two fragments that qualify as diagnostic evidence. As a repercussion of this criterion, PFASs lacking analytical reference standards or library spectra containing more than two MS/MS fragments (such as perfluoroalkyl sulfonamides and ultrashort-chain PFASs) may not be confirmed to level 2 confidence with a solely mass spectrometry-based approach. In the absence of analytical reference standards, complementary analytical approaches should be utilized to improve the identification confidence of these compounds, and upon confident identification, their MS/MS spectra should be prioritized for inclusion in widely available libraries.

Level 3: Candidate Structures. Many PFASs may be prioritized for identification through suspect screening. However, matching a formula to a suspect list alone is insufficient for level 3 confidence; MS/MS spectra or homologues must provide some evidence for candidate PFASs identified with level 3 confidence. Ideally, PFASs identified at level 3 will be supported with coidentified homologues; however, if no homologue confidently is detected, the identification may reach level 3 confidence with sufficient MS/MS evidence and a retention time consistent with the proposed structure. At levels 3a-c, a sufficient number of MS/MS fragments should align the structure with a subclass of PFASs with similar nonfluorinated moieties (see High-Resolution MS/MS Spectra).[11] In some cases, it may be that this extent of fragmentation cannot be achieved, particularly for compounds that have isomers and that also do not fragment appreciably under standard conditions.

https://doi.org/10.1021/acs.estlett.2c00206
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

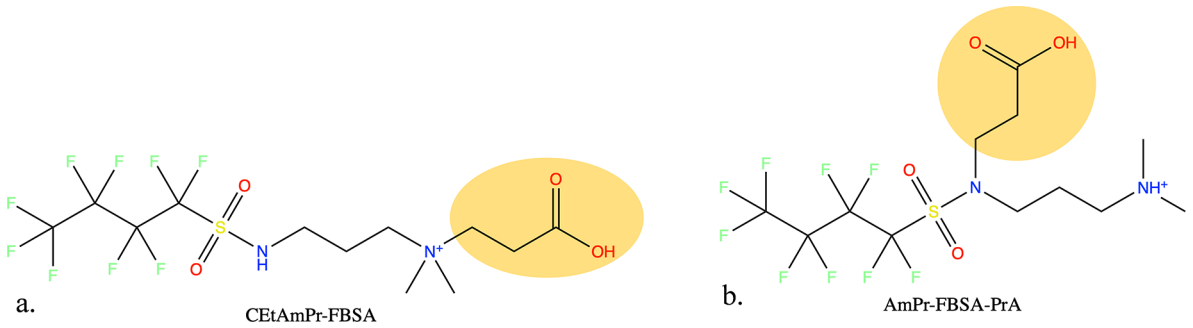


Figure 2. (a) CEtAmPr-FBSA (CID 139595456) and (b) AmPr-FBSA-PrA (CID 162420439) are (headgroup) positional isomers which differ by the position of the highlighted propanoic acid group.

Circumstantial candidates at levels 3c and 3d arise from compelling experimental or (bio)chemical evidence indicative of a specific structure. Since levels 3c and 3d describe different methods of determining a candidate structure from circumstantial evidence, the two confidence levels should generally be considered comparable to each other. However, consecutive letters have been chosen for the ease of presentation and reporting.

Level 3a: Positional Isomer Candidates. For positional isomers, the presence of a particular functional group is confirmed by fragmentation, but the position of the functional group is ambiguous. To avoid conflation with constitutional isomers (which have the same molecular formula but distinct functional groups), fragmentation should specifically indicate the presence of the functional group in question. Analysts should note the positional isomer candidates when identifying PFASs at level 3a. For example, the following could be used: (1) "We identified UPFOS with level 3a confidence; the location of the unsaturated bond could not be identified within the scope of this study." (2) "We identified the feature at m/z = 457.083 with level 3a confidence; carboxyethyl dimethyl ammoniopropyl perfluorobutane sulfonamide (CEtAmPr-FBSA) and dimethyl ammoniopropyl perfluorobutane sulfonamido propanoic acid (AmPr-FBSA-PrA) are the two positional isomer candidates." Authors should annotate[18] the analyte and fragments to the greatest extent possible.

Positional isomerization may occur in the head or tail of PFAS structures. If the identity of an analyte can be confirmed with an analytical reference standard despite the existence of structures with potentially indifferentiable tail fragments (e.g., Cl-PFOS), it may be identified with level 1b confidence. However, tail group isomers without an indistinguishable analytical reference standard should be identified at level 3a if the position of any functional groups (e.g., H or Cl substitution, unsaturated bond, ether) cannot be confirmed (Figure S2).

PFASs with positional isomerization in their headgroups (e.g., CEtAmPr-FBSA and AmPr-FBSA-PrA; Figure 2) should be identified at level 3a if the position of functional groups cannot be confirmed.

