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Identification of Novel Perfluoroalkyl Ether Carboxylic Acids (PFECAs) and Sulfonic Acids (PFESAs) in Natural Waters Using Accurate Mass Time-of-Flight Mass Spectrometry (TOFMS)

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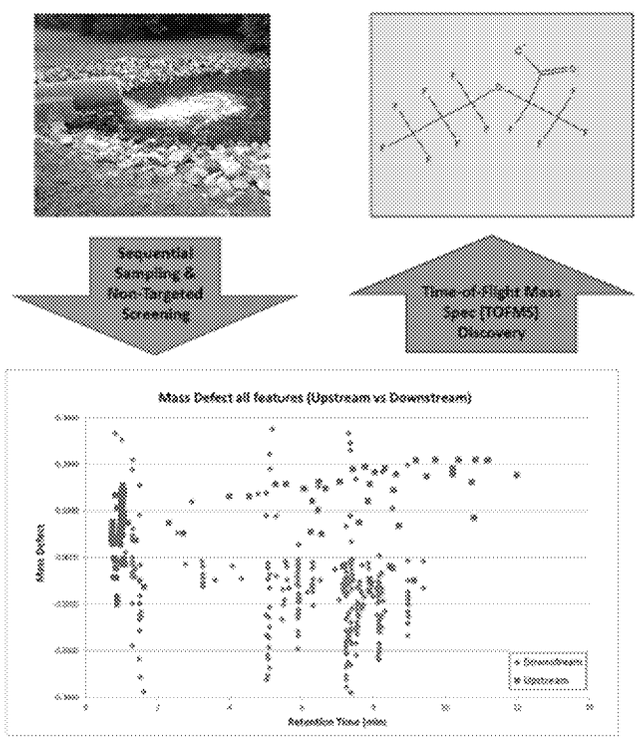
Supporting Information

ABSTRACT: Recent scientific scrutiny and concerns over exposure, toxicity, and risk have led to international regulatory efforts resulting in the reduction or elimination of certain perfluorinated compounds from various products and waste streams. Some manufacturers have started producing shorter chain per- and polyfluorinated compounds to try to reduce the potential for bioaccumulation in humans and wildlife. Some of these new compounds contain central ether oxygens or other minor modifications of traditional perfluorinated structures. At present, there has been very limited information published on these "replacement chemistries" in the peer-reviewed literature. In this study we used a time-of-flight mass spectrometry detector (LC-ESI-TOFMS) to identify fluorinated compounds in natural waters collected from locations with historical perfluorinated compound contamination. Our workflow for discovery of chemicals included sequential sampling of surface water for identification of potential sources, nontargeted TOFMS analysis, molecular feature extraction (MFE) of samples, and evaluation of features unique to the sample with source inputs. Specifically, compounds were tentatively identified by (1) accurate mass determination of parent and/or related adducts and fragments from in-source collision-induced dissociation (CID), (2) in-depth evaluation of in-source adducts formed during analysis, and (3) confirmation with authentic standards when available. We observed groups of compounds in homologous series that differed by multiples of CF_2 (m/z 49.9968) or CF_2O (m/z 65.9917). Compounds in each series were chromatographically separated and had comparable fragments and adducts produced during analysis. We detected 12 novel perfluoroalkyl ether carboxylic and sulfonic acids in surface water in North Carolina, USA using this approach. A key piece of evidence was the discovery of accurate mass in-source n-mer formation (H^+ and Na^+) differing by m/z 21.9819, corresponding to the mass difference between the protonated and sodiated dimers.



INTRODUCTION

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) have unique physical and structural properties that make them extremely resistant to chemical and thermal degradation. As a result, PFASs have been used in a wide range of consumer

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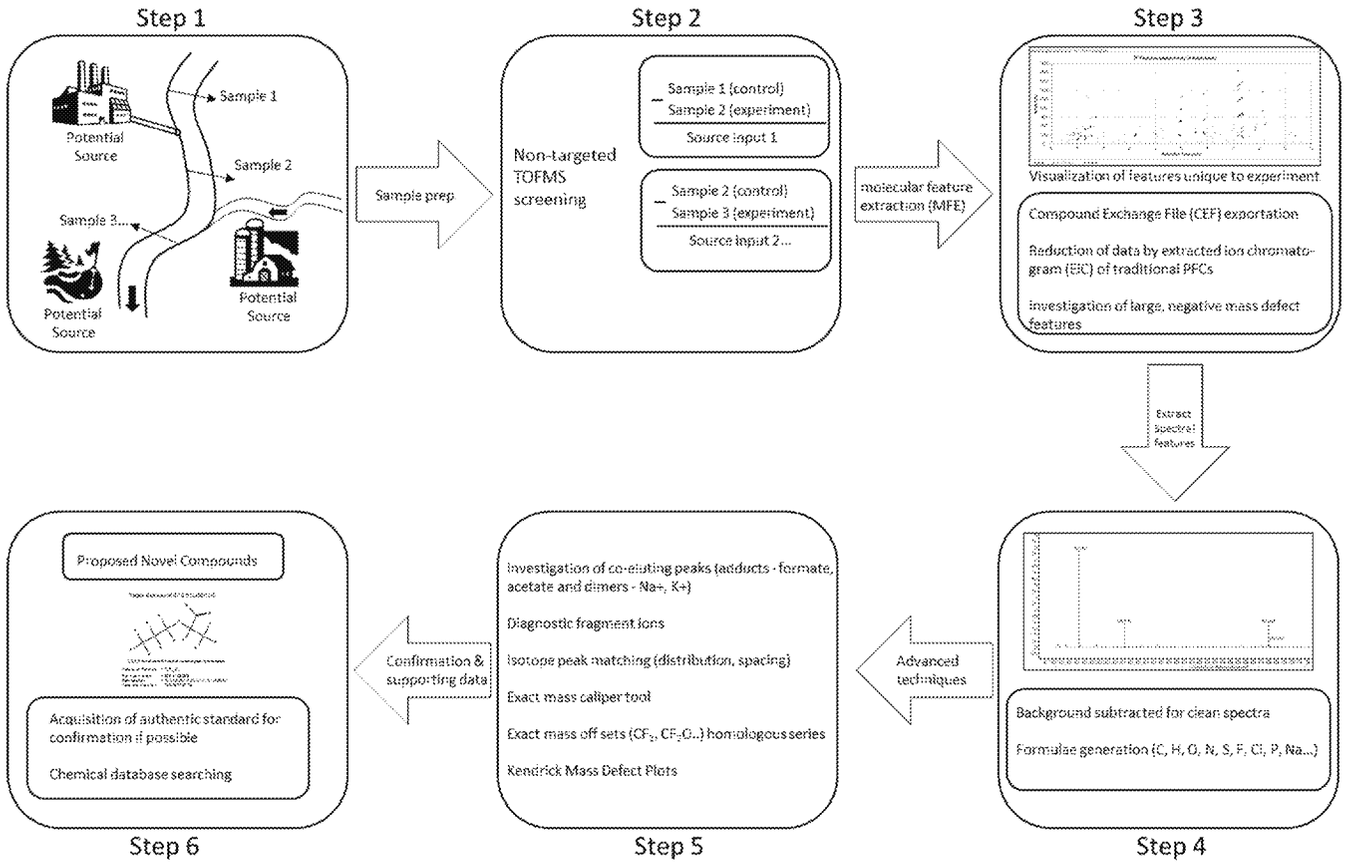

Figure 1. Sample workflow for TOFMS discovery.

products and industrial applications.[1] Between 1950 and 2000, the eight-carbon PFASs, including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS), were among the most commonly produced and used perfluorinated compounds. PFASs have been detected in many environmental and biological matrices across the globe for a number of years,[2] and this ubiquity has led to mounting concerns about exposure and potential toxicity. As a result, many international regulatory efforts have been enacted to reduce, substitute, or eliminate long-chain (8 consecutive perfluorinated carbons or longer) PFASs from products and waste streams. In the United States, eight major fluorochemical manufacturers entered into a voluntary stewardship agreement with the U.S. Environmental Protection Agency (EPA) to phase out the use and production of long-chain perfluorinated chemistries by 2015.[3] To take their place, manufacturers have started using alternative chemistries that may include both shorter-chain perfluorinated (<C8) and/or polyfluorinated materials. For example, some of these replacement compounds still have the traditional perfluorinated carbon regions, but they are broken into shorter units by insertion of ether oxygens at regular intervals. These ether linked compounds are still technically classified as perfluorinated if all carbons are substituted with fluorine and polyfluorinated if any carbon is occupied by a hydrogen rather than a fluorine.[4] The rationale is that the addition of oxygens would likely make the replacement chemistry more labile to degradation, and thus more favorable for usage. However, to date the chemical manufacturers have disclosed little information publicly concerning the chemical structures and potential toxicities of the replacement PFASs now being produced. This makes it extremely difficult for independent research groups to evaluate their environmental distributions and potential toxicities.

A rare exception is a peer-reviewed publication on 4,8-dioxa-3H-perfluorononanoate (ADONA) (Supporting Information Figure S1), a polyfluorinated alternative to PFOA which is said to have a "more favorable toxicological profile".[5] This single journal article was published by its manufacturer, but standards for this new material have not been made available (to our knowledge) for comprehensive ecological or toxicological evaluation. Manufacturers typically submit more detailed information concerning compound structure, potential toxicity, environmental fate, and projected production volumes to regulatory authorities prior to large-scale production (e.g., the U.S. EPA's premanufacture notice process), but this information is held as confidential business information and cannot be disclosed to the general research community.

One example of industrial producers manufacturing replacement compounds is outlined in an executed draft consent order from the state of West Virginia to DuPont Corporation allowing the discharge of a new fluorinated compound into the Ohio river, at the Washington Works facility (West Virginia).[6] This is the same facility that has been the source of historical PFOA contamination of local surface and groundwater due to industrial manufacture and discharge and is the center of a court-ordered investigation by the C8 science panel to determine if PFOA exposure in the local human population has led to adverse health effects.[7] A number of recent epidemiological studies have indicated that exposure to PFOA at this location has been associated with adverse human health outcomes.[8,9]

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Typical environmental occurrence investigations have for years focused on targeted analysis for specific analytes of interest. Highly precise and sensitive LC and GC MS/MS methods have been developed to quantify contaminants of concern. A more recent approach for environmental sample analysis has been the use of high-resolution mass spectrometry (HRMS) for these efforts. Some researchers have used nontargeted or suspect screening effectively in demonstrating many chemical classes in wastewater effluent without the use of reference standards.[11−13] These approaches have begun to rely more heavily on the use of databases, accurate mass searches, and HRMS molecular formula prediction capabilities for discovery. Confirmation with authentic standards still remains the "gold standard". Schymanski et al.[14] reported on five levels of confidence in identifying small molecules using HRMS ranging from exact mass matches, through molecular formula prediction, tentative candidate structures, library match searches, and finally confirmation with a reference standard. This is the type of approach that we use in this work to identify previously undescribed PFASs in surface water.

As it is mostly unknown what is being produced to replace traditional perfluorinated compounds, a series of sampling events was conducted to collect water samples with determination of new per- and polyfluorinated substances as a goal. In Nakayama et al.,[10] a sampling of surface water in the Cape Fear River Basin in North Carolina indicated sources of perfluorinated compounds intermittently spread throughout the drainage basin. A follow-up investigation with more focused sampling to determine sources was not conducted. However, based on this early work, there was some indication that there were PFASs sources in the Fayetteville, NC area. More recent surface water sampling trips in this region indicated continued elevated concentrations of traditional perfluorinated compounds, supporting the Nakayama et al.[10] findings. One such source was known to be downstream of the industrial effluent discharge of a fluorochemical production facility. Further investigation of these samples for identification and occurrence of potential replacement chemistries was undertaken.

MATERIALS AND METHODS

Sample Workflow for Discovery. A diagram of the workflow we used for novel compound discovery in surface water samples is shown in Figure 1. It is a six-step process that starts with sequential sampling of water, followed by nontargeted screening, visualization of detected peaks, further investigation of suspect features, advanced TOFMS techniques, and, last, compound confirmation with authentic standards when available. Using this approach, it becomes possible to detect chemicals that enter a waterway from a source (point or nonpoint) between sampling locations. This can then be used for such purposes as source elucidation, compound discovery, or both. In the following sections we will be referring to Figure 1 and the specific step in the process to which we are referring.

Sample Collection. In the summer of 2012, water samples were collected at locations on the Cape Fear River and its tributaries, with some locations having previously been found to contain measurable PFASs.[15] Grab samples were taken from the bank and bridge crossings with a lab-made dip sampler or a stainless steel Kemmerer sampler, and stored in a 1-L HDPE bottle following the procedures described in Nakayama et al.[16] Samples ($n = 9$) were acquired from the main flow of the Cape Fear River including from just south of the city of Fayetteville, NC to the bridge crossing in the town of Tar Heel, NC. Duplicate samples, trip spikes, and trip blanks were included in this sampling. This sampling also included tributaries to the Cape Fear River along the same stretch of river where reclaimed wastewater treatment plant (WWTP) and industrial effluent streams occurred (Figure 1, step 1).

Sample Analysis for Traditional PFASs. Samples were prepared and analyzed according to the methods of Nakayama et al.[16] In brief, water samples were stabilized with addition of nitric acid and stored at ambient temperature prior to analysis (<7 days). Each water sample was measured in a graduated cylinder, and the sampling bottle was washed with 10 mL of methanol to solubilize analytes that may have become sorbed to the container wall during shipping and storage. The water was then placed back in the methanol-washed bottle with the methanol rinsate, spiked with a mixture of internal standards, and filtered through a glass fiber filter. A volume, typically 500 mL, of water was concentrated on a Waters WAX SPE cartridge using a positive displacement pump. The cartridge was eluted with basic methanol (0.3% NH_4OH) and the eluent was concentrated via evaporation under N_2 gas to 1 mL and prepared for analysis. A procedural blank was prepared as described above using 500 mL of in-house DI water. Traditional PFASs (C_4−C_{10} perfluorocarboxylic acids (PFCAs); perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), and PFOS) analysis was performed using a Waters Acquity ultra performance liquid chromatograph interfaced with a Waters Quattro Premier XE triple quadrupole mass spectrometer (UPLC-MS/MS) (Waters, Milford, MA, USA).

TOFMS Investigation of Novel Fluorinated Compounds. The extraction method used for traditional PFASs analysis was also used for elucidation of novel compounds. Elevated concentrations of traditional PFASs in samples were used as an indication of samples that were likely closer to sources (Figure S2). Sample analysis was performed using an Agilent 1100 series HPLC interfaced with a 6210 series Accurate-Mass LC-TOF system (Agilent Technologies, Palo Alto, CA). The mass spectrometer was operated in electrospray ionization (ESI) negative mode and any drift in the mass accuracy of the TOF was continuously corrected by infusion of two reference compounds (purine (m/z 119.03632) and the acetate adduct of hexakis (1H,1H,3H-tetrafluoropropoxy) phosphazine (m/z 980.016375)) via dual-ESI sprayer. Chromatographic separation was accomplished using an Eclipse Plus C8 column (2.1 × 50 mm, 3.5 μm; Agilent). The method consisted of the following conditions: 0.2 mL/min flow rate; column at 30 °C; mobile phases: A: ammonium formate buffer (0.4 mM) and DI water/methanol (95:5 v/v), and B: ammonium formate (0.4 mM) and methanol/DI water (95:5 v/v); gradient: 0−15 min a linear gradient from 75:25 A/B to 15:85 A/B; with a 4 min post time for equilibration.

Identification of Suspect Features. The total ion chromatogram (TIC) of each water sample was subjected to the software molecular feature extraction algorithm (MFE) and was restricted to the 100 most intense features m/z 50−1700 based on peak height. A molecular feature is defined as a single accurate mass with a specific retention time and an integrated area count. Compounds identified in the solvent and procedural blank samples were used to develop a mass exclusion list to subtract from subsequent unknown samples. After molecular features were identified, they were exported as a Compound Exchange File (.CEF) and imported into Mass Profiler to compare upstream with downstream samples.

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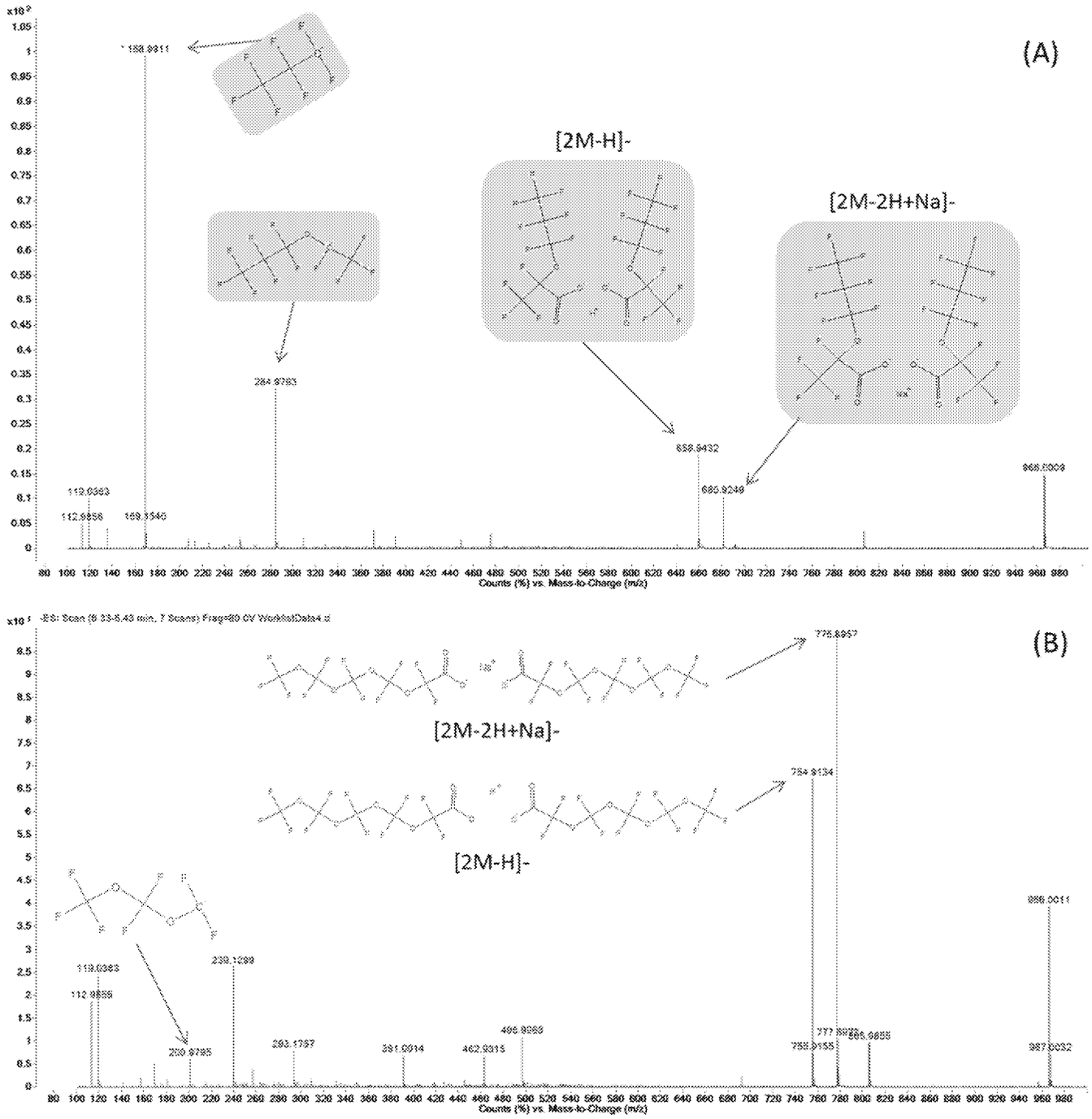

Figure 2. ESI negative spectra for two example perfluorinated ether carboxylic acids found in water: (A) $C_6HF_{11}O_3$ (retention time 5.9−6.0 min) and (B) $C_6HF_{11}O_6$ (retention time 6.5−6.6 min). Note: in spectrum (A and B) m/z 112.9856, 119.0363, and 966.0007 are reference masses for continuous mass calibration. Peaks not pointed out are not associated with the compound of interest.

Pairwise comparisons of sequential samples in river flow (Figure 1 step 2) were used to identify those requiring further investigation. For instance, features found in the downstream sample that were not found in the upstream sample indicate a source (point or nonpoint) exists between the two sampling locations (Figure 1 step 3). Further investigation of peaks with larger area counts using the "identify compounds" feature in the software and additional analyses of samples were used for confirmation. Perfluorinated compounds already identified with traditional UPLC-MS/MS analysis were eliminated from this remaining list further narrowing the unknown compounds requiring follow-up scrutiny.

Poly- and perfluorinated compounds tend to have a negative mass defect due to the presence of multiple fluorine and oxygen atoms. Negative mass defect means that the exact mass of a compound is less than the nominal mass.[17] For instance, the exact mass of perfluorooctanoate ($C_8F_{15}O_2$) is 412.9664, whereas the nominal mass is 413 Da. In this instance negative mass defect compounds only found in the downstream sample were further explored. An additional characteristic of samples that are contaminated with per- and polyfluorinated com-

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Table 1. Accurate Mass of Polyfluorinated Compounds and In-Source Artifacts Found in Extracted Water Samples

number	formula	CAS no.	name	$[M]^a$	$[M - H]^-$ m/z	$[2M - 2H + Na]^-$ m/z	$[2M - H]^-$ m/z
Monoether PFECAs							
1	$C_3HF_5O_3$			179.9846	178.9773	380.9438	358.9619
2	$C_4HF_7O_3$			229.9813	228.9740	480.9372	458.9553
3	$C_5HF_9O_3$	863090-89-5		279.9782	278.9709	580.9310	558.9491
4	$C_6HF_{11}O_3$	13252-13-6	undecafluoro-2-methyl-3-oxahexanoic acid	329.9750	328.9677	680.9247	658.9427
5	$C_7HF_{13}O_3$			379.9718	378.9645	780.9182	758.9363
6	$C_8HF_{15}O_3$			429.9686	428.9613	880.9118	858.9299
Polyether PFECAs							
7	$C_7HF_{13}O_7$	39492-91-6	perfluoro-3,5,7,9,11-pentaoxadodecanoic acid	443.9515	442.9442	908.8776	886.8957
8	$C_6HF_{11}O_6$	39492-90-5	perfluoro-3,5,7,9-butaoxadecanoic acid	377.9598	376.9525	776.8942	754.9123
9	$C_5HF_9O_5$	39492-89-2	perfluoro-3,5,7-propaoxaoctanoic acid	311.9681	310.9608	644.9108	622.9289
10	$C_4HF_7O_4$	39492-88-1	perfluoro-3,5-dioxahexanoic acid	245.9764	244.9691	512.9274	490.9455
PFESAs							
11	$C_7HF_{13}O_5S$	66796-30-3[b]		443.9337	442.9264		
12	$C_7H_2F_{14}O_5S$			463.9399	462.9326		
Other							
	Na^+			22.9892			
	H^+			1.0073			
	CF_2O			65.9917			
	CF_2			49.9968			

[a]Indicates the monoisotopic mass of the neutral species. [b]CAS number for Nafion copolymer.

pounds is the presence of multiple peaks that differ by exactly ± m/z 49.9968 and/or 65.9917, corresponding to a difference of CF_2 and CF_2O, respectively. This happens because PFAS synthesis is an industrial process that yields a distribution of products that differ primarily in chain length and/or degree of isomerization. Compounds identified as having a negative mass defect and differing from another peak by ± m/z 49.9968 and 65.9917 are highly likely to be polyfluorinated compounds. Selected samples were also analyzed by QTOF to aid with confirmation of proposed structures (generously provided by colleagues mentioned in the Acknowledgments).