Per convention, branched and linear PFAS isomers do not constitute positional isomers (Figure S2). Distinguished by their chromatographic signal, branched and linear isomers within a sample should be identified together at the highest confidence level determined for any single isomer, which may be higher than level 3. For example, branched perfluoroheptanesulfonate (PFHpS) found alongside linear PFHpS which matches a linear PFHpS analytical standard may be identified with level 1b confidence, even if every branched isomer cannot be confirmed with a matched analytical standard. The observation of both branched and linear isomers should always be reported: the co-occurrence of linear and branched isomers usually indicates synthesis by electrochemical fluorination,[19] and the relative abundance of branched and linear isomers can inform PFAS forensics.[20,21]

Useful biochemical or physicochemical information may be obtained for isomeric structures identified at level 3a.[22] Regulatory action on the basis of environmental detections with level 3a confidence may be justified, in part because these isomers frequently occur as mixtures. Further, concerns that regulation may be impractical for compounds that cannot routinely be identified with at least level 2 confidence would be mitigated by regulating PFASs as a class.[23]

Level 3b: Fragmentation-Based Candidates. PFASs identified with level 3b confidence have candidate structures informed by their MS/MS spectra. At level 3b, the MS/MS fragments need not be diagnostic (see High-Resolution MS/MS Spectra) but rather aligned with a specific PFAS subclass. Without diagnostic fragments or evidence of particular functional groups, constitutional isomer candidates of PFASs on suspect lists may often be identified at level 3b.

Level 3c: Circumstantial Candidates by Fragmentation Evidence. Circumstantial evidence that can facilitate identification of candidates may derive from knowledge of the experimental design and/or subclass of PFASs investigated (e.g., identifying transformation products based on a known parent compound or vice versa). Candidate spectra at level 3c should have subclass-aligned fragmentation. Additional evidence that may be used in the identification includes the following:

- In silico predictions of subclass-aligned MS/MS fragments for the proposed structure.[24]
- Fractionation of species by positive or negative charge through anion or cation exchange solid-phase extraction.[25]
- Detection of possible zwitterionic PFASs in both positive and negative ionization modes.
- Chromatography indicative of electrochemical fluorination. In such cases, often a branched isomer peak (or peaks) is followed by a linear isomer peak.
- The abundance of homologues that are separated by $-(CF_2CF_2)-$ (i.e., 99.9936 Da). Elevated concentrations of only even- or odd-length homologues are indicative of fluorotelomerization. Homologue lengths are typically more uniformly distributed in electrochemical fluorination-based mixtures.[26,27]
- A positive mass defect, which may indicate the presence of nonfluorinated functional groups in the structure.

https://doi.org/10.1021/acs.estlett.2c00206
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

Level 3d: Circumstantial Candidates by Homologue Evidence. For candidate structures with little fragmentation-based evidence, distinct patterns of homologues can constitute sufficient circumstantial evidence to enable identification with level 3d confidence. The presence of homologues, identified with level 2 confidence or greater, and the candidate's retention time consistent with the homologous series (see Retention Time) provide strong circumstantial evidence for a candidate that otherwise lacks sufficient MS/MS data. For example, if perfluorohexane sulfonamide (FHxSA) and perfluorooctane sulfonamide (FOSA) have been identified with level 2 confidence and a feature m/z matches the formula for perfluoroheptane sulfonamide (FHpSA) with a retention time between that of FHxSA and FOSA, the feature may be identified at level 3d.

Level 4: Unequivocal Molecular Formula. At level 4 confidence, the mass and isotopic pattern of the analyte must allow the unequivocal determination of a molecular formula, but unlike a level 3 identification, MS/MS data and homologues are structurally inconclusive or nonexistent. It can be difficult to determine unequivocal molecular formulas for fluorine-containing compounds, especially as the m/z increases, due to the light mass and the monoisotopic nature of fluorine. Features at level 4 may have the same formula as a compound (or compounds) on a PFAS suspect list but require additional evidence for identification with level 3 confidence. Nonetheless, researchers are encouraged to note if the analyte mass matches a PFAS on a suspect list, particularly if other evidence (e.g., experimental conditions and analytical techniques) supports the possible presence of the mass-matched structure.

Level 5: Exact Masses of Interest. Feature prioritization is an initial step to identify potential novel and nontarget PFASs. Therefore, level 5 identifications with only an exact mass are often the practical starting point for identification with greater confidence. Features prioritized by suspect screening[1,6] and data filtering for exact masses of interest are identified at different sublevels.

Because an exact mass is not indicative of a specific PFAS, level 5 identifications are often less useful for reporting and are indicated below the red line in Table 1. Level 5 analytes should generally only be reported in exceptional cases (e.g., remarkably high abundance, association with toxicity, increasing abundance over time). As a result, highly interesting level 5 identifications may inform community prioritization efforts, making it possible to find collaborators who have complementary data available that may increase the confidence level.

Possible methods for improving confidence in level 5 identifications include varying the fragmentation mode and/or collision energy, increasing the mass-on-column value, modifying source parameters, and using orthogonal chromatography to enhance separation and to reduce artifacts/interference.

Level 5a: PFAS Suspect Screening Exact Mass Match. Suspect matches are features with the same exact mass as features on a suspect list (within a certain error tolerance) but which lack any conclusive structural or formulaic information (see Accurate Mass). Suspect screening can be conducted either with software that compares masses to a suspect list[6,28] or with methods developed in-house.

Level 5b: Nontarget PFAS Exact Mass of Interest. Data filtering involves the use of software[6] to prioritize detected exact masses that have an elevated likelihood of being novel PFASs. Common prioritizations through filtering data include the following:

- Features with a mass defect between −0.11 and 0.12.[5] For PFAS studies, it is convenient to filter features by their CF_2-normalized mass defect, frequently 0.85−1 in negative ionization mode[29,30] or 0−0.15 in positive ionization mode.[17]
- Features with at least three homologues detected. Data filtering for homologous series is commonly facilitated by Kendrick mass defect plots.[5,30,31]
- Features with at least two fragments consistent with fluorinated substructures.[5]
- PFASs of different subclasses with identical Kendrick mass defects can be distinguished using the Z^* value, which can be calculated using the nominal mass.[7]

CONFIDENCE CRITERIA

Accurate Mass. The high resolving power and mass accuracy of HRMS provide selective and sensitive PFAS detection in various matrices. We recommend reporting the mass error (i.e., the difference between measured and theoretical exact mass) because accuracy influences several identification confidence criteria. Accurate masses are required to determine the mass defect, which can be used to prioritize potential PFASs and identify homologous series. The measured mass of features prioritized by suspect screening must be accurate with the proposed exact mass and within a certain tolerable error of PFASs on a suspect list.[32]

Researchers should endeavor to use analytical reference standards as benchmarks for mass accuracy because mass errors are often consistent within a subclass (e.g., the mass error for most negatively ionizable PFASs is typically close to that of PFOS for a given instrument). A 5−10 ppm mass accuracy threshold is common; however, accuracy standards may change as HRMS technology improves. When reporting mass error for a given annotation among several samples, we recommend including variability by reporting either a range of values or an average with a measure of spread.

Isotopic Pattern Match. Matching an isotopic pattern is an important tool for confident molecular formula identification by HRMS. However, for features at low abundance, particularly features rich in elements lacking abundant minority isotopes (e.g., fluorine), a reliable isotopic pattern may not be distinguishable. In such cases, the (mono)isotopic pattern should be considered a match if it is consistent with the proposed structure at the measured abundance.

Retention Time. Ideally, researchers should compare an analyte's retention time to those of any homologues of the proposed structure: in reversed-phase liquid chromatography, retention times should increase at predictable intervals consistent with the increasing uninterrupted perfluorinated chain length. The analyte retention time should also be compared to those of analytical reference standards. This comparison can facilitate the exclusion of some false positives (e.g., from in-source fragmentation) by providing an approximate retention time for parent compounds of a particular mass and indicate retention time shifts due to matrices and methods.

However, homologues may not always be detected, and the retention times of analytical reference standards are not reliable benchmarks for PFASs with complex and multiply charged headgroups. For example, fluoroalkyl sulfonamides commonly elute considerably later than perfluoroalkyl sulfonic acids with corresponding—or even longer—perfluoroalkyl chain lengths (e.g., $RT_{FHxSA} > RT_{PFHpS}$), despite their fairly similar

https://doi.org/10.1021/acs.estlett.2c00206
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