TOFMS Experimental Workflow. Once an unknown compound with negative mass defect was noted, it was isolated from other mass spectral data in the following way: An extracted ion chromatogram (EIC) was generated from the total ion chromatogram (TIC) using the m/z identified. The EIC was then used to do a background subtraction of the spectral region both immediately before and after the detected peak (typically ±0.1 min) (Figure 1 step 4). The center of the detected peak was extracted for the mass spectral information, and the background spectrum was subtracted for a clean spectrum. This was necessary to eliminate, to the best of our ability, competing spectral peaks that were coeluting and not associated with the spectrum being investigated. This would include, but is not limited to, spectral signals originating from the reference compounds (constantly infused to maintain a lock on mass assignments) and their fragments. Once suspect spectral signals were isolated, a series of experiments were conducted to induce in-source fragmentation ions to aid with compound identification based on the work of Ferrer and Thurman.[18] Specifically, a series of methods with differing fragmentor voltages (80−190 V) were run in sequence to look for diagnostic ions that emerged as fragmentor voltage increased. In addition, common adducts such as Na^+, NH_4^+, acetate, etc. were used to aid with compound identification. Detected spectral features were subjected to molecular formula generation using the elements (C, H, O, N, S, P, Cl and F).[19] Formulas generated were scored based on accurate mass, isotope abundance (compound mass distributions attributable to elemental mass distributions e.g., 1.1% ^{13}C, 0.2% ^{18}O, etc.) and isotope distribution (for example, slight differences in isotope mass such as when an m+2 peak is from two ^{13}C or a single ^{18}O) . Only scores >75% were considered for further exploration. Figure 1 steps 4 and 5 outline the advanced TOFMS techniques used to discover novel chemical species.

RESULTS

With the approach described above, sites were identified for further investigation when a large increase in number and magnitude (area counts) of unknown compounds was found in downstream samples. In addition, a very large increase (greater than 2 orders of magnitude) in concentration of historically measured PFAAs also persisted for many river miles downstream of a certain point (Figure S2) consistent with observations in Nakayama et al.[10] Interestingly, the profile of the historically measured PFASs contributing to the total PFASs found at each location was seen to dramatically change near this location (Figure S3). The major compounds contributing to this increase were perfluoropentanoic acid (PFPeA), followed by perfluoroheptanoic acid (PFHpA) and perfluorobutanoic acid (PFBA). It was evident that there was a significant source (total historically measured PFASs concentration increased by >100 times) of per- and polyfluorinated compounds near this location. Plotting the samples (CFR004 upstream vs CFR002 downstream) in MassProfiler for visualization indicated that there were 77 features that were unique to the downstream sample (Figure S4) with many (n = 69) having negative mass defects. In this figure (S4) the size of the symbol is proportional to the area counts of the molecular feature. Traditional PFASs with known concentrations (in Figure S2) are included for comparison purposes. Peaks with larger areas were investigated first under the assumption that these concentrations would be among the highest. Additional

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pairwise comparisons were done on all sequential samples, however results indicated that samples downstream of CFR003 were simply dilutions of a source input. Samples were chosen for additional scrutiny where there was sufficient analytical signal without saturation of the detector which compromises mass accuracy.

Dimers and Fragments. It became evident that in multiple instances several of the larger peaks had a number of common characteristics: (1) negative mass defect, (2) multiple related chromatographic peaks differing by ± m/z 49.9968 and/or 65.9917 (± a CF_2 group and/or a CF_2O group), and (3) apparent noncovalent homodimers linked by either a proton or sodium ion. For example, at a retention time of 5.9–6.0 min, two large peaks emerged from the spectra with m/z of 658.9462 and 680.9256 (Figure 2A). After careful evaluation, it became apparent that these were the proton-bound and sodium-bound in-source homodimers of undecafluoro-2-methyl-3-oxahexanoic acid ($C_6HF_{11}O_3$) (Table 1). These differed by m/z 21.9819, the difference between the Na^+ and H^+ versions of the dimer. It is important to note that the $[M - H]^-$ peak (m/z 328.9677), which one might expect to be the most prominent, was barely distinguishable from the background signal at this point because the in-source ionization conditions so heavily favored the formation of these dimers. Given the prominence of the $C_6HF_{11}O_3$ compound in this sample, we postulated that a homologous series of related perfluorinated ether carboxylic acids (PFECAs), differing from the first compound by either the addition or deletion of CF_2 units (m/z 49.9968) might also be present. An EIC for each of the hypothetical masses, based on sequential addition or deletion of CF_2 units (m/z 49.9968) was extracted from the TIC, with the resulting series of related PFECAs presented in Figure S5. Although not specifically shown in this Figure, we also observed the presence of the analogous sodium and proton bound dimers for each compound identified in this homologous series of PFECAs ($C_{(n=3-8)}HF_{(n=5-15)}O_3$ (Table 1). To obtain additional evidence supporting the proposed structure of the compounds eluting at 5.9 min, additional experiments were conducted by altering the fragmentor voltage in order to try to form diagnostic ions from the m/z of 658.9427 and 680.9247 dimers eluting at this time. Figure S6 shows diagnostic dimer and fragment ions resulting from in-source CID that are consistent with the structure proposed for the $C_6HF_{11}O_3$ compound. The major ions found are also shown in Figure S10. Extracted spectra comparable to those shown in Figure S6, differing only by the loss of CF_2 groups, were investigated as well (Figure S7) lending credence to the postulated structure(s) and discussed in more detail in the following section.

Confirmation of Structure. An online search of the formula and proposed structure of the $C_6HF_{11}O_3$ compound eluting at 5.9 min led to a tentative match in a "grey-literature" citation that mentions a new-generation processing aid for the production of high-performance fluoropolymers identified only as C3 dimer acid/salt (CAS 13252-13-6) (Figure S6).[6] With a CAS number, it was possible to purchase an authentic standard (Synquest Laboratories, Alachua, FL) to compare for retention times and mass spectral ionization patterns. The authentic standard compared very well with the tentatively identified compound found in water samples (±0.051 min RT; ± m/z 0.0002 (0.51 ppm) (Figure 1 step 6). Additional authentic standards for other compounds in this homologous series were not commercially available. However, common spectral patterns and adducts were observed differing only by repeating CF_2 units, as supporting information for compound identification of a homologous series of PFECAs (Figure S7).

Additional Perfluorinated Ether Acids. Figure S4 shows that there are a number of features that are present in the water sample downstream of a potential input location that are not in the upstream sample. Evident in Figure S4 are a pronounced series of peaks that appear as vertical lines, likely from coeluting and related chemicals (see later discussion on in-source n-mer formation). A number of these peaks exhibited negative mass defects. Further investigation of the TOFMS spectra indicated two peaks coeluting at 6.5 min (m/z 776.8942 and 754.9123) with a mass difference of m/z 21.9819, again suggesting the presence of in-source proton- and sodium-bound homodimers (Figure 2B). Knowing this information, we postulated an exact mass of m/z 376.9525 for the $[M - H]^-$ ion (Table 1). We further postulated a homologous series based on addition and deletion of CF_2 units, but exact masses corresponding to ± m/z 49.9968 were not detected. Given reports of industrial producers making greater use of ether oxygens to limit the size of perfluorinated regions within a given molecule, we postulated that a homologous series could also be based on the repeating units of CF_2O, with an exact mass offset of ± (m/z 65.9917).[17,20] Searching the sample for m/z 376.9525 ± this hypothetical CF_2O offset did yield another homologous series (Figure S8). In addition, as was previously the case, each of these new compounds was also found to form sodium-bound and proton-bound homodimers, providing further support for the proposed structures (Table 1). Confirmation of these compounds with authentic standards was not possible. It should be noted that in the homologous series shown, the chemical $C_3HF_5O_3$ (m/z 178.9773) is a common feature (Table 1) to the previously identified homologous series. Taken together, the exact mass of the proposed structures, the offset by a CF_2O, and the exact mass of the in-source dimers formed, all support the occurrence of this additional homologous series. One compound in this homologous series ($C_4HF_7O_4$ $[M - H]$ 244.9691 Table 1) was subjected to QTOF analysis leading to a series of accurate mass fragments consistent with the proposed structure (Figure S9). The accurate mass fragments show the sequential breakage of the ether oxygen bonds and the resulting fragment ions.

In-Source *n*-mer Formation. Multiple peaks originally thought to be of polymeric origin (m/z 1176.8353 and 1576.7769) were determined to be sodium-bound n-mers of m/z 376.9525 already identified (Figure S12). The retention time of these peaks (6.5 min) indicates this is an in-source phenomenon, as they coelute (Figure S4). In-source artifacts occur in the ESI source and can include such things as the formation of adducts (formate, Na^+, H^+, NH_4^+), fragments, dimers, trimers, and combinations of the above. Peaks that do not coelute, or are chromatographically separated, are generally considered not to be in-source artifacts. The observed spectra (Figure S12) include the formation of n-mers[17] including 2 and 3 bound sodium cations in addition to the compounds shown in Figure S11. The formation of other n-mers with >1 bound sodium was seen as well for additional PFECAs found in this work (data not shown). Recent work by Trier et al.[17] indicates that the formation of these polyfluorinated surfactant clusters occurs in the MS source in the gas phase and are pH and concentration dependent.[17] However, this phenomenon may also be instrument-specific as sample dilution in this study did not lead to loss of these in-source dimers being formed. Similar