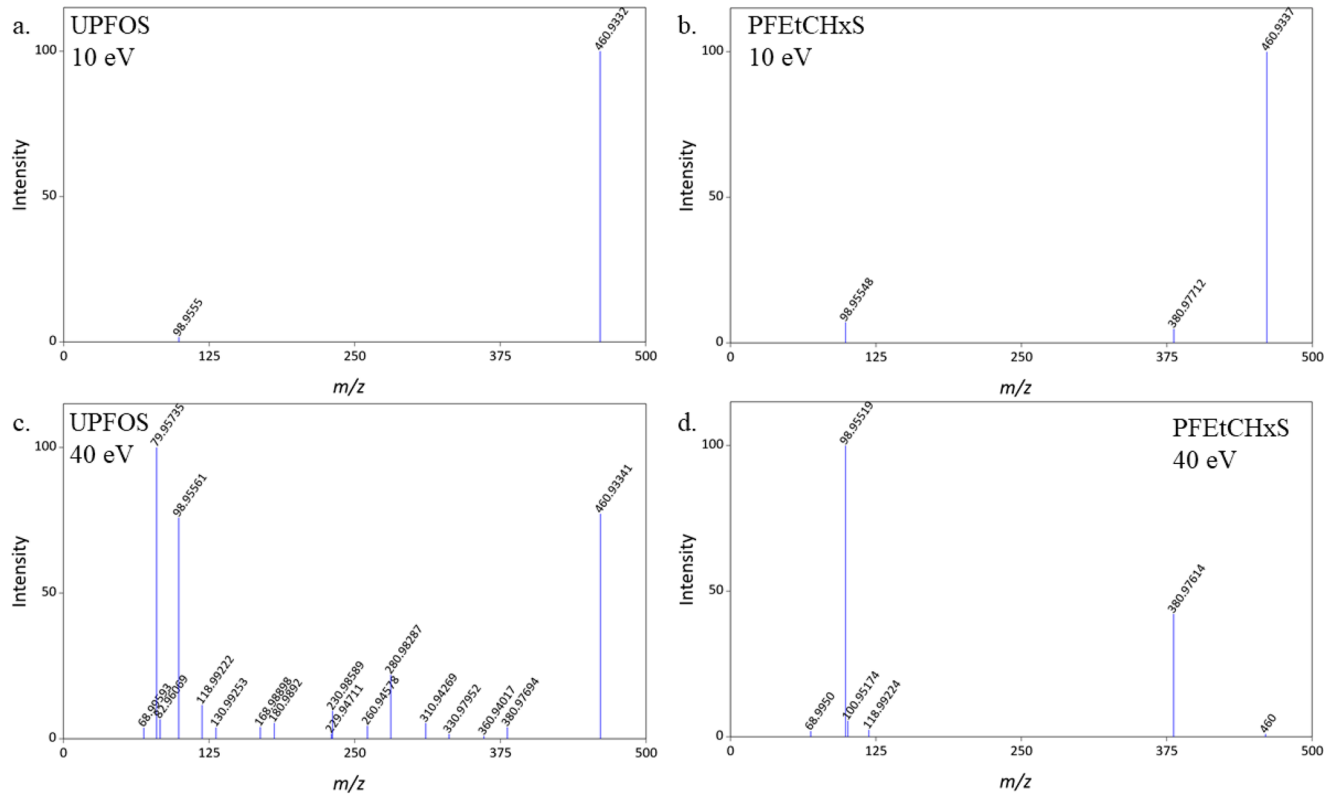


Figure 3. Mass spectra for (a, c) UPFOS and (b, d) PFEtCHxS collected via QTOF-MS with collision-induced dissociation at 10 and 40 eV. UPFOS spectrum collected from liver tissue of mouse dosed with aqueous film-forming foam (AFFF). PFEtCHxS spectrum collected from vehicle mouse liver tissue spiked with PFEtCHxS.

structures.[33] The retention times of PFASs with even more complex head groups (e.g., betaines, sulfonamido carboxylic acids) are very difficult to predict from the retention times of analytical reference standards.[17] Fluorinated chain lengths are convenient proxies for expected retention time, but other properties, such as the degree of branching and the charge and hydrophobicity of headgroups, influence retention time as well.[34,35]

High-Resolution MS/MS Spectra. To qualify as diagnostic for level 2 identification, MS/MS fragments must be attributable to specific chemical substructures. Examples include annotated fragments[18,36] found in the literature and empirically derived library spectra for species within the proposed subclass. Evidence of in-source fragmentation or adduct formation (e.g., a retention time inconsistent with the precursor mass, as is common for some perfluoroalkyl ether substances) should be evaluated before considering a fragment as diagnostic structural evidence.[37] Diagnostic fragments should not be determined solely via in silico prediction; however, novel HRMS systems could generate fragments not yet cataloged in library spectra, in which case the diagnostic fragment could be justified and validated with fragmentation chemistry knowledge, including in silico tools[24] combined with manual verification.

Finally, the entire MS/MS spectrum should be evaluated to provide context for determining that diagnostic fragments provide unequivocal evidence of a specific structure. For example, the FO_3S^- (m/z = 98.96) and $C_8F_{15}^-$ (m/z = 380.98) fragments are not solely sufficient to distinguish UPFOS from PFEtCHxS (both with precursor m/z = 460.93; Figure 1). Therefore, other complementary data in the MS/MS spectra (e.g., the *presence* or *absence* of fragments at m/z = 79.96, 230.99, and/or 280.98) are necessary for these fragments to be considered diagnostic (Figure 3).[38]

Detailed reporting of diagnostic fragments by PFAS researchers and the sharing of this information in a FAIR (findable, accessible, interoperable, and reusable) and open manner would greatly facilitate the exchange of PFAS fragment information.[39]

Fragments that do not meet the standards of diagnostic evidence may still be valuable to identification efforts if they are subclass aligned. To qualify as subclass aligned, the observed fragments must be associated with chemical substructures from the proposed PFAS subclass. Such fragments may be attributable to any of several related parent structures with common functional groups. For example, a fragment at m/z = 58.0651 (corresponding to $C_3H_8N^+$) may be attributable to many headgroups (including those with ammonio propyl, dimethyl ammonio, and betaine moieties)[17] and is therefore aligned with subclasses containing these headgroups, but not diagnostic. To qualify as evidence supporting a level 3c identification, the observed fragments may also be subclass aligned based on in silico fragmentation predictions for the proposed structure.

COMMUNICATING CONFIDENCE

There are relatively many new HRMS users in the field of PFAS research. These researchers must contend with a limited number of analytical PFAS reference standards. It is therefore essential to the large and growing body of environmental PFAS science that the certainty of PFAS identifications made through HRMS is communicated clearly and uniformly. The identification scale which we present is consistent with the norms for communicat-

https://doi.org/10.1021/acs.estlett.2c00206
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

ing confidence in the identification of small organic molecules by HRMS but also incorporates PFAS-specific conventions and tools. Our scale is therefore intended to contextualize and augment existing identification confidence scales[5,8] rather than supersede them. The approaches discussed in this Global Perspective are not unique to PFAS studies, so researchers in other fields employing HRMS for organic molecule identification could adopt some of the conventions which we propose for more clearly communicating confidence (e.g., the strength of homologue evidence may also be useful in the identification of chlorinated paraffins or nonfluorinated surfactants). The criteria in our scale are adaptable to advances in HRMS technology and should remain relevant as HRMS becomes more commonplace and sophisticated. Our scale can clarify the level of certainty in PFAS identification and, in doing so, facilitate more confident identifications as researchers are better able to build on previous work.

KEY MESSAGES

(1) Nontargeted HRMS analysis is an important component of PFAS research because of the small number of analytical PFAS standards and the rapid rate of novel PFAS discovery.
(2) More reliable and harmonized identification and reporting is needed for PFASs identified via HRMS.
(3) Guidance specific to PFASs is necessary due to their characteristics which can both facilitate and complicate nontarget identification. For example, PFASs are frequently found in homologous series. Likewise, branched and linear PFAS isomers are conventionally grouped together for regulatory and research purposes.
(4) We propose identification criteria and sublevels for PFAS identification which clarify and contextualize previous guidance on communicating confidence in small molecule identification via HRMS.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.2c00206.

Figure S1: Confidence of PFAS identification example workflow. Figure S2: Guidance for classification and identification of PFASs with some evidence of structure from MS/MS spectrum but multiple isomers possible. (PDF)

AUTHOR INFORMATION

Corresponding Author

Christopher P. Higgins − *Department of Civil and Environmental Engineering, Colorado School of Mines, Golden, Colorado 80401, United States; Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, Woolloongabba, QLD 4102, Australia;* orcid.org/0000-0001-6220-8673; Phone: 720-984-2116; Email: chiggins@mines.edu

Authors

Joseph A. Charbonnet − *Department of Civil and Environmental Engineering, Colorado School of Mines, Golden, Colorado 80401, United States; Department of Civil, Construction and Environmental Engineering, Iowa State University, Ames, Iowa 50011, United States;* orcid.org/0000-0001-8766-6072

Carrie A. McDonough − *Department of Civil Engineering, Stony Brook University, Stony Brook, New York 11794, United States;* orcid.org/0000-0001-5152-8495

Feng Xiao − *Department of Civil Engineering, University of North Dakota, Grand Forks, North Dakota 58202, United States;* orcid.org/0000-0001-5686-6055

Trever Schwichtenberg − *Department of Chemistry, Oregon State University, Corvallis, Oregon 97331, United States;* orcid.org/0000-0001-7882-4665

Dunping Cao − *Department of Chemistry, Oregon State University, Corvallis, Oregon 97331, United States*

Sarit Kaserzon − *Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, Woolloongabba, QLD 4102, Australia;* orcid.org/0000-0002-6744-1279

Kevin V. Thomas − *Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, Woolloongabba, QLD 4102, Australia;* orcid.org/0000-0002-2155-100X

Pradeep Dewapriya − *Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, Woolloongabba, QLD 4102, Australia;* orcid.org/0000-0002-6383-1843

Benjamin J. Place − *National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States;* orcid.org/0000-0003-0953-5215

Emma L. Schymanski − *Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Belvaux 4362, Luxembourg;* orcid.org/0000-0001-6868-8145

Jennifer A. Field − *Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon 97331, United States;* orcid.org/0000-0002-9346-4693

Damian E. Helbling − *School of Civil and Environmental Engineering, Cornell University, Ithaca, New York 14850, United States;* orcid.org/0000-0003-2588-145X

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.estlett.2c00206

Notes

Certain commercial equipment, instruments, software, or materials are identified in this communication to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This manuscript development was partially supported by the Strategic Environmental Research and Development Program (SERDP ER20-1375, ER20-1056, and ER20-1156) and the U.S. EPA (83967001-0 and RD839660). E.L.S. acknowledges funding support from the Luxembourg National Research Fund (FNR) for project A18/BM/12341006 and the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 101036756 for ZeroPM. S.K., K.V.T., and P.D. acknowledge funding from the Australian

National Health and Medical Research Council (NHMRC, Grant Identification No. APP1185347).