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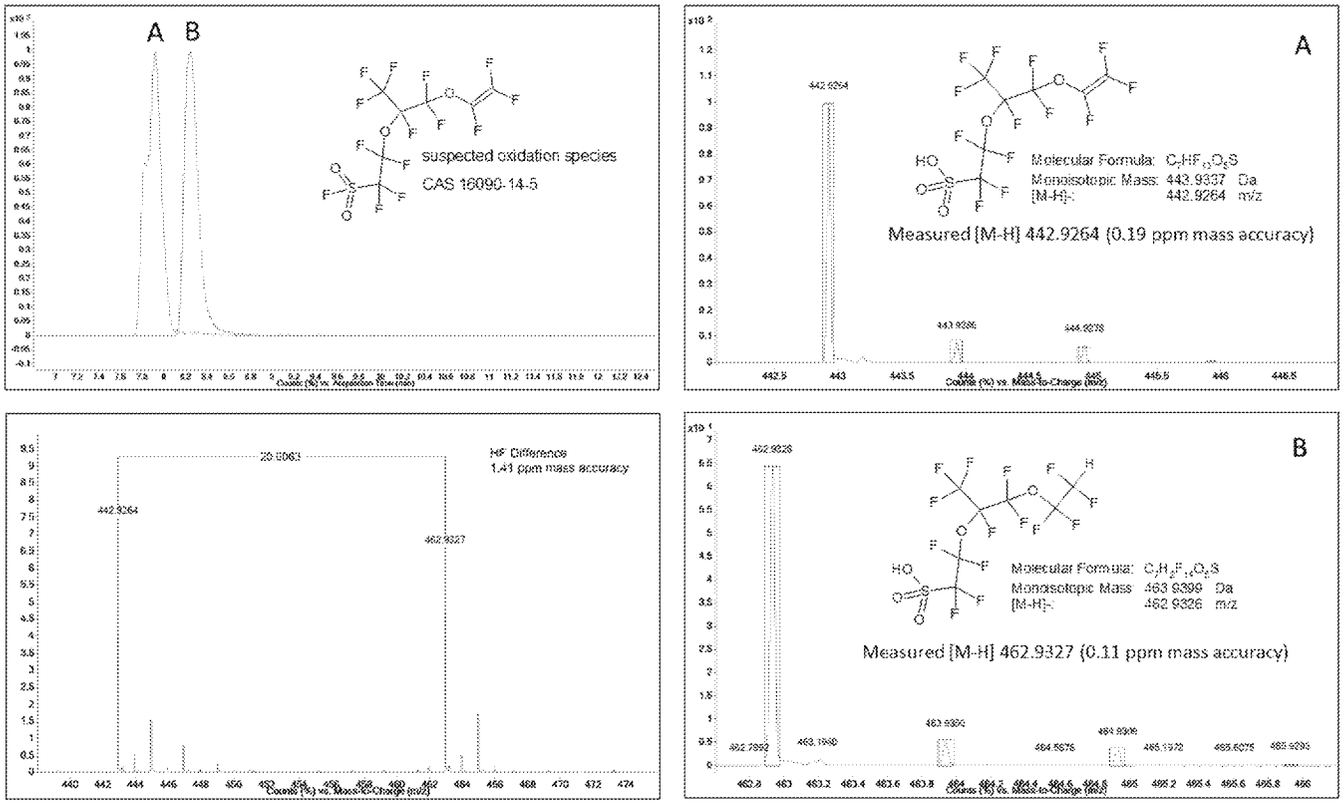

Figure 3. Suspected perfluoro ether sulfonic acids identified in water. Oxidation species are likely products of CAS 16090-14-5, a perfluorinated ether sulfonyl fluoride. Chromatogram shown of two resolved peaks (A,B) and isotope cluster matching of measured versus exact mass. Red boxes indicate the exact mass for the suspected formulas, compared to the accurate mass profile spectrum. Mass difference between two species is an HF (m/z 20.0063−1.41 ppm mass accuracy).

sodium- and proton-bound, as well as mobile phase, modifier (i.e., formate) adducts have been shown by Trier et al.[17] for perfluorocarboxylic acids and mono-PAPs (perfluorinated alkyl phosphates).

Perfluoroalkyl Ether Sulfonic Acid (PFESA) Identification. Records of groundwater monitoring at a fluorochemical manufacturing plant with wells in close proximity to our sampling site indicate PFOA in μg/L concentrations.[21] This document also indicated a sulfonated tetrafluoroethylene based fluoropolymer-copolymer with the trade name Nafion was also produced at this facility. Our data provide evidence that materials related to this compound are also emitted into the river at this point. Figure 3 shows the chemical structures of the suspected oxidation species and resulting transformation products tentatively identified in our samples. An EIC of m/z 442.9264, which is the exact mass of the $[M - H]^-$ moiety of the copolymer containing the sulfonate group (IUPAC name 1,1,2,2-tetrafluoro-2-({1,1,1,2,3,3-hexafluoro-3-[(trifluorovinyl)-oxy]-2-propanyl}oxy)ethanesulfonic acid), suggests this compound is present in the water. In addition, spectral evidence supports the detection of a related compound which is consistent with the addition of an HF ($[M - H]^-$ m/z 462.9327) (IUPAC name 1,1,2,2-tetrafluoro-2-{[1,1,1,2,3,3-hexafluoro-3-(1,1,2,2-tetrafluoroethoxy) propan-2-yl]oxy}-ethanesulfonic acid). The two suspected compounds differ by the mass of HF (m/z 20.0062−1.41 ppm mass accuracy) (Figure 3). These compounds elute as two discrete chromatographic peaks, likely explained by the position occupied by the H and F additions. It is possible that these chemicals originate from the oxidation of ethanesulfonyl fluoride, 2-[1-[difluoro-[(t(fluoroethenyl)oxy]methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro- (CAS 16090-14-5) which may have been in use at this industrial location.[22] These compounds would thus be classified as perfluoroalkyl ether sulfonic acids (PFESA).[4] However, unlike previous perfluorinated ether carboxylic acid compounds found in this study, in-source proton- and sodium-bound dimers were not found for these perfluorinated ether sulfonates. In addition, a homologous series based on CF_2 or CF_2O additions was not found. We have not been able to obtain an authentic standard for confirmation of this proposed structure.

DISCUSSION

It has been well-known for a number of years that, due to the toxicity and persistence of perfluorinated compounds, industrial producers are moving toward shorter chain per- and polyfluorinated compound homologues. However, it is not well-known which compounds are being produced as replacements for historic perfluorinated compounds (e.g., PFOS and PFOA). Peer-reviewed literature showing the chemical structure of manufactured per- and polyfluorinated replacement compounds is sparse. An example of one such publication from 3M Corporation indicated that the compound ADONA (4,8-dioxa-3H-perfluorononanoate) is being made to replace ammonium perfluorooctanoate (APFO) as an emulsifier in the manufacture of fluoropolymers.[5] The data presented in this study and Figure S1 indicate that the manufacture and release of per- and polyfluorinated compounds with CF_2 units interspersed with ether oxygen linkages likely is ongoing. A more recent study suggests that polyfluoroalkyl ether

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compounds are being produced and that the number and spacing of ether oxygen linkages and size of the resulting per- and polyfluorinated compound may be manufacturer-specific including other major fluorochemical producers (DuPont, 3M, Solvay, and Asahi).[20] However, there are little to no data in the peer-reviewed literature on most of these compounds. The use of HRMS appears to be an ideal technique to use for discovery and occurrence of new PFASs being released into the environment.

As illustrated in this research, identification of previously undescribed compounds in environmental matrices can be made difficult by the formation of a multitude of in-source artifacts such as in-source gas phase adducts, *n*-mers, and the presence of a homologous series of per- and polyfluorinated compounds. D'Agostino and Mabury identified novel fluorinated surfactants in aqueous film-forming foams (AFFF) and commercial surfactant concentrates using high-resolution mass spectrometry.[23] Their results imply that there are a number of additional fluorinated compounds being produced and emitted into the environment that are unknown to most analysts. The use of TOFMS or other high-resolution mass spectrometers and the workflow we demonstrated (Figure 1) appears to be a useful way to determine occurrence and emissions of new and emerging fluorinated compounds for environmental occurrence efforts.

Schymanski et al. report on identifying small molecules via HRMS, with five levels of confidence.[14] As has been noted earlier, one of the pitfalls of discovery work is the lack of authentic standards at times. As such the highest level of confidence for confirmed structure (Level 1) can be made to one compound in this effort, with the remaining compounds falling in the categories of tentative candidate(s) (Level 3) or probable structure (Level 2).[14] An additional line of evidence we present that Schymanski et al. appear not to is the presence of a homologous series (CF_2, CF_2O) as evidence for compound identification.

The manufacture of shorter-chain perfluorinated and polyfluorinated compounds may have an advantageous effect on biological persistence and bioaccumulation. Two recent publications deemed "The Helsingor Statement"[24] and the "Madrid Statement"[25] explore the state of the science related to poly- and perfluorinated alkyl substances. Shorter straight-chain homologues of perfluorocarboxylic acids ($<C_6$) and sulfonic acids (<PFBS) have been shown to be cleared quickly from mammalian species tested and thus are not expected to bioaccumulate as readily as PFOS and PFOA.[26] However, the environmental persistence of these shorter chain perfluorinated compounds is likely to be no different from that of the related longer-chain acids. The conventional wisdom is the perfluorinated ether compounds will be more labile than the *n*-alkyl perfluorinated homologues with ether oxygen linkages being more susceptible to chemical or microbial attack. However, as the CF_2 bond in perfluorinated compounds is not at all open to microbial attack due to its bond strength, even the slightest degree of degradation of a per- or and polyfluorinated ether compound would be considered "more" labile. Environmental degradation or toxicology studies associated with the compounds identified in this study were not found by the authors in the peer-reviewed literature at the time of writing this Article. Although data are scarce, the few perfluoropolyethers (PFPEs) to have undergone any kind of degradation studies show little to no biodegradation or hydrolysis.[28] These data mainly come from the European Chemical Agency (ECHA) database of registered substances and associated information.[27] Past studies have demonstrated poor removal efficiency for historical PFASs from source water to drinking water in conventional systems (i.e., treatments that do not include granular activated charcoal (GAC)). It is unknown if these PFECAs and PFESAs are removed by conventional WWTP processes.

Environmental Implications. We demonstrate the presence of a series of novel perfluorinated ether carboxylic and sulfonic acid(s) found to be in natural waters using a nontargeted workflow (Figure 1). The compounds consist of a homologous series of per- and polyfluorinated compounds with repeating units of CF_2 or CF_2O subunits. LC-TOFMS investigations of accurate mass dimers and *n*-mers (proton- or sodium-bound) in addition to diagnostic fragment ions support these findings. Further unidentified compounds with negative mass defects are likely also of per- and polyfluorinated origin, but have not yet been identified and are undergoing further analytical scrutiny. Nontargeted screening and discovery of novel species in samples such as reported here is an ongoing process. Additional environmental samples from other locations may be needed to identify additional chemistries.

Once a xenobiotic compound is identified in the environment, it falls upon the scientific community to begin monitoring efforts to find the extent of contamination of newly discovered species. In addition, toxicological and degradation investigations of identified compounds can commence once a compound has been identified. The procurement of authentic compounds for use in these types of investigations may be a major limiting factor in conducting such investigations, as most of these compounds appear not to be commercially available. More research will need to be conducted on these perfluorinated ether carboxylic and sulfonic acids concerning environmental occurrence, toxicology, and degradation potential.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b01215.

Additional information including tables, figures, chemicals structures, and spectra (PDF)

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Notes

The authors declare no competing financial interest.

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Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Supporting Information for:

Identification of novel perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs) in natural waters using accurate mass time-of-flight mass spectrometry (TOFMS)

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5. Agilent Technologies Inc., Wilmington, DE

This document includes one table, and 12 SI figures, on 13 pages.

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Table S1. Water samples description and GPS coordinates.