REFERENCES

(1) Place, B. *Suspect List of Possible Per- and Polyfluoroalkyl Substances (PFAS)*; National Institute of Standards and Technology, 2021. DOI: 10.18434/mds2-2387.

(2) Wang, Z. *S25, OECDPFAS, List of PFAS from the OECD*; Zenodo, 2018. DOI: 10.5281/zenodo.3653165.

(3) *Environmental Fate and Effects of Poly- and Perfluoroalkyl Substances (PFAS)*; Report No. 8/16; CONCAWE: Brussels, 2016.

(4) Liu, Y.; D'Agostino, L. A.; Qu, G.; Jiang, G.; Martin, J. W. High-Resolution Mass Spectrometry (HRMS) Methods for Nontarget Discovery and Characterization of Poly- and per-Fluoroalkyl Substances (PFASs) in Environmental and Human Samples. *Trends in Analytical Chemistry* **2019**, *121*, 115420.

(5) Koelmel, J. P.; Stelben, P.; McDonough, C. A.; Dukes, D. A.; Aristizabal-Henao, J. J.; Nason, S. L.; Li, Y.; Sternberg, S.; Lin, E.; Beckmann, M.; Williams, A. J.; Draper, J.; Finch, J. P.; Munk, J. K.; Deigl, C.; Rennie, E. E.; Bowden, J. A.; Godri Pollitt, K. J. FluoroMatch 2.0—Making Automated and Comprehensive Non-Targeted PFAS Annotation a Reality. *Anal Bioanal Chem.* **2022**, *414*, 1201−1215.

(6) Jacob, P.; Wang, R.; Ching, C.; Helbling, D. E. Evaluation, Optimization, and Application of Three Independent Suspect Screening Workflows for the Characterization of PFASs in Water. *Environ. Sci.: Processes Impacts* **2021**, *23*, 1554−1565.

(7) Young, R. B.; Pica, N. E.; Sharifan, H.; Chen, H.; Roth, H. K.; Blakney, G. T.; Borch, T.; Higgins, C. P.; Kornuc, J. J.; McKenna, A. M.; Blotevogel, J. PFAS Analysis with Ultrahigh Resolution 21T FT-ICR MS: Suspect and Nontargeted Screening with Unrivaled Mass Resolving Power and Accuracy. *Environ. Sci. Technol.* **2022**, *56* (4), 2455−2465.

(8) Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environ. Sci. Technol.* **2014**, *48* (4), 2097−2098.

(9) Bugsel, B.; Bauer, R.; Herrmann, F.; Maier, M. E.; Zwiener, C. LC-HRMS Screening of per- and Polyfluorinated Alkyl Substances (PFAS) in Impregnated Paper Samples and Contaminated Soils. *Anal Bioanal Chem.* **2022**, *414* (3), 1217−1225.

(10) Wang, Y.; Yu, N.; Zhu, X.; Guo, H.; Jiang, J.; Wang, X.; Shi, W.; Wu, J.; Yu, H.; Wei, S. Suspect and Nontarget Screening of Per- and Polyfluoroalkyl Substances in Wastewater from a Fluorochemical Manufacturing Park. *Environ. Sci. Technol.* **2018**, *52* (19), 11007−11016.

(11) 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. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. *Integrated Environmental Assessment and Management* **2011**, *7* (4), 513−541.

(12) Benskin, J. P.; Bataineh, M.; Martin, J. W. Simultaneous Characterization of Perfluoroalkyl Carboxylate, Sulfonate, and Sulfonamide Isomers by Liquid Chromatography−Tandem Mass Spectrometry. *Anal. Chem.* **2007**, *79* (17), 6455−6464.

(13) Beauchamp, C. R.; Camara, J. E.; Carney, J.; Choquette, S. J.; Cole, K. D.; DeRose, P. C.; Duewer, D. L.; Epstein, M. S.; Kline, M. C.; Lippa, K. A.; Lucon, E.; Molloy, J.; Nelson, M. A.; Phinney, K. W.; Polakoski, M.; Possolo, A.; Sander, L. C.; Schiel, J. E.; Sharpless, K. E.; Toman, B.; Winchester, M. R.; Windover, D. *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; National Institute of Standards and Technology, 2021. DOI: 10.6028/NIST.SP.260-136-2021.

(14) Liu, Y.; Pereira, A. D. S.; Martin, J. W. Discovery of $C_5 - C_{17}$ Poly- and Perfluoroalkyl Substances in Water by In-Line SPE-HPLC-Orbitrap with In-Source Fragmentation Flagging. *Anal. Chem.* **2015**, *87* (8), 4260−4268.

(15) Horai, H.; Arita, M.; Kanaya, S.; Nihei, Y.; Ikeda, T.; Suwa, K.; Ojima, Y.; Tanaka, K.; Tanaka, S.; Aoshima, K.; Oda, Y.; Kakazu, Y.; Kusano, M.; Tohge, T.; Matsuda, F.; Sawada, Y.; Hirai, M. Y.; Nakanishi, H.; Ikeda, K.; Akimoto, N.; Maoka, T.; Takahashi, H.; Ara, T.; Sakurai, N.; Suzuki, H.; Shibata, D.; Neumann, S.; Iida, T.; Tanaka, K.; Funatsu, K.; Matsuura, F.; Soga, T.; Taguchi, R.; Saito, K.; Nishioka, T. MassBank: A Public Repository for Sharing Mass Spectral Data for Life Sciences. *J. Mass Spectrom.* **2010**, *45* (7), 703−714.

(16) *MassBank-data*; MassBank Consortium, 2021. DOI: 10.5281/zenodo.5775684.

(17) 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. Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater. *Environ. Sci. Technol.* **2017**, *51* (4), 2047−2057.

(18) Glossary. *Benchmarking and Publications for Non-targeted Analysis (BP4NTA)*. https://nontargetedanalysis.org/glossary/ (accessed 2021−12−16).

(19) Benskin, J. P.; Yeung, L. W. Y.; Yamashita, N.; Taniyasu, S.; Lam, P. K. S.; Martin, J. W. Perfluorinated Acid Isomer Profiling in Water and Quantitative Assessment of Manufacturing Source. *Environ. Sci. Technol.* **2010**, *44* (23), 9049−9054.

(20) Benskin, J. P.; De Silva, A. O.; Martin, J. W. Isomer Profiling of Perfluorinated Substances as a Tool for Source Tracking: A Review of Early Findings and Future Applications. In *Reviews of Environmental Contamination and Toxicology Vol. 208*; De Voogt, P., Ed.; Springer: New York, 2010; Vol. *208*, pp 111−160. DOI: 10.1007/978-1-4419-6880-7_2.

(21) Charbonnet, J. A.; Rodowa, A. E.; Joseph, N. T.; Guelfo, J. L.; Field, J. A.; Jones, G. D.; Higgins, C. P.; Helbling, D. E.; Houtz, E. F. Environmental Source Tracking of Per- and Polyfluoroalkyl Substances within a Forensic Context: Current and Future Techniques. *Environ. Sci. Technol.* **2021**, *55*, 7237.

(22) Cheng, W.; Doering, J. A.; LaLone, C.; Ng, C. Integrative Computational Approaches to Inform Relative Bioaccumulation Potential of Per- and Polyfluoroalkyl Substances Across Species. *Toxicol. Sci.* **2021**, *180* (2), 212−223.

(23) Kwiatkowski, C. F.; Andrews, D. Q.; Birnbaum, L. S.; Bruton, T. A.; DeWitt, J. C.; Knappe, D. R. U.; Maffini, M. V.; Miller, M. F.; Pelch, K. E.; Reade, A.; Soehl, A.; Trier, X.; Venier, M.; Wagner, C. C.; Wang, Z.; Blum, A. Scientific Basis for Managing PFAS as a Chemical Class. *Environmental Science & Technology Letters* **2020**, *7*, 532.

(24) Getzinger, G. J.; Higgins, C. P.; Ferguson, P. L. Structure Database and *In Silico* Spectral Library for Comprehensive Suspect Screening of Per- and Polyfluoroalkyl Substances (PFASs) in Environmental Media by High-Resolution Mass Spectrometry. *Anal. Chem.* **2021**, *93* (5), 2820−2827.

(25) D'Agostino, L. A.; Mabury, S. A. Identification of Novel Fluorinated Surfactants in Aqueous Film Forming Foams and Commercial Surfactant Concentrates. *Environ. Sci. Technol.* **2014**, *48* (1), 121−129.

(26) Ellis, D. A.; Martin, J. W.; De Silva, A. O.; Mabury, S. A.; Hurley, M. D.; Sulbaek Andersen, M. P.; Wallington, T. J. Degradation of Fluorotelomer Alcohols: A Likely Atmospheric Source of Perfluorinated Carboxylic Acids. *Environ. Sci. Technol.* **2004**, *38* (12), 3316−3321.

(27) Spaan, K. M.; van Noordenburg, C.; Plassmann, M. M.; Schultes, L.; Shaw, S.; Berger, M.; Heide-Jørgensen, M. P.; Rosing-Asvid, A.; Granquist, S. M.; Dietz, R.; Sonne, C.; Rigét, F.; Roos, A.; Benskin, J. P. Fluorine Mass Balance and Suspect Screening in Marine Mammals from the Northern Hemisphere. *Environ. Sci. Technol.* **2020**, *54* (7), 4046−4058.