Sample ID	Description	Latitude	Longitude
CFR 001	Cape Fear river Tar Heel, NC	34.74525	-78.78574
CFR 002	Cape Fear river below Huske lock and dam #3	34.83026	-78.82246
CFR 003	unnamed tributary	34.83179	-78.82375
CFR 004	Cape Fear river above Huske lock and dam #3	34.83544	-78.82347
CFR 005	Cape Fear river below Rockfish creek	34.96820	-78.81579
CFR 006	Rockfish Creek	34.95610	-78.84424
CFR 007	Rockfish Creek WWTP effluent	34.96834	-78.82765
CFR 008	Cape Fear River at Fayetteville boat ramp access	34.99669	-78.85076
CFR 009	Regional drinking water sample	34.94199	-78.92422

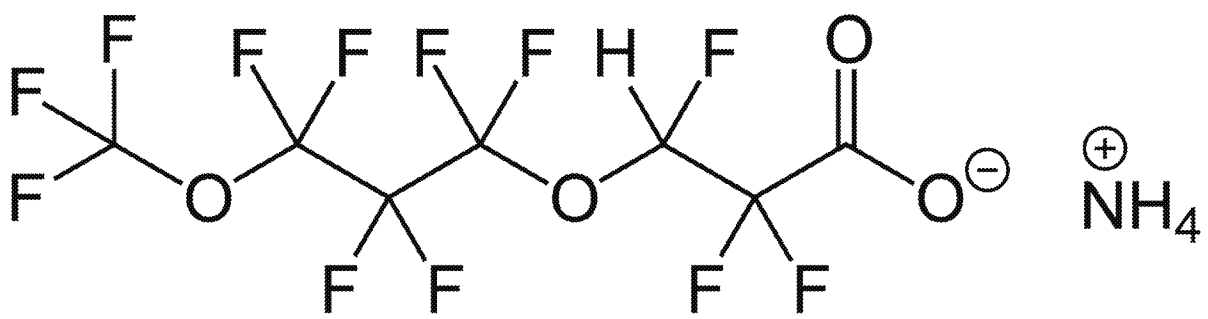

Figure S1. The structure of ADONA (4,8-dioxa-3H-perfluorononanoate) a polyfluorinated compounds used to replace classic perfluorinated compounds such as APFO.

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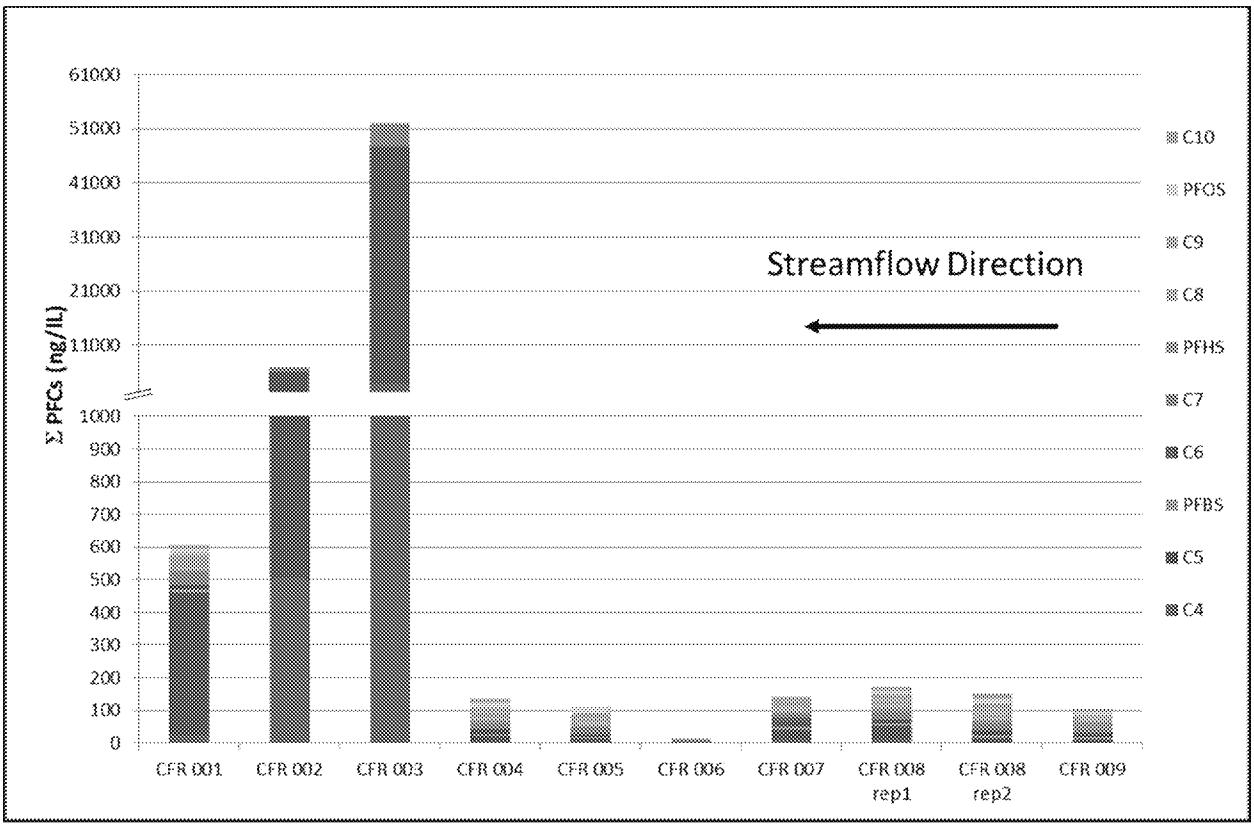


Figure S2. PFAAs found in water samples from the Cape Fear River. Note the y-axis is a split scale.

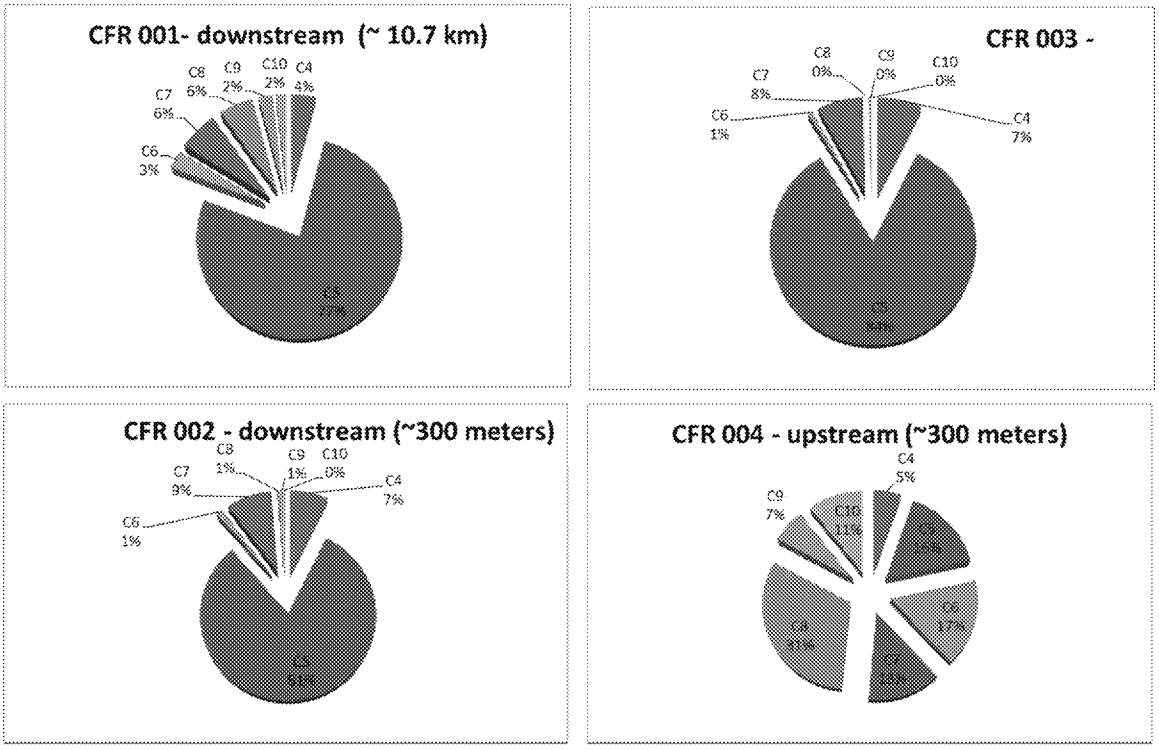


Figure S3. Proportion of PFAAs contribution to the total for select water samples from the Cape Fear River.

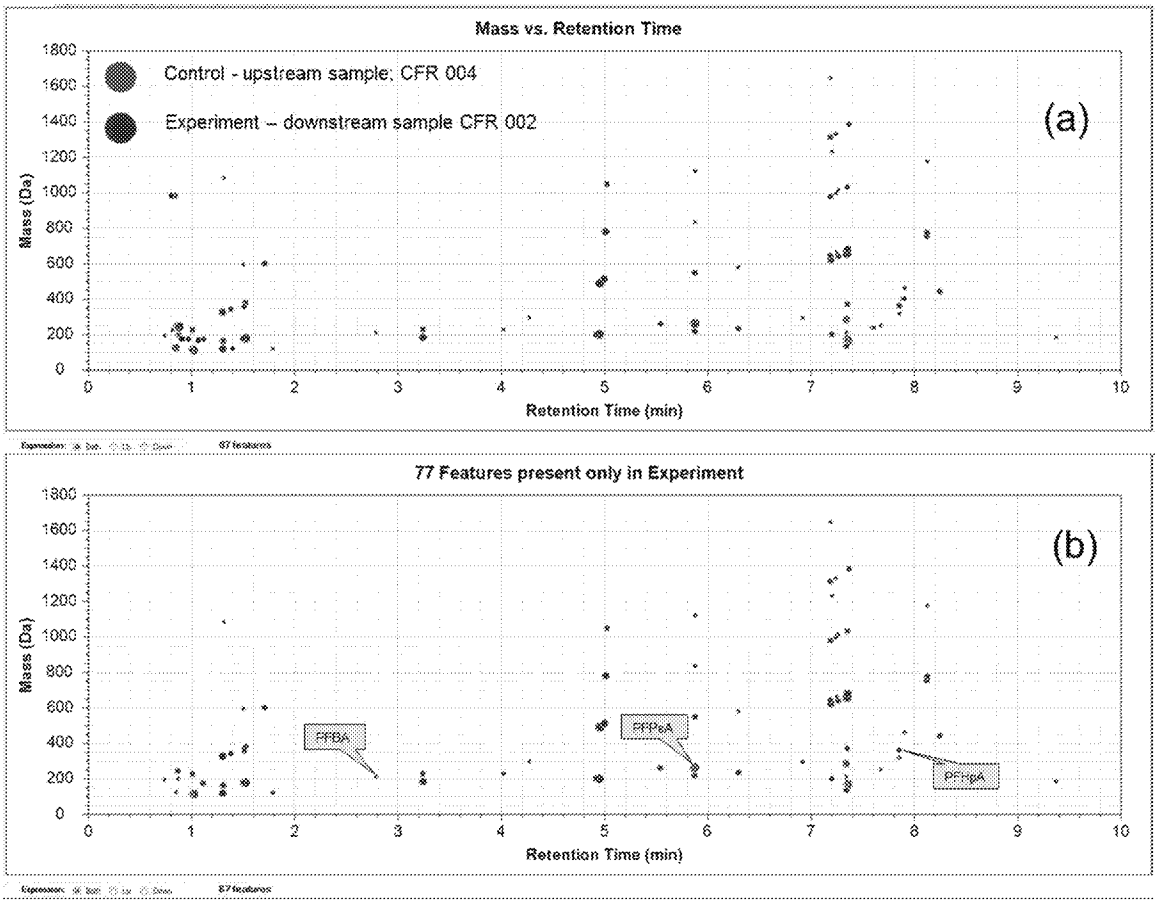


Figure S4. Mass Profiler visualization of molecular features found in (a) control and experimental sample and (b) 77 features unique only to experimental sample, with select peaks previously identified (PFBA, PFPeA and PFHpA). Note: The size of the symbol in this plot is proportional to the area of the peak. Pronounced co-eluting peaks that appear as vertical lines are likely related (i.e. fragments, M-H-, *n-mers*).

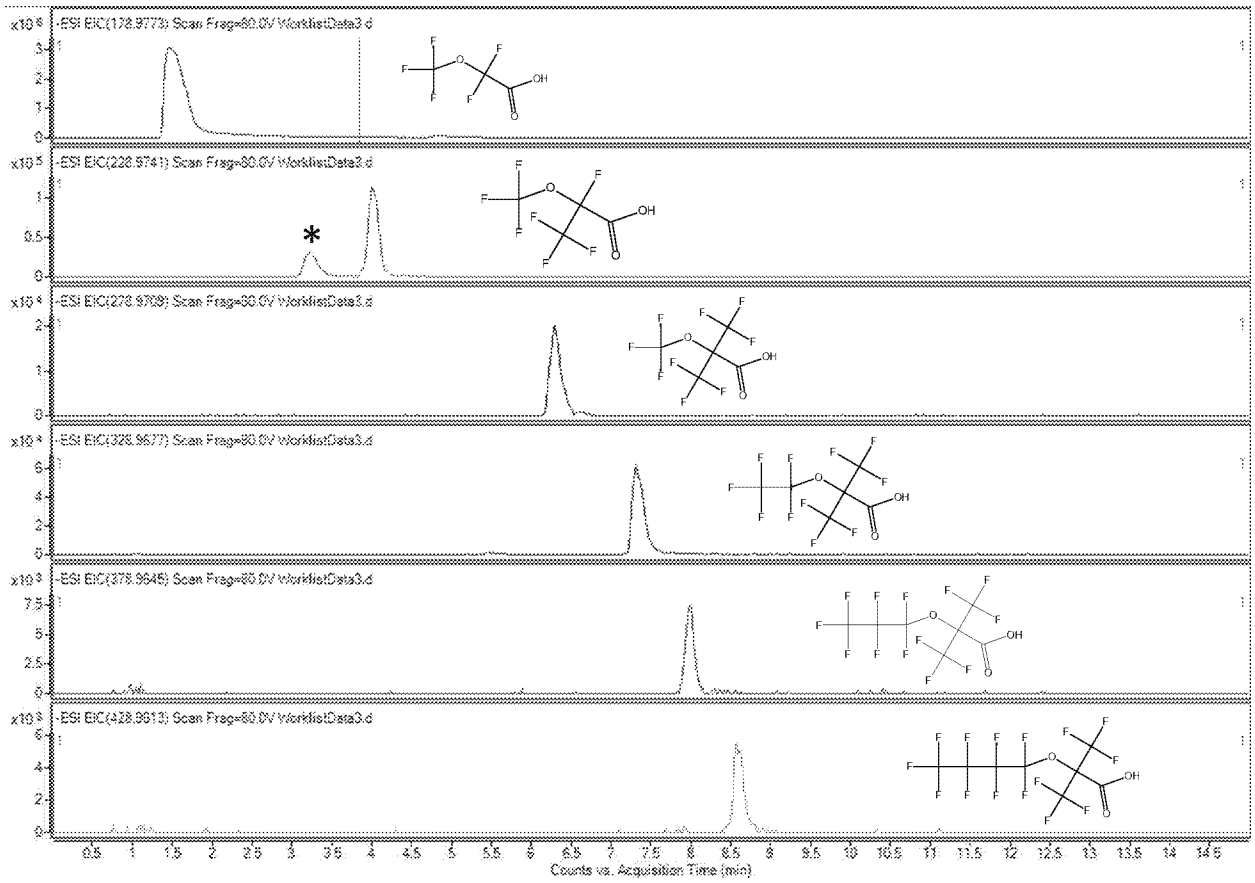


Figure S5. Extracted Ion Chromatogram (EIC) of a suspected homologous series of perfluorinated ether carboxylic acids. Note: The chromatographic peak indicated by an * is the substance associated with this homologous series based on the H^+ and Na^+ dimer co-elution at this retention time. The second later eluting peak in this chromatogram is a similar m/z, possibly an isomer.

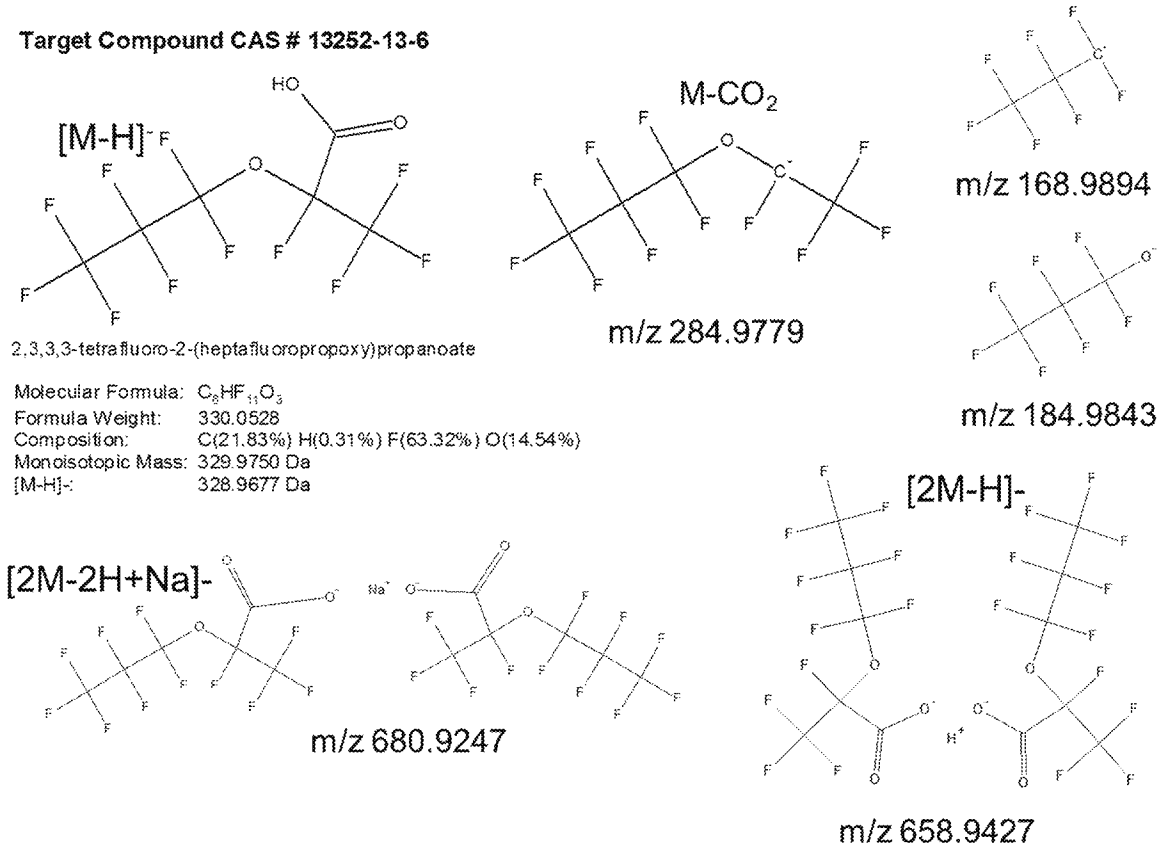
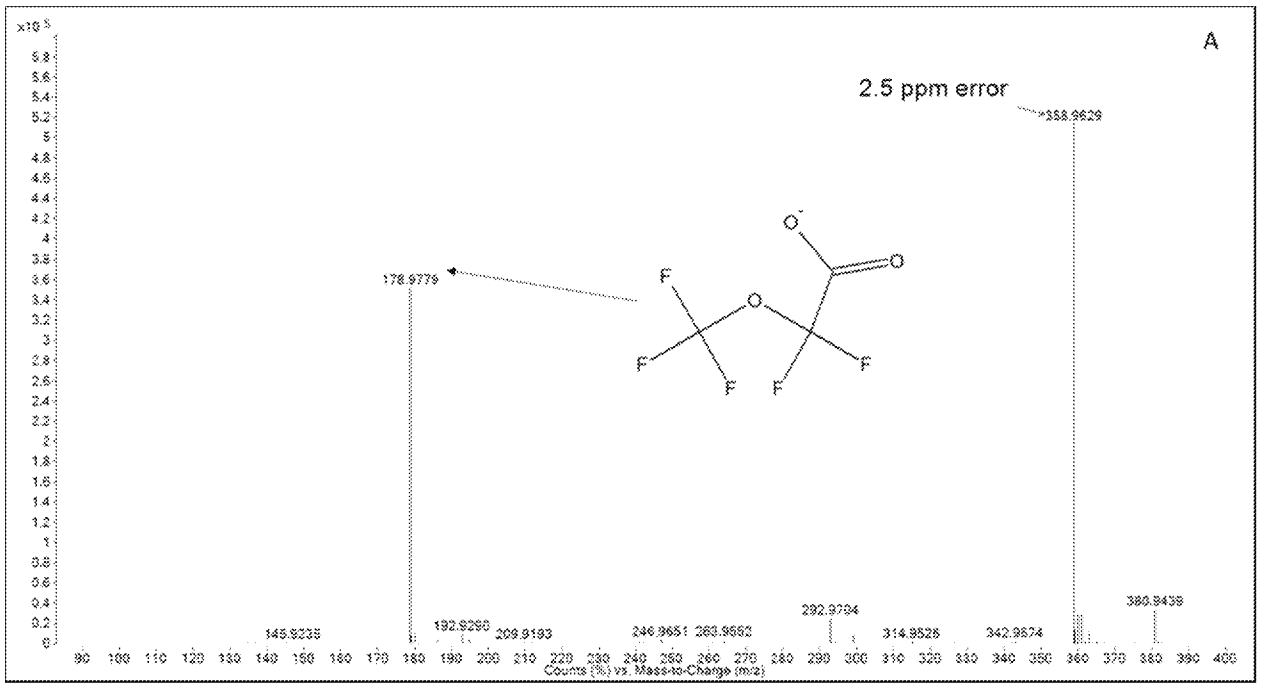
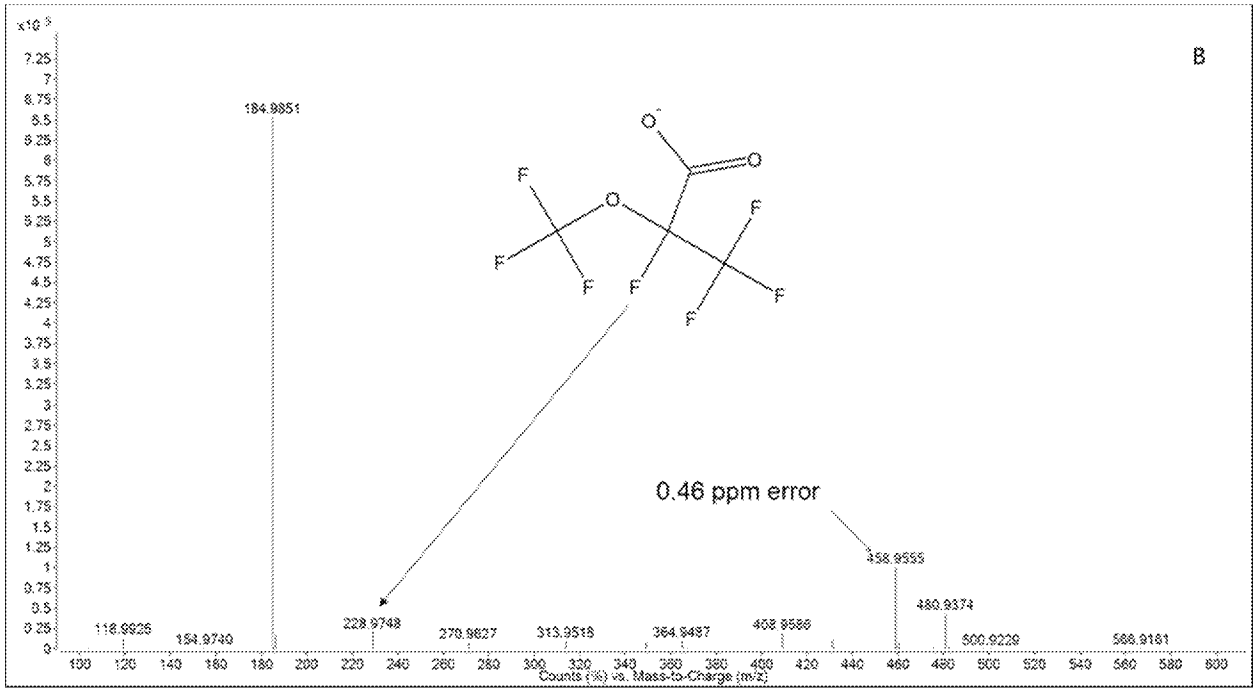


Figure S6. Identified perfluorinated ether carboxylic acid and diagnostic fragment and dimer ions.

92


A
2.5 ppm error
358.9629
178.9779
380.9439
292.9704
314.9528
342.9674
Counts (%) vs. Mass-to-Charge (m/z)

93


B
184.9851
0.46 ppm error
358.9533
480.9374
228.9748
Counts (%) vs. Mass-to-Charge (m/z)

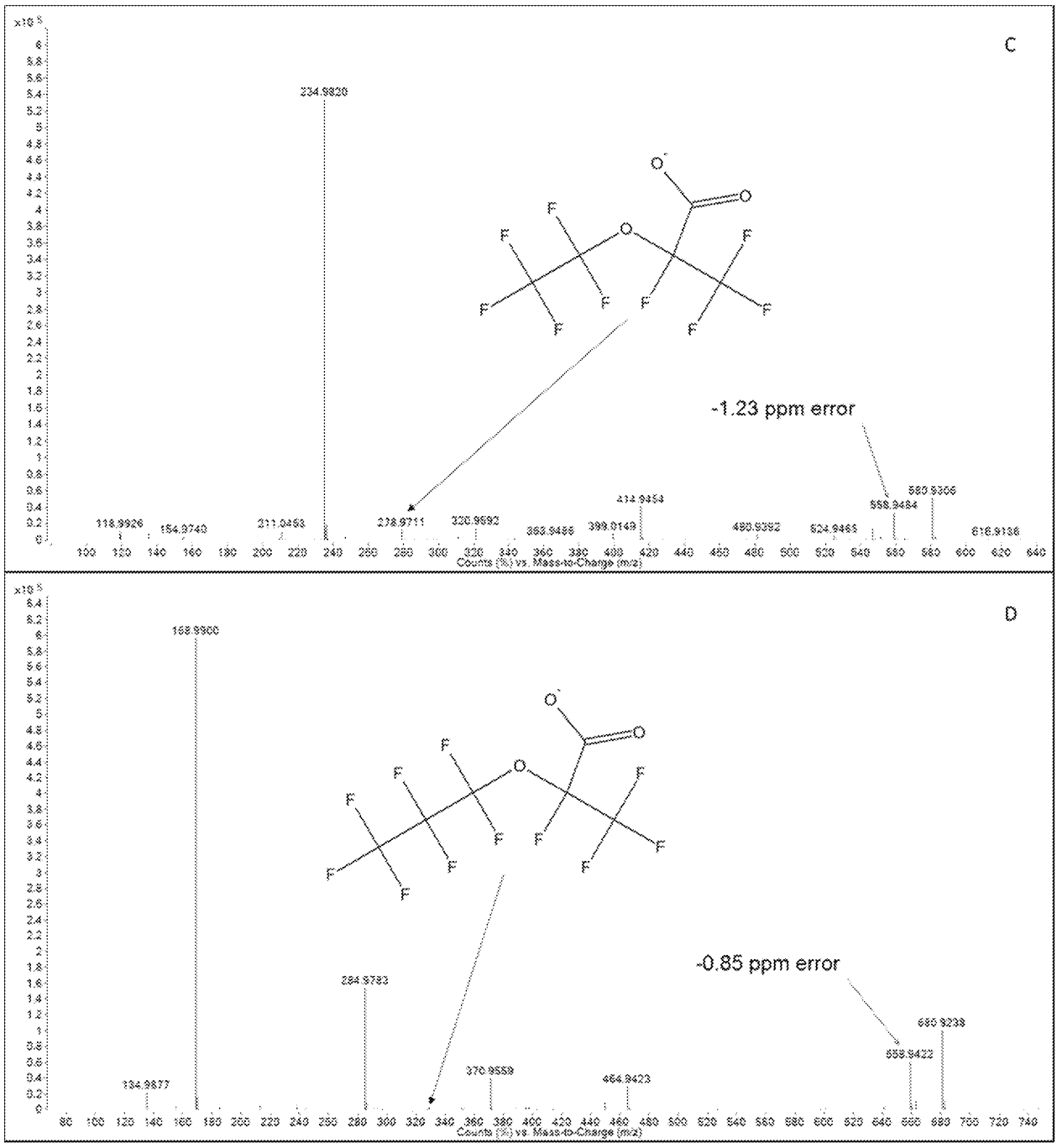


Figure S7 Spectra for Figure S5 chromatographic plot EICs. The spectra shown are for the first four eluting mono ether PFECAs shown in Figure S5. Plot A corresponds to m/z 178.9773; Plot B corresponds to m/z 228.9741; Plot C corresponds to m/z 278.9709; Plot D corresponds to m/z 328.9677. Error values shown are for the corresponding $[2M-H]^-$ (Table 1)

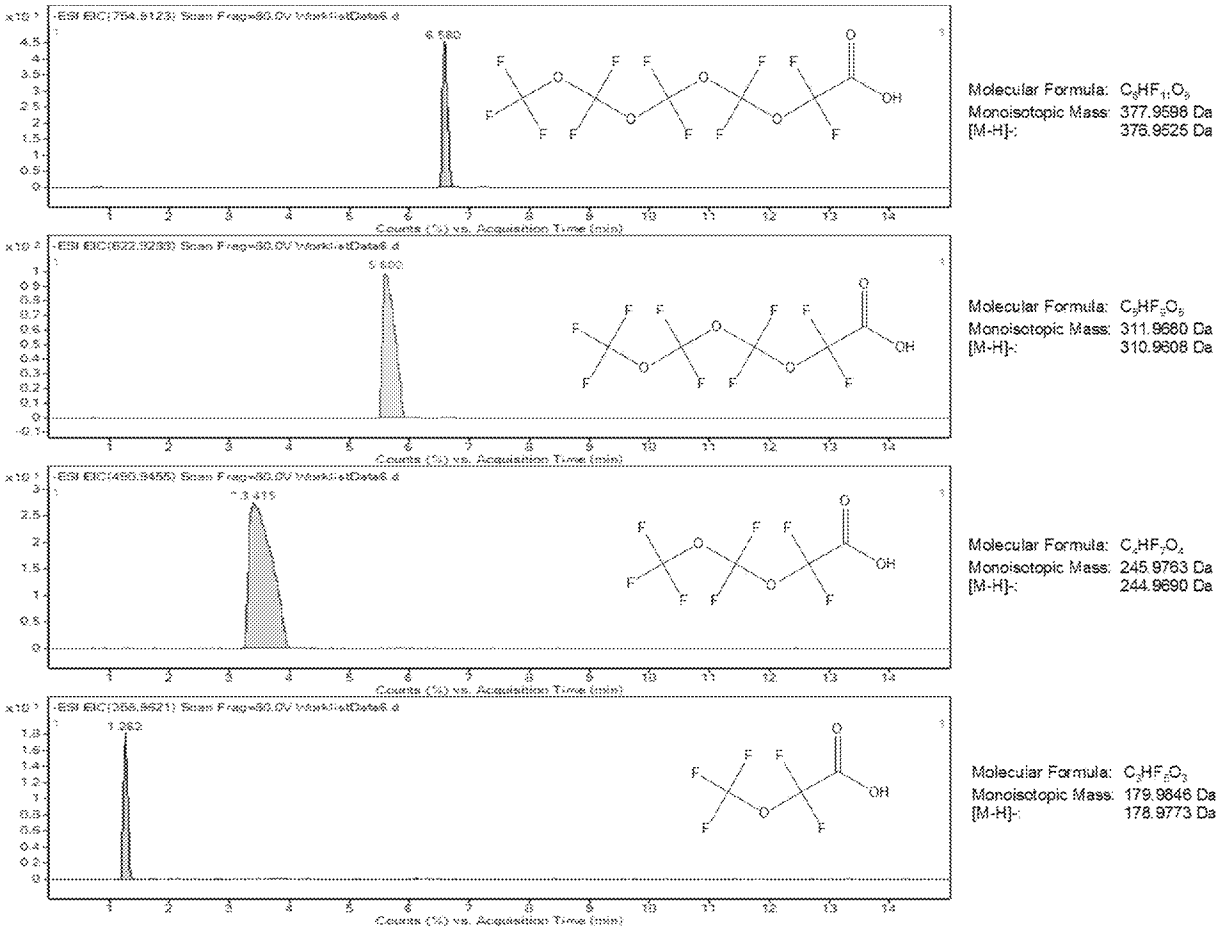


Figure S8. Extracted ion chromatogram of additional perfluorinated ether carboxylic acids homologous series. Proton bound dimer EIC shown for chromatogram. Monomer structure and exact mass shown

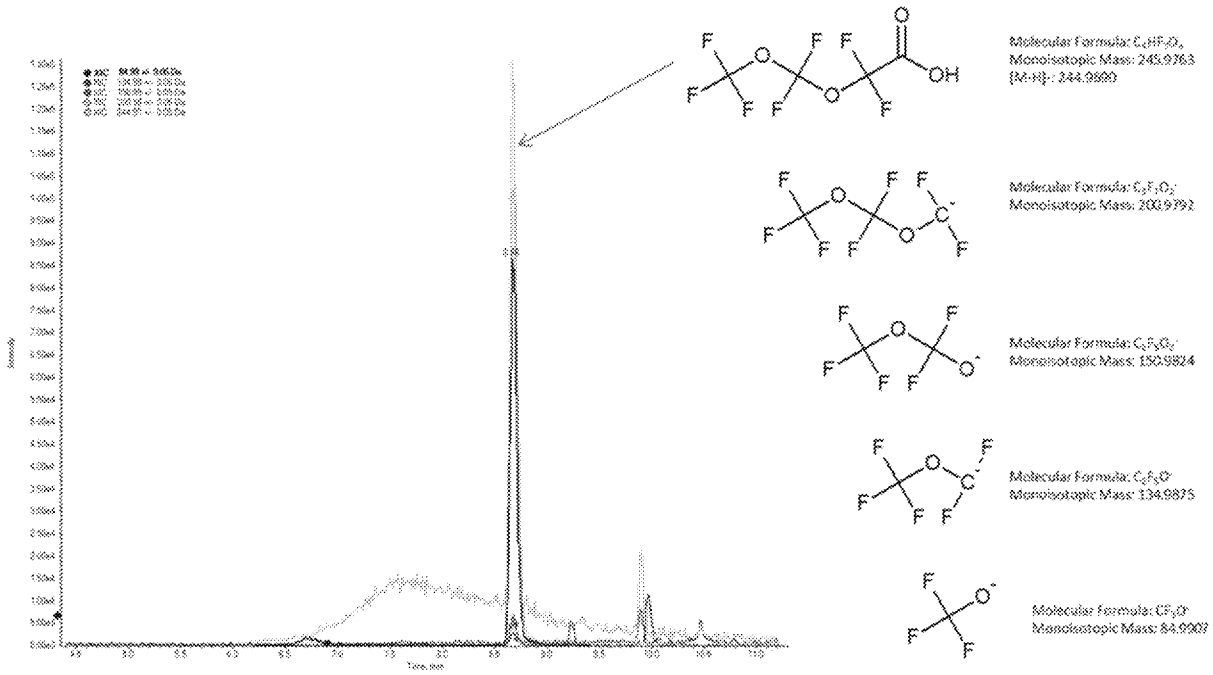
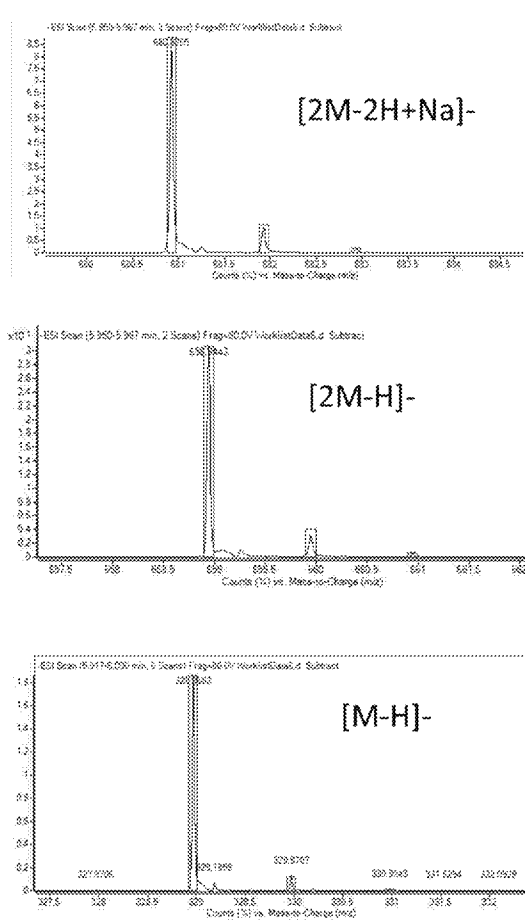
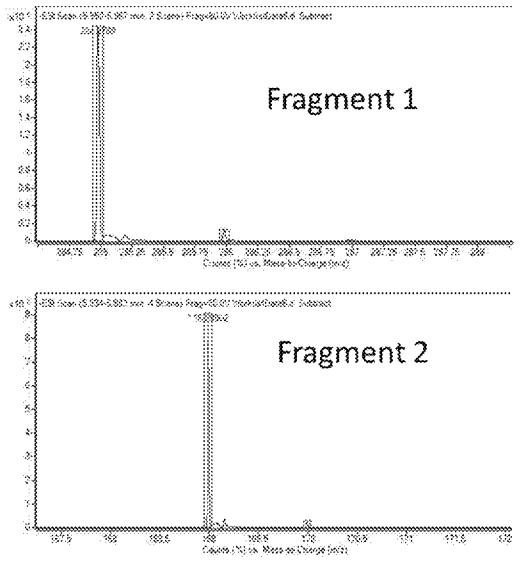


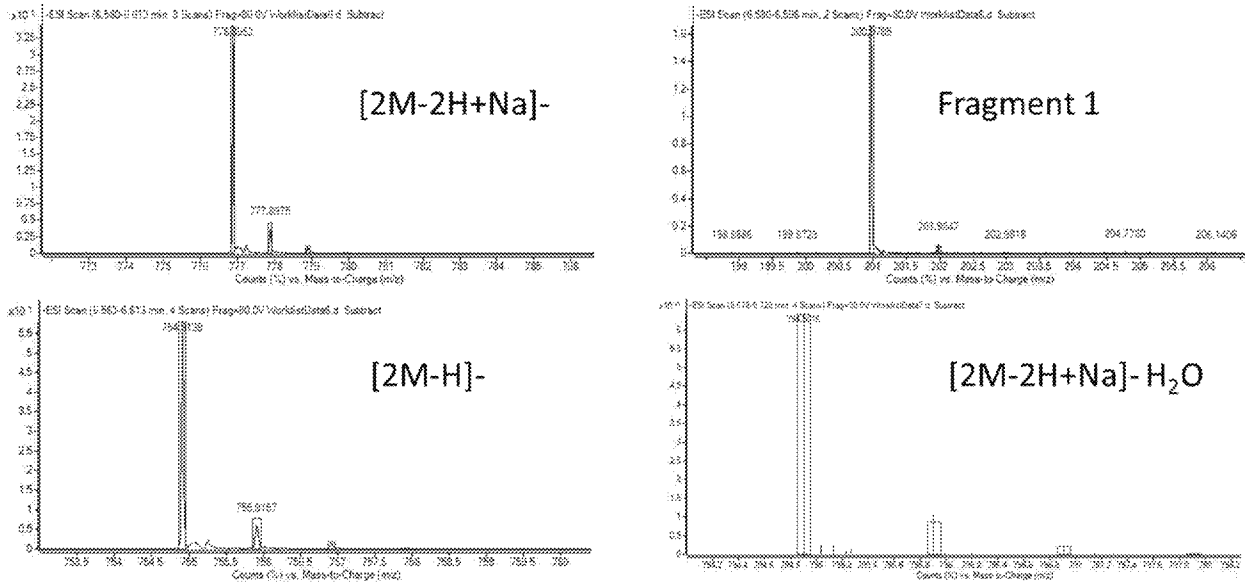
Figure S9. QTOF fragment ions for $C_4HF_7O_4$ (m/z 244.9691) pefluoroether carboxylic acid.





	Formula	Measured m/z	Exact m/z	Error (ppm)	Score
[2M-2H+Na]-	$C_{12}F_{22}NaO_6^-$	680.9255	680.9247	-1.2	98.11
[2M-H]-	$C_{12}HF_{22}O_6^-$	658.9433	658.9427	-2.36	92.96
[M-H]-	$C_6F_{11}O_3^-$	328.9683	328.9677	-1.8	97.85
Fragment 1	$C_5F_{11}O^-$	284.9789	284.9779	-3.49	96.35
Fragment 2	$C_5F_7^-$	168.9902	168.9894	-4.75	84.41

Figure S10. Spectrum, isotope pattern matching and identified adducts and fragment of molecular feature (m/z 328.9683) found in water. The table shows the formula, measured m/z, the exact m/z, the error associated with the measurement and the software scoring of the isotope cluster. The red boxes around each spectrum peak indicate the agreement between the measured and theoretical isotope cluster. The scoring is a bundled measurement of the accurate mass of the monoisotopic peak versus theoretical, the isotope abundance and the isotope spacing.



	Formula	Measured m/z	Exact m/z	Error (ppm)	Score
[2M-2H+Na]-	$C_{12}F_{22}NaO_{12}^-$	776.8952	776.8942	-1.31	95.92
[2M-H]-	$C_{12}HF_{22}O_{12}^-$	754.9139	754.9122	-2.22	92.66
[M-H]-	$C_6F_{11}O_6^-$	Not Observed	376.9525	---	---
Fragment 1	$C_3F_7O_2^-$	200.9785	200.9792	3.71	95.18
[2M-2H+Na]- H_2O	$C_{12}H_2F_{22}NaO_{13}^-$	794.9016	794.9047	3.95	84.15

Figure S11. Spectrum, isotope pattern matching and identified adducts and fragment of molecular feature (m/z 376.9525) found in water. The table shows the formula, measured m/z, the exact m/z, the error associated with the measurement and the software scoring of the isotope cluster. The red boxes around each spectrum peak indicate the agreement between the measured and theoretical isotope cluster. The scoring is a bundled measurement of the accurate mass of the monoisotopic peak versus theoretical, the isotope abundance and the isotope spacing.

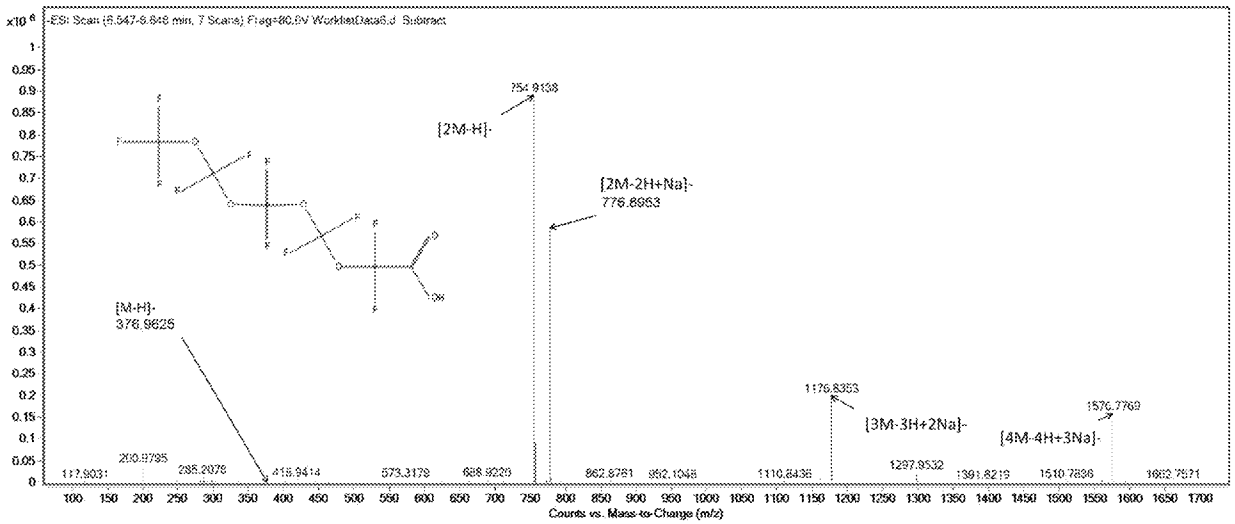


Figure S12. Spectrum of novel perfluorinated ether carboxylic acids showing proton bound and sodium bound *n*-mers found. The structure shown is the parent compound. The various in-source n-mers formed are shown with shorthand nomenclature for dimers, trimers and tetramers.