(28) Helmus, R.; ter Laak, T. L.; van Wezel, A. P.; de Voogt, P.; Schymanski, E. L. PatRoon: Open Source Software Platform for Environmental Mass Spectrometry Based Non-Target Screening. *J. Cheminform* **2021**, *13* (1), 1.

(29) Barzen-Hanson, K. A.; Field, J. A. Discovery and Implications of C_2 and C_3 Perfluoroalkyl Sulfonates in Aqueous Film-Forming Foams and Groundwater. *Environmental Science & Technology Letters* **2015**, *2* (4), 95−99.

https://doi.org/10.1021/acs.estlett.2c00206
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

(30) Myers, A. L.; Jobst, K. J.; Mabury, S. A.; Reiner, E. J. Using Mass Defect Plots as a Discovery Tool to Identify Novel Fluoropolymer Thermal Decomposition Products: Thermal Decomposition Products of PCTFE. *J. Mass Spectrom.* **2014**, *49* (4), 291−296.
(31) Jacob, P.; Barzen-Hanson, K. A.; Helbling, D. E. Target and Nontarget Analysis of Per- and Polyfluoralkyl Substances in Wastewater from Electronics Fabrication Facilities. *Environ. Sci. Technol.* **2021**, *55* (4), 2346−2356.
(32) Maizel, A. C.; Shea, S.; Nickerson, A.; Schaefer, C.; Higgins, C. P. Release of Per- and Polyfluoroalkyl Substances from Aqueous Film-Forming Foam Impacted Soils. *Environ. Sci. Technol.* **2021**, *55* (21), 14617−14627.
(33) Rodowa, A. E.; Knappe, D. R. U.; Chiang, S.-Y. D.; Pohlmann, D.; Varley, C.; Bodour, A.; Field, J. A. Pilot Scale Removal of Per- and Polyfluoroalkyl Substances and Precursors from AFFF-Impacted Groundwater by Granular Activated Carbon. *Environ. Sci.: Water Res. Technol.* **2020**, *6* (4), 1083−1094.
(34) Schwichtenberg, T.; Bogdan, D.; Carignan, C. C.; Reardon, P.; Rewerts, J.; Wanzek, T.; Field, J. A. PFAS and Dissolved Organic Carbon Enrichment in Surface Water Foams on a Northern U.S. Freshwater Lake. *Environ. Sci. Technol.* **2020**, *54* (22), 14455−14464.
(35) Brusseau, M. L.; Yan, N.; Van Glubt, S.; Wang, Y.; Chen, W.; Lyu, Y.; Dungan, B.; Carroll, K. C.; Holguin, F. O. Comprehensive Retention Model for PFAS Transport in Subsurface Systems. *Water Res.* **2019**, *148*, 41−50.
(36) Koelmel, J. P.; Paige, M. K.; Aristizabal-Henao, J. J.; Robey, N. M.; Nason, S. L.; Stelben, P. J.; Li, Y.; Kroeger, N. M.; Napolitano, M. P.; Savvaides, T.; Vasiliou, V.; Rostkowski, P.; Garrett, T. J.; Lin, E.; Deigl, C.; Jobst, K.; Townsend, T. G.; Godri Pollitt, K. J.; Bowden, J. A. Toward Comprehensive Per- and Polyfluoroalkyl Substances Annotation Using FluoroMatch Software and Intelligent High-Resolution Tandem Mass Spectrometry Acquisition. *Anal. Chem.* **2020**, *92*, 11186.
(37) Strynar, M.; Dagnino, S.; McMahen, R.; Liang, S.; Lindstrom, A.; Andersen, E.; McMillan, L.; Thurman, M.; Ferrer, I.; Ball, C. Identification of Novel Perfluoroalkyl Ether Carboxylic Acids (PFECAs) and Sulfonic Acids (PFESAs) in Natural Waters Using Accurate Mass Time-of-Flight Mass Spectrometry (TOFMS). *Environ. Sci. Technol.* **2015**, *49* (19), 11622−11630.
(38) McDonough, C. A.; Choyke, S.; Barton, K. E.; Mass, S.; Starling, A. P.; Adgate, J. L.; Higgins, C. P. Unsaturated PFOS and Other PFASs in Human Serum and Drinking Water from an AFFF-Impacted Community. *Environ. Sci. Technol.* **2021**, *55*, 8139.
(39) Schymanski, E. L.; Bolton, E. E. FAIR Chemical Structures in the Journal of Cheminformatics. *J. Cheminform* **2021**, *13* (1), 50.

https://doi.org/10.1021/acs.estlett.2c00206
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX