

# Detection and Quantitation of Per- and Polyfluoroalkyl Substances in Sea Foam and the Corresponding Sea Water

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## ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants found in various matrices, including water and sea foam. This study utilized both quantitative and non-targeted analysis to investigate PFAS contamination in sea foam, bulk water, and blank samples as previous work has suggested PFAS can accumulate in higher concentrations in sea foam than the bulk water. High-resolution mass spectrometry (HRMS) was employed to identify and quantify PFAS, with strict quality control measures applied to confirm results. Quantitative analysis detected up to 42 PFAS compounds, with particularly high concentrations in condensed sea foam samples, highlighting their role in accumulating PFAS. In one foam sample, PFOS, was observed at ca. 8,000,000 ng/L whereas, PFO5DoA, a compound predominantly found in North Carolina, was detected at levels exceeding 20,000 ng/L in some samples. Notably, emerging and regionally relevant PFAS - including unsaturated perfluorooctane sulfonic acid (UPFOS), hexafluoropropylene oxide trimer acid (HFPO-TeA), and 3-oxaperfluorononane sulfonic acid (F53) - were identified through non-targeted analysis. Comparisons between bulk water and sea foam samples revealed distinct PFAS concentration patterns, and significantly higher concentrations in the sea foam. The study underscores the need for continued monitoring of PFAS in coastal environments to assess potential human and ecological exposure risks.

## KEYWORDS

PFAS contamination, Sea foam accumulation, High-resolution mass spectrometry (HRMS), Quantitative analysis, Non-targeted analysis, Per- and polyfluoroalkyl substances (PFAS), Emerging PFAS compounds

## SYNOPSIS

This study investigates PFAS contamination in sea foam and bulk water using HRMS. Results reveal high PFAS concentrations in sea foam, identifying emerging compounds and emphasizing the need for coastal environmental monitoring and exposure assessment.

## INTRODUCTION

Per and poly-fluoroalkyl substances (PFAS) are a group of synthetic molecules that are ubiquitously found in biological and environmental forms of media.<sup>1</sup> Many PFAS have surface-active properties that make these compounds especially persistent and toxic to humans and the environment.<sup>2</sup> These same surface-active properties mean PFAS are very proficient at forming foams in aqueous media. When PFAS are released into natural waterways excessive foams have been observed that, in some cases, have been directly linked to a preferential partitioning of PFAS to the surface microlayer of water bodies.<sup>3</sup> These foam-causing PFAS are suspected to originate from sources such as landfill leachate runoff, chemical manufacturer discharge, and airport discharge (presumably from firefighting foam).<sup>4</sup> In an evaluation of PFAS-containing foam found in the state, Michigan's Department of Health and Human Services concluded these foams may present a public health hazard as a result of ingestion or dermal exposure to the foam, due to the high levels of PFAS they contain and issued a foam advisory warning residents to avoid contact with foam found on rivers and lakes.<sup>5</sup>

North Carolina has been especially affected by PFAS contamination due to chemical manufacturing that occurs along the Cape Fear River. Many reports have identified and quantitated various PFAS at remarkable levels in environmental samples as well as biological fluids of residents.<sup>6-10</sup> Communities in this region remain concerned about historic and new exposures to

PFAS impacting their way of life. Recently, nine United Nations human rights experts called out major PFAS producers, as well as state and federal regulators, for failing to protect residents, especially in North Carolina, from business-related human rights abuses. These studies, combined with community advocacy efforts have influenced legislature at the federal level as GenX, a PFAS found predominantly in NC, has been assigned an U.S. EPA Maximum Contaminant Level of 10 parts per trillion (ppt). Some of the PFAS identified in these studies are particularly pertinent to North Carolina as these compounds are predominantly produced/discharged from chemical manufacturing plants located in this area. These compounds include (but are not limited to) GenX, PFMOAA, PEPA, PMPA, PFO2HxA, PFO3OA, PFO4DA,<sup>11</sup> PFO5DoA,<sup>11</sup> Nafion Byproduct 1 (PS Acid), Nafion Byproduct 2, NVHOS, Hydro-EVE.<sup>12</sup> In a consent order between the North Carolina Department of Environmental Quality, Cape Fear River Watch and Chemours, Chemours was required to provide reverse osmosis drinking water systems for homes in Bladen County with drinking water wells contaminated with greater than 70 ppt total PFAS or 10 ppt of any individual PFAS from a select list of PFAS (including PFMOAA, PMPA, PFO2HxA, PEPA, PFO3OA, PFO4DA, Nafion Byproduct 1 [PS Acid], Nafion Byproduct 2, PFO5DoA, PFHpA and GenX) known to occur primarily in this region.<sup>13</sup>

In recent years, residents of coastal North Carolina, particularly around the Cape Fear River estuary, reported an increased frequency and intensity of foam “blooms” on the beaches. The grassroots community group, Clean Cape Fear, reached out to the Superfund Research Program and its associated Community Engagement Core at North Carolina State University for assistance in identifying and quantitating PFAS in these foams.<sup>14</sup> These studies (though not peer-reviewed) found many PFAS at ranges likely exceeding µg/L levels. Sample collection protocols, however, precluded the ability to perform any quantitative analysis. The lack of paired bulk water samples

did not allow for comparison of any enrichment of PFAS in the foams, and the lack of blank collection samples hindered the ability to assess sample handling/processing contamination. This present study was therefore designed and executed to provide quantitative results for known PFAS, as well as provide a broad range detection strategy for identifying more novel compounds that may be present.

## **MATERIALS AND METHODS**

### **Standards**

In this study 49 PFAS were quantified. See Supplemental Materials (Tables S1 and S2) for a complete listing of standards and heavy isotope labeled internal standards acquired from CIL and other vendors. Briefly, this method quantitated the following PFAS compounds: 4:2 FTS, 6:2 FTS, 8:2 FTS, 10:2 FTS, NEtFOSAA, NMeFOSAA, PFOSA (a.k.a FOSA), FHxSA, MeFOSA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDaA, PFTrDA, PFTeDA, N-AP-FHxSA, N-CMAMP-6:2 FOSA (6:2 FTAB), N-TAMP-FHxSA, Hydro-EVE, PFMOAA, HFPO-DA (GenX), PEPA, PFO3OA, PFO4DA, PFO5DoA, NaDONA, Nafion Byproduct 1 (PS Acid), Nafion Byproduct 2, NVHOS, F53B Major (9Cl-PF3ONS), and F53B Minor (11Cl-PF3OUdS). Heavy isotope internal standards were used whenever available.

### **Sample Collection**

Samples were collected around coastal North Carolina as foam blooms were observed (Table 1, Fig. 1). Notable locations include the Cape Fear Regional Jetport (Fig. 1 purple shape), the Wilmington International Airport (Fig. 1 teal shape), and the Military Ocean Terminal Sunny Point

(MOTSU) (Fig 1. orange shape). MOTSU is the largest military terminal in the world and serves as a major ammunition depot for the U.S. military. Collection locations ranged from sandy beach to water alongside a dock further upriver. Based on collection location, all samples are expected to contain salt water as the Cape Fear River has been estimated to contain predominantly salt water as far north as the port of Wilmington.<sup>15</sup> Three sample types were collected at each collection site. The first sample was a “Field Blank”. For this, a clean 1L HDPE bottle was taken to the collection site, and while wearing gloves the cap was removed for two minutes and then the lid was placed back on. The HDPE bottles used were pre-rinsed with methanol in the lab to remove any potential residual PFAS from the manufacturing process. Additionally, these blank samples serve to track any PFAS that may be contributed by these bottles and the overall methodology at large. The second sample type was “Bulk Water” collection. For this sample type, while wearing gloves, a clean 1L HDPE (with the cap securely on), was submerged into the water as close to the intended foam collection site as possible. Once submerged the cap was removed until the bottle was filled and then the cap was screwed back on while still submerged. This protocol was intended to prevent any incidental contamination of the bulk water sample with any microlayer liquid or foam from on top of the water. The outside of the bottle was dried with a paper towel. The final sample collected was the actual foam sample. For this a 1L HDPE bottle was taken to the site and, while wearing gloves, the lid was removed. Using a small black plastic serving spoon (confirmed to be PFAS free), foam was scooped into the bottle with emphasis on collecting only foam (and not surrounding water or debris). Foam was collected until there was no more foam in the vicinity or the 1L bottle was full and the cap was then placed securely back on the bottle. The outside of the bottle was dried with a paper towel and the coordinates of this particular collection site were

labeled on the bottle. Samples were stored at 4 °C until processing, which in some cases was several weeks later.

### **Sample Preparation**

#### *Field Blanks:*

For field blank 1L HDPE bottles, 10 µL of a 400 ng/mL internal standard mixture (4 ng of each IS compound) was added to each bottle followed by 20 mL of LC-MS grade water (Fisher Scientific). The bottle was shaken vigorously and poured into a 50 mL polypropylene (PP) conical tube. This sample was extracted using a Waters weak anion exchange (WAX) 6cc cartridge. Briefly, cartridges were initially cleaned using 4 mL of methanolic ammonium hydroxide (0.5% - 25 mL of 22% NH<sub>4</sub>OH in 1 L of methanol). After this the cartridge was further conditioned with 4 mL of methanol and then 4 mL of water. Then, the aqueous sample was loaded onto the cartridge by passing it through at a rate of ~1 drop/second. The cartridge was then cleaned with an additional 4 mL of 25 mM acetate buffer (25 mL of 1M acetate buffer in 1 L of water). Finally, the cartridge was dried and 15 mL conical tubes (Falcon brand, Fisher catalog 14-959-70C) were placed below cartridges for collection of eluate. Following this, 10 mL of LC-MS grade methanol was added to the original collection bottle and the bottle was shaken vigorously. This methanol portion was then poured into the 50 mL conical tube (used in a previous step). The tube was capped and vortexed. These steps help to avoid loss of hydrophobic PFAS to the walls of the containers that came into contact with the sample. This methanol was then poured into the extraction cartridge for this sample and the flow through was collected. Four mL of methanolic ammonium hydroxide (0.5%) was then run through the cartridge to elute the remaining PFAS. These two methanol eluates were both collected in the same 15 mL conical tube. This solution was fully dried and then reconstituted in 4 mL of 50:50 methanol:water by adding the methanol portion first.<sup>16</sup> By their nature, the blank

collection samples had no associated volume and so back-calculation of these concentrations was dubious. A volume of 20 mL was chosen as this was the volume of water initially added to the empty bottles. Back calculation of bulk water concentrations was performed by using a dilution factor that considered the final elution volume (4mL), and initial volume measured for each sample (20 mL). Each sample's on-instrument concentration was therefore divided by 5 to reach the original sample concentrations.

*Bulk water:*

Ten microliters of a 400 ng/mL internal standard mixture (4 ng of each of the 23 IS compounds) was added to each bottle. Exact volumes of bulk water samples were measured using individual 1 L PP graduated cylinders (volumes reported in Table S3). Some samples had debris, sand, or fine silt. When samples were transferred from collection bottle to graduated cylinder this material was avoided via decanting. Samples were then poured back into the original collection bottles. Samples were extracted in an identical manner to the field blanks, except siphon tubes were used to ease loading of the 1 L samples. Additional 50 mL PP conical tubes (Falcon brand, Fisher catalog 14-432-22) and a disposable polypropylene (PP) transfer pipet (Thermo Scientific, Fisher catalog 13-711-9D) were used to get the last of the sample while avoiding as much debris and silt as possible. All plastic labware was tracked for each sample. Upon reaching the [neutral] methanol elution, this volume of methanol was added to each piece of plasticware that came in contact with the samples in order to re-solubilize any hydrophobic PFAS that may have been lost. As described previously, this methanol was then eluted through the SPE cartridge and combined with the subsequent basic methanol eluent. Eluates were dried fully and reconstituted in 4 mL of 50:50 methanol:water by adding the methanol portion first. Back calculation of bulk water concentrations



was performed by using a dilution factor that considered the final elution volume (4mL) and initial volume measured for each sample (ca. 1 L). For example, the on-instrument measured concentration of bulk water sample 1 was divided by 238.75 (955 mL / 4 mL) to reach the original sample concentration for each PFAS measured.

#### *Sea Foam:*

For sea foam samples, all foam had condensed into a liquid in the 1 L HDPE bottles. A 5 mL pipet (Eppendorf, Fisher catalog 13-690-033) was used to measure a specific volume of the condensed sea foam. Any remaining condensed sea foam was discarded. Volumes of condensed sea foam are noted in Table S3. After measuring the specific volume, the samples were transferred to 50 mL conical tubes and 10  $\mu$ L of a 400 ng/mL internal standard mixture (4 ng of each IS compound) was added to each sample. Otherwise, sea foam samples were extracted the same as previous sample types. Pipets used to measure specific volume of condensed foam were washed with the neutral methanol eluent as were the falcon tubes used. Combined methanolic eluates were dried fully and reconstituted in 4 mL of 50:50 methanol:water by adding the methanol portion first. Back calculation of bulk water concentrations was performed by using a dilution factor that considered the final elution volume (4mL), and initial volume measured for each sample. For example, the on-instrument measured concentration of sea foam sample 1 was divided by 3.5 (14 mL / 4 mL) to reach the original sample concentration for each PFAS measured.

#### *Extraction Controls:*

A positive control was created by using 5 mL of nano pure water and spiking in 400  $\mu$ L of a 10,000 ng/L (4 ng) sample mixture. A negative control was created by using just 5 mL of nano

pure water. Ten microliters of a 400 ng/mL internal standard mixture (4 ng of each IS compound) was added to each control (positive and negative). These controls were created in the lab and were used to track the quality of each extraction batch.

## **Data Analysis**

### *Quantitative Analysis:*

All quantitative analyses were performed on an Thermo Fisher Scientific Orbitrap Exploris 240 (Bremen, Germany) in conjunction with a Thermo Scientific Vanquish Horizon LC system (Germering, Germany). A Phenomenex Kinetex 2.6  $\mu\text{m}$  particle, F5 100 Å, 100  $\times$  2.1 mm analytical column (part number 00D-4723-AN) was used for separation. Calibration curves for all analytes ranged from 0.5 ng/L to 5,000 ng/L. NIST SRM 8446 and SRM 8447 were combined and run throughout the batch. Complete method details and method validation information can be found in previous works<sup>17</sup> and in Supplemental Materials (LC-MS Methods section). Quantitative data processing was performed using TraceFinder version 5.1 (Thermo Fisher). For all quantitative sea foam results, multiple quality metrics were tracked to insure identification confidence. Exact mass precursor ions had to match within 4 ppm of the expected mass. The isotope pattern of the identified compound was matched to theoretically predicted isotopic patterns for each compound (at least two isotopes with a “fit threshold %” of at least 90). Product ion spectra were required to match at least one (exact mass) product ion observed in the MS2 spectra from analytical standards (specifically from the acquired calibrators run with that batch). Additionally, retention times and peak shapes were required to match calibrators for each result to be considered positive.

### *Non-targeted Analysis:*

All non-targeted analysis (NTA) measurements were performed on the same system as the quantitative work (i.e., Orbitrap Exploris 240). A slightly altered mass analysis experiment was used that incorporated data dependent analysis (DDA) acquisition as well as Acquire X, where the same sample is injected multiple times and each successive injection is used to add each selected exact mass to an exclusion list that is applied to subsequent scans, such that the same ion is not selected for MS2 analysis multiple times. In this way AcquireX, via multiple linked injections, should be capable of collecting product ion information on lower abundance compounds that would otherwise be missed by a single DDA injection/acquisition. Compound Discoverer (version 3.3) was used for data processing. More complete method details can be found in a previous work<sup>18</sup> and complete compound discoverer settings can be found in the supplemental materials (Compound Discoverer Settings excel file). A previously reported spectral library was incorporated into the Compound Discoverer workflow as an mzVault library to aid in identifications of compounds especially with regards to MS2 product ion spectra matching.<sup>19</sup>

## RESULTS AND DISCUSSION

### Quantitative Analysis:

All quantitative results were qualified using several metrics. Exact mass matching precursor extracted ion chromatograms had to have matching retention times and shapes to be considered positive. Once these three basic metrics (exact mass, retention time, peak shape) were qualified the resulting precursor and product ion data from these peaks were checked for additional information. Although these additional quality metrics were not required for result to be “confirmed” in this study, these additional metric help contribute to the certainty of the result. These additional metrics included checking that the precursor isotope pattern matched the

theoretical pattern based on the formula. Additionally, product ion matching was checked to confirm that at least one product ion matched what was expected based on calibrators run with that batch. Occasionally, if the precursor signal was of a very low abundance an isotope pattern was not able to be properly confirmed. Due to the timing of triggered product ion scans, there were occasions where product spectra were collected after the precursor peak had already eluted and therefore the product ion data did not include the expected product ions. This most often happened if the precursor abundance was very low but did also occur on several sea foam samples where concentrations were so high that saturation and suppression affected the quality of product ion data. Information on which of these quality metrics passed/failed for each sample can be found with the quantitative results in the Supplemental Materials (Quantitative Results excel file).

Concentrations of PFAS in the blank samples (when normalized to an original volume of 20mL) showed no PFAS greater than a concentration of 7 ng/L, and only PFOS showed confirmed positive results (i.e., precursor mass match, retention time match, chromatographic peak shape). These PFOS positives are likely the result of contamination from some step in the sample preparation process or from the LC-MS instrument. One blank (Sample #2) showed a response for the zwitterion N-TAmP-FHxSA that had matching retention time, peak shape and product ions and a quantitative value of 16.79 ng/L (Figure S1). This result had passing retention time, peak shape, exact mass and matching product ions. However, due to the low signal abundance an isotope pattern could not be matched to the theoretically predicted isotope pattern. Two other samples (samples 3 and 6) showed very low levels of this same zwitterion. It is unclear how these blank samples contained this PFAS as there were also not any high concentration injections run before this sample (i.e., there was no evidence of carryover in the chromatographic system). One blank sample also had extremely low levels of FBSA and FHxSA, while PFDoA, PFOA, and PFTeDA

all showed a positive hit in this single blank sample. All of these results were very near the limits of quantitation for the individual analytes and none of the results (with the exception of the PFOS hits) had any additional confirmatory evidence (e.g., isotope pattern match or product ion matches).

Bulk water samples showed many more confirmed positive results for 37 PFAS ranging from (relatively) low concentrations for 6:2 FTS, FOSA, HFPO-DA (GenX), Hydro-EVE, Nafion Byproduct 2, N-CMAMp, NEtFOSAA, NVHOS, PFBA, PFBS, PFDA, PFDoA, PFDS, PFHpA, PFHpS, PFHxA, PFHxS, PFMOAA, PFNA, PFNS, PFO3OA, PFO4DA, PFO5DoA, PFPeA, PFPeS, PFTeDA, PFTTrDA, PFUdA and PS Acid to higher concentrations for PFOA and PFOS (Figure 2). Some of these identifications are legacy PFAS that have likely been in the environment for a long period of time and may not necessarily be the result of any recent contamination events. However, several of these PFAS (including HFPO-DA, Hydro-EVE, Nafion Byproduct 2, NVHOS, PFMOAA, PFO3OA, PFO4DA, PFO5DoA, and PS Acid) occur predominantly in North Carolina as a result of a specific manufacturing facility.<sup>7</sup> Sample 10 had high levels of many different classes of PFAS. Interestingly, sample 7, collected from nearly the same location 5 months prior, had a very disparate profile of PFAS concentrations. These samples highlight the time dependent variation that can occur with testing moving bodies of water for environmental contamination.<sup>20</sup> In Fig. 2 (H), which is shown as a log scale, the 70 ppt concentration specified in the Chemours consent order is highlighted. Although this concentration is meant to apply to only a select number of PFAS, it is shown here for comparison.

The sea foam samples were evaluated next, and their concentrations were normalized to their condensed liquid form. When considering these condensed liquid concentrations, many PFAS had extremely high concentrations (Figures 3 and 4). This includes three PFAS from the fluorotelomer

sulfonic acid, perfluoroalkylcarboxylic acid, perfluoroalkylsulfonic acid, perfluoroalkyl sulfonamide, zwitterionic, perfluoroethercarboxylic acid and perfluoroethersulfonic acid classes. Four PFAS actually showed lower concentrations in condensed foam when compared to their bulk water counterparts and included NVHOS, PFBA, PFMOAA, and PFO3OA. All of these compounds are quite hydrophilic with masses less than 311 Da. Concentration factors were calculated by dividing the average concentrations of PFAS in condensed foam and dividing by their average concentration in the associated bulk water sample. A correlation was observed in this data that suggested that PFAS with masses close to 500 Da tended to concentrate to a greater degree (Fig. S2).

In Fig. 4 (H), which is shown as a log scale, the 70 ppt concentration specified in the Chemours consent order is highlighted. All foam samples had total PFAS values above this limit. In one case (foam 10), the levels were 1.2 million times this level. Foam sample 10 had PFOS in excess of 8 million ng/L. In addition, various regional North Carolina PFAS such as PFO5DoA was in excess of 10,000 ng/L in 4 of the 13 samples and Nafion Byproduct 2 was in excess of 1,000 ng/L in 7 of the 13 samples tested. These levels are the highest ever reported for water based environmental foams exceeding previous values recorded in Michigan<sup>4</sup> and Wisconsin<sup>3</sup>.

### **Non-targeted Analysis:**

NTA results were filtered to keep only the features that matched the following criteria: annotated mass  $\pm$  5 ppm, retention time between 2 min and 18 min (for a 20 min method), mass defect between -0.116 and 0.268, and >2 fluorine atoms. There were also several criteria related to database matching (see Supplemental Materials, Compound Discoverer Settings excel file). Upon filtering based on these criteria, 516 features remained. These features were scored according to

the Shymanski scale<sup>21, 22</sup> and database matches were manually curated and confirmed. Level 1 hits (highest confidence) were ignored as these PFAS compounds were already identified and quantitated in the quantitative analysis portion of this study. All level 1-3 results can be found in Supplemental Materials (Full NTA Results excel file). Several of these prioritized hits are discussed in the following sections.

#### *Unsaturated perfluorooctane sulfonic acid (UPFOS) - level 2a*

A precursor ion with a mass of 460.9337 Da was identified at a retention time of 10.3 min in 10 out of 13 samples (Fig. 5). This mass corresponds to the formula C<sub>8</sub>HF<sub>15</sub>O<sub>3</sub>S with a mass error of 0.71 ppm. The product ion spectrum from Sample 12 matches an unsaturated form of perfluorooctane sulfonic acid (UPFOS), as listed in the Duke Spectral Library. While concentration estimates are challenging without calibration standards, the relative abundance of UPFOS was approximately an order of magnitude higher than other high-confidence identifications (excluding level 1 entries). Notably, the highest UPFOS levels were found in Samples 12, 6, and 11, collected near the Cape Fear Regional Jetport.

UPFOS (CAS 2089109-43-1, DTXSID501032594) is listed as a level 1 entry in the CompTox Chemicals Dashboard and has been previously documented.<sup>23-27</sup> Barzen-Hanson et al. reported UPFOS in various AFFF formulations and commercial products. In 2021, McDonough et al. detected UPFOS in the serum of adult residents of El Paso County, Colorado. Key product ions—380.9765, 280.9797, 180.9899, 98.9557, and 79.9573—observed in their study were also present in the samples analyzed here. Additionally, McDonough et al. found UPFOS to be bioaccumulative in mice in a 2020 study.<sup>28</sup> In a review of the literature, no studies evaluating the toxicity of this compound were found.

*Hexafluoropropylene oxide trimer acid (HFPO-TeA dimer) - level 2a*

A precursor ion at 660.9389 Da was also detected, along with its isotopic envelope, and matching the chemical formula C<sub>12</sub>HF<sub>23</sub>O<sub>5</sub> (Fig 6). The retention time of 12.6 min aligns with expectations based on the molecular weight of this compound. The resulting product ion spectrum contained several exact mass ions consistent with the Duke Spectral Library entry for hexafluoropropylene oxide trimer acid (HFPO-TeA) and with previous findings reported by McDonough<sup>26</sup> and Barzen-Hanson et al.<sup>23</sup> This compound was detected in 6 of the 13 foam samples analyzed in this study.

HFPO-TeA, a perfluoroalkyl ether carboxylic acid (CAS 65294-16-8, DTXSID70276659), has been widely documented. It is commonly associated with fluorochemical manufacturing<sup>29, 30</sup> and has been detected in various environmental matrices near production sites in China.<sup>31, 32</sup> Li et al. demonstrated HFPO-TeA bioaccumulation through trophic transfer within estuarine food webs in China.<sup>29</sup> Several research groups in Florida have reported HFPO-TeA in matrices linked to landfills.<sup>33-35</sup> HFPO-TeA is a homolog of HFPO-DA (GenX), which has been widely observed in environmental and biological samples in North Carolina.<sup>10, 36</sup> HFPO-DA's presence led to a consent order between Chemours, the North Carolina Department of Environmental Quality (NCDEQ), and environmental groups.<sup>13</sup> This compound has been linked to endocrine disruption,<sup>37</sup> oxidative stress and inflammation,<sup>38</sup> and reproductive toxicity<sup>39</sup> in *in vitro* and/or *in vivo* models.

*3-oxaperfluorononane sulfonic acid (F53) - level 2a*

A precursor ion with a mass of 514.9257 was also identified, corresponding to the chemical formula C<sub>8</sub>HF<sub>17</sub>O<sub>4</sub>S, with a mass error of 1.05 ppm and a retention time of 11.20 min (Fig. 7). The isotopic pattern aligned closely with predictions for this formula. The product ion spectrum



matched an entry in the Duke Spectral Library for “potassium 3-oxaperfluorononane sulfonate,” commonly referred to as “F-53 (6:2 F-PFAES).” Structurally, this compound is similar to F53B minor, differing only in the absence of a chlorine substitution on the end of the alkyl chain. This perfluoro ether sulfonic acid compound was detected in 3 out of 13 samples, where it appeared in relatively high abundance.

F-53 (CAS 754925-54-7, DTXSID60904575) is a “level 1, expertly curated” entry in the CompTox Chemical Dashboard. Dudášová et al. identified F-53 as a suspect candidate in 2024 from a bream liver sample collected from the Rhine River in Germany.<sup>40</sup> Their analysis utilized direct infusion on an ion cyclotron resonance-mass spectrometer (ICR-MS). Reports of F-53 in environmental samples remain limited. However, F-53 is referenced in several patents for PFAS compounds used in photoresists, highlighting its industrial relevance.<sup>41-45</sup> In our review of the literature, we found no studies evaluating the toxicity of this compound.

#### *Perfluorohexane sulfinic acid (PFHxSI) - level 2a*

A precursor ion at 382.9419 Da, along with its isotope pattern, matched to the chemical formula  $C_6HF_{13}O_2S$ , with a mass error of 0.51 ppm (Fig. 8). The retention time of 9.2 min aligned well with estimates based on standards. The product ion spectrum collected from foam sample 10 corresponded to “perfluorohexanesulfinate zinc salt” in the Duke spectral library, with the three most abundant ions—318.9798, 168.9894, and 118.9926—also observed in the sample's spectrum. Notably, retention times across other samples where this compound was identified were all within three seconds of each other.

Perfluorohexanesulfinate (PFHxSI; CASN 115416-67-6, DTXSID601032911) is the sulfinic acid analog of perfluorohexane sulfonic acid (PFHxS), differing by its R-S(=O)OH

headgroup as opposed to the  $\text{R-S(=O)}_2\text{OH}$  structure of PFHxS. This compound is a Level 1 entry on the CompTox Chemical Dashboard due to its expert curation. Bao et al. (2023) identified PFHxSI in soils from a former fluorochemical plant in China, publishing product ion spectra showing matching ions—318.98, 168.99, and 82.96—as observed here (see Fig. 8).<sup>46</sup> Additionally, Wang et al. reported PFHxSI in AFFF-contaminated groundwater in Pennsylvania.<sup>47</sup> Further, Yeung et al. (2009) developed a method for extracting PFAS and related sulfinic acids, including PFHxSI, using a Waters Oasis WAX SPE setup.<sup>48</sup> In our review of the literature, we found no studies evaluating the toxicity of this compound.

#### *1H-Perfluoro-2,5-dioxahexane - level 2b*

A precursor ion at 250.9761 Da and its associated isotopic envelope matched to the chemical formula of  $\text{C}_4\text{HF}_9\text{O}_2$  (Fig. 9). The retention time of 11.52 min was peculiar; an earlier RT would be expected for this low of a mass, indicating this could be an in-source fragment of some sort. However, peak shapes and retention times were consistent for the three unknowns and quality control in which this compound was identified. The product ion spectrum collected from foam sample 10 matched to “1-(Difluoromethoxy)-1,1,2,2-tetrafluoro-2-(trifluoromethoxy)ethane” also known as “1H-Perfluoro-2,5-dioxahexane” in the Duke spectral library. The two most abundant product ions in the spectral library entry – 184.9843 and 84.9907 – were also observed here.

1H-Perfluoro-2,5-dioxahexane (CASN 40891-98-3, DTXSID801022354) is a halogenated dioxolane that lacks the more common acidic headgroups of other PFAS detailed here. Pavlovic et al identified this compound (under the name “1-(Difluoromethoxy)-1,1,2,2-tetrafluoro-2-(trifluoromethoxy)ethane”) in roe deer in Italy.<sup>49</sup> Their findings indicated that 1H-Perfluoro-2,5-dioxahexane was significantly more abundant in the liver of these deer compared to their muscle

tissue. 1H-Perfluoro-2,5-dioxahexane is described in several patents as having properties (e.g., vapor pressure, etc.), that give it anesthetic activity.<sup>50, 51</sup> In our review of the literature, we found no studies evaluating the toxicity of this compound.

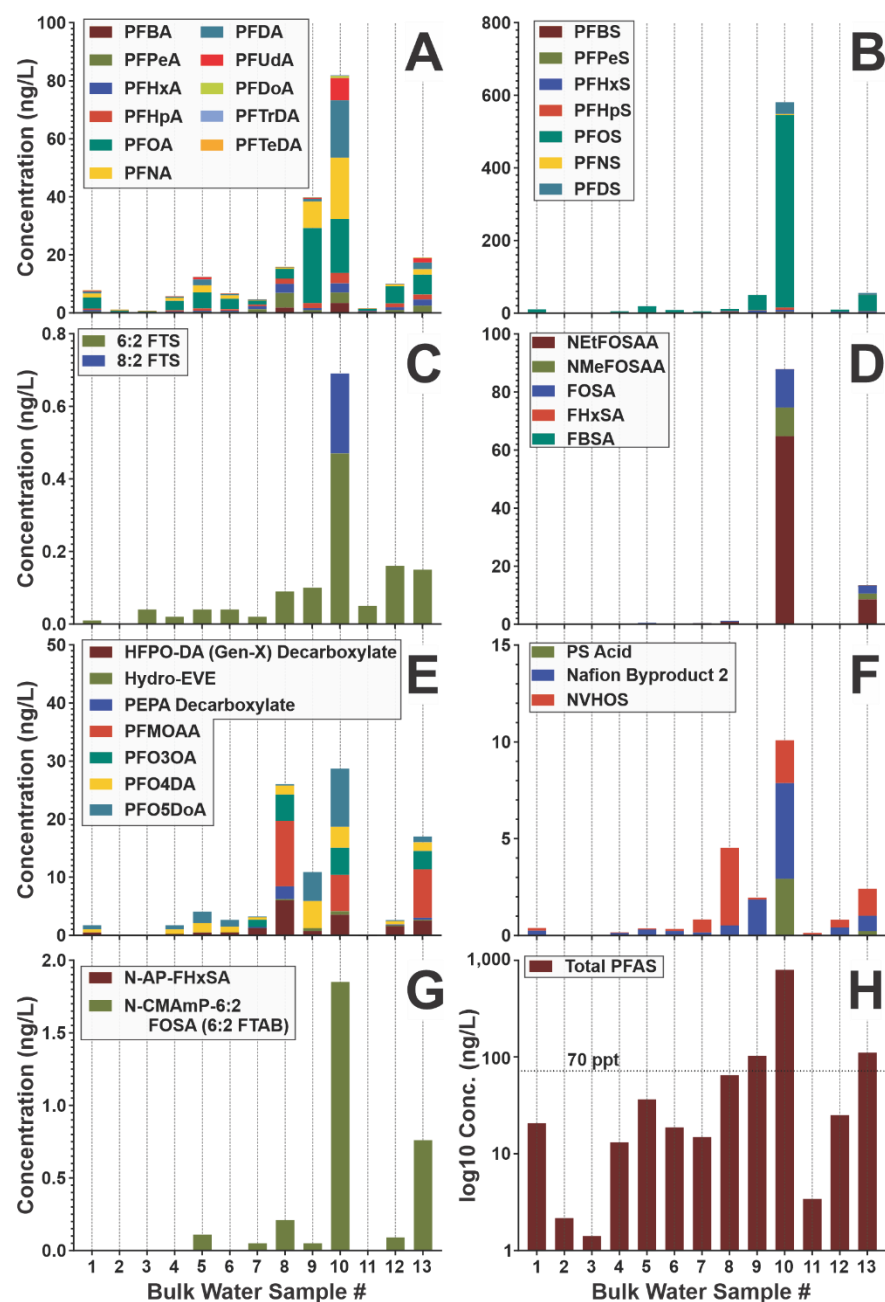
This study provides a comprehensive analysis of PFAS contamination in sea foam, bulk water, and blank samples using both quantitative and non-targeted approaches. 35 PFAS were detected in both the sea foam and sea water. Three hydrophilic PFAS (PEPA, PFBA and PFMOAA) were detected only in water and five more hydrophobic PFAS (10:2 FTS, F53B Major, MeFOSA, N-AP-FHxSA, and N-TAmP-FHxSA) were detected only in sea foam. Interestingly, 34 of the 35 PFAS detected in both matrices had higher concentrations in the sea foam than the sea water. Furthermore, several emerging PFAS were identified in sea foam, including UPFOS, HFPO-TeA, F53, PFHxSI, and 1H-Perfluoro-2,5-dioxahexane. The findings indicate that sea foam serves as a concentrating reservoir for PFAS, with certain compounds showing significantly higher levels compared to bulk water. The identification of novel and regionally relevant PFAS underscores the need for continued monitoring and investigation into their environmental fate and potential human exposure risks.

Sample #	Date/Time	Location (Coordinates, decimal form)
1	7/3/22, 2:15 PM	33.843220, -77.962183
2	8/10/22, 5:36 PM	33.962530, -77.921559
3	8/10/22, 8:11 PM	34.045504, -77.885807
4	10/1/22, 10:10 AM	33.912809, -78.267105
5	10/1/22, 11:11 AM	33.913679, -78.158537
6	10/1/22, 12:15 PM	33.896111, -78.054668
7	10/22/22, 4:19 PM	34.051034, -77.918011
8	11/12/22, 5:00 PM	34.238243, -77.951212
9	11/13/22, 3:45 PM	33.908524, -78.109810
10	3/19/23, 2:30 PM	34.048385, -77.921128
11	1/28/23, 12:14 PM	33.909972, -78.119330
12	2/15/23, 5:15 PM	33.908549, -78.109552
13	3/4/23, 6:30 PM	34.153852, -77.944081

**Table 1:** Sampling information for the samples collected.

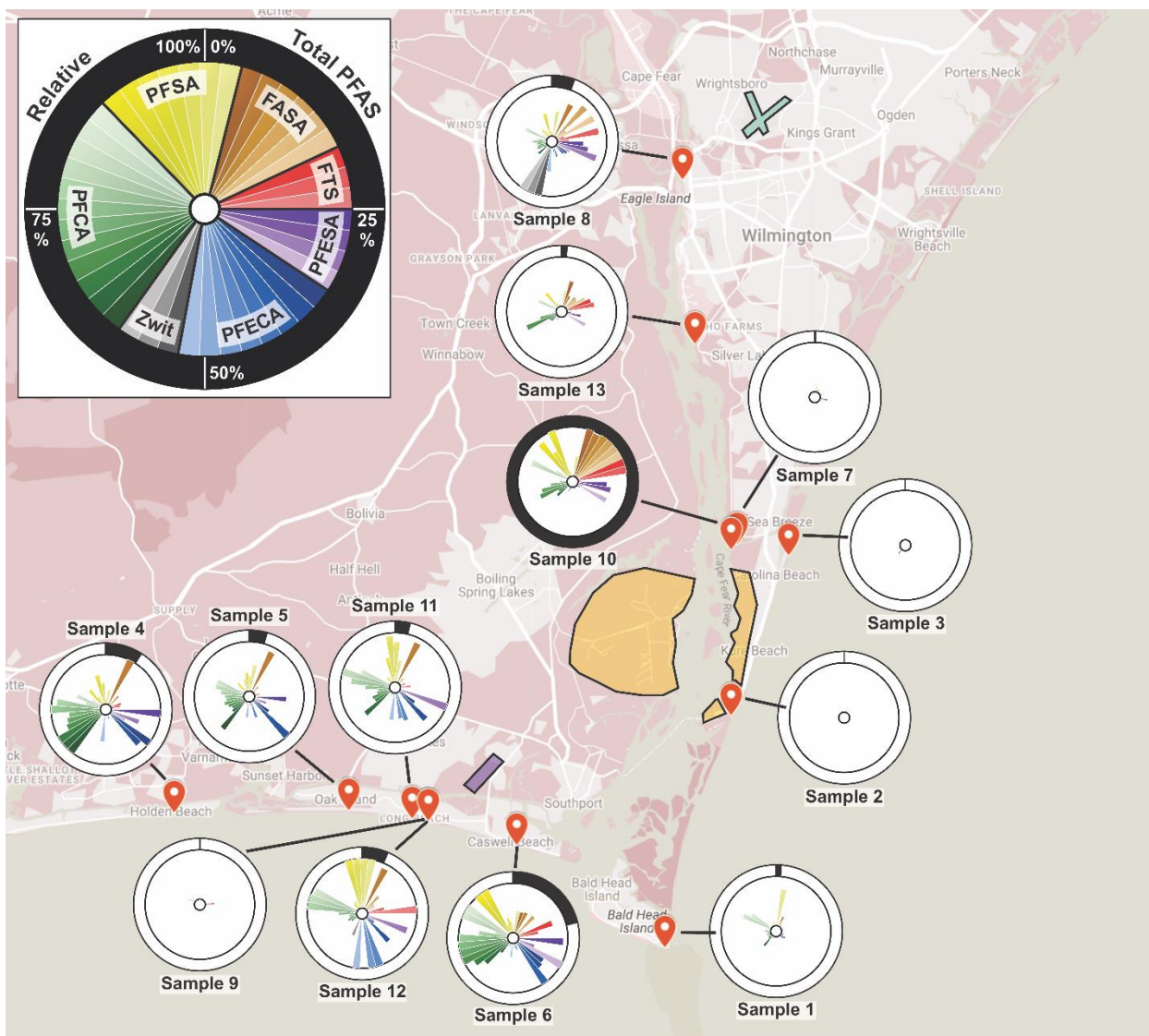


**Figure 1:** Map of sample collection locations along the coast of North Carolina and within the Cape Fear River estuary. The Cape Fear Regional Jetport (purple shape), the Wilmington International Airport (teal shape), and the Military Ocean Terminal Sunny Point (MOTSU, orange shape) are shown for proximity considerations with relation to sampling locations. Inset: Photograph of sea foam collected at site labeled “Sample 10”.

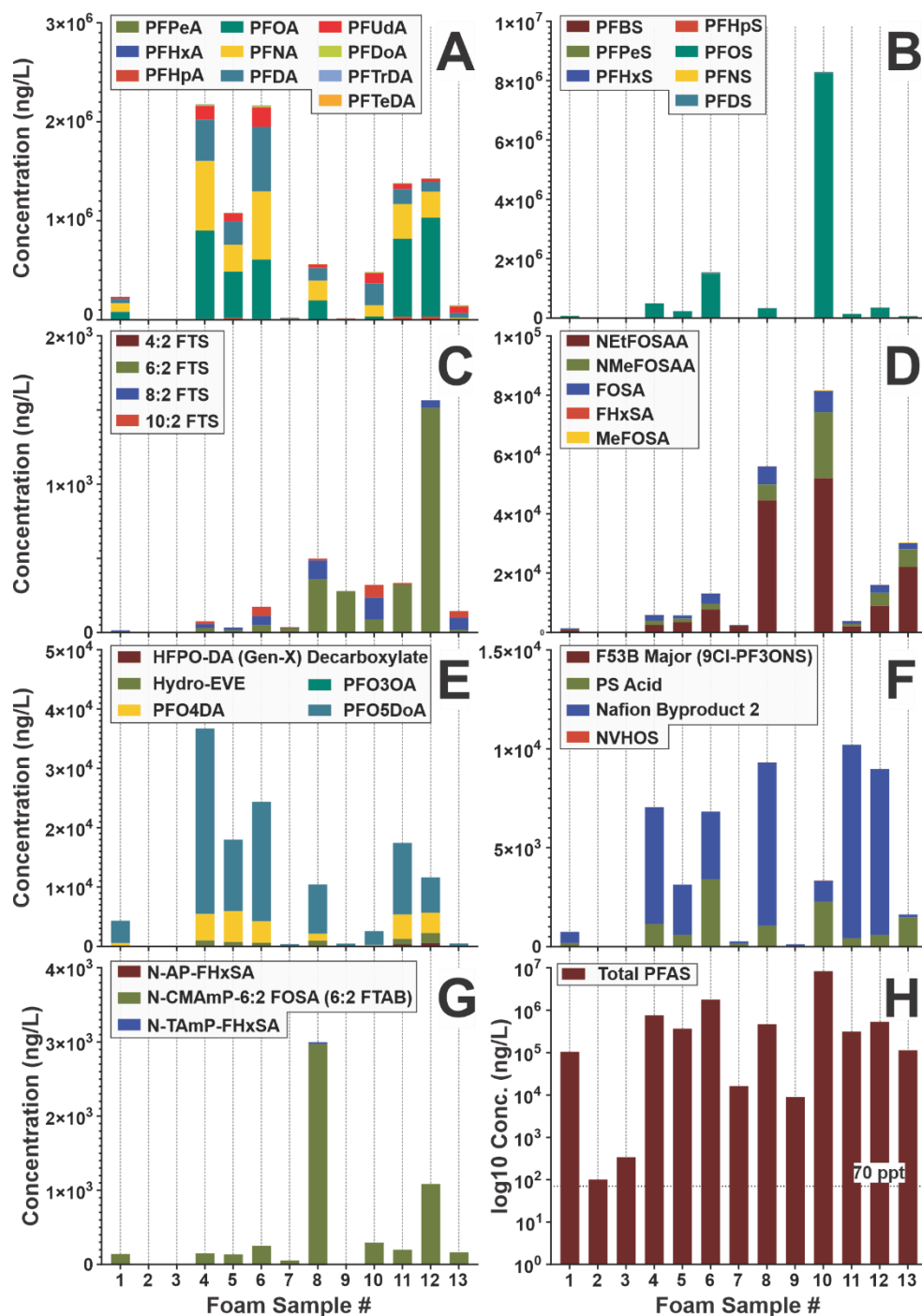


**Figure 2:** Quantitative results for the bulk water samples collected with every sea foam sample. Results are separated by class: (A) perfluoroalkyl carboxylic acids, (B) perfluoroalkyl sulfonic acids. (C) fluorotelomer sulfonates, (D) Sulfonamides, (E) perfluoroalkyl ether carboxylic acids, (F) perfluoroalkyl ether sulfonic acids, (G) Zwitterions, (H) log<sub>10</sub> total sum of all PFAS quantitated.



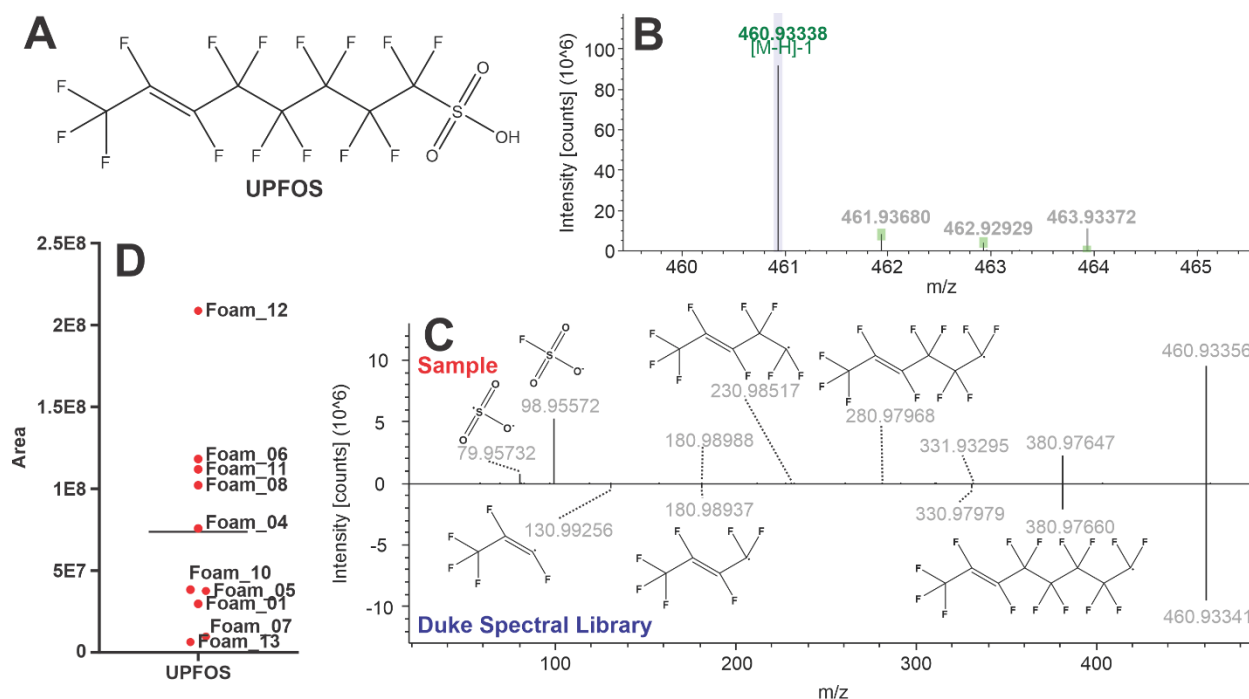


**Figure 3:** Quantitative results for seafoam samples represented by ToxPi graphs. Results are color-coded by class. Maximum concentrations are relative to the highest concentration observed for each analyte. A black circle behind the ToxPi graph indicates the sum total of PFAS observed (relative to the highest total PFAS observed in sample 10 and going clockwise starting at 12 o'clock on the circle). Notable locations nearby include the Cape Fear Regional Jetport (purple shape), the Wilmington International Airport (teal shape), and the Military Ocean Terminal Sunny Point (MOTSU) (orange shape).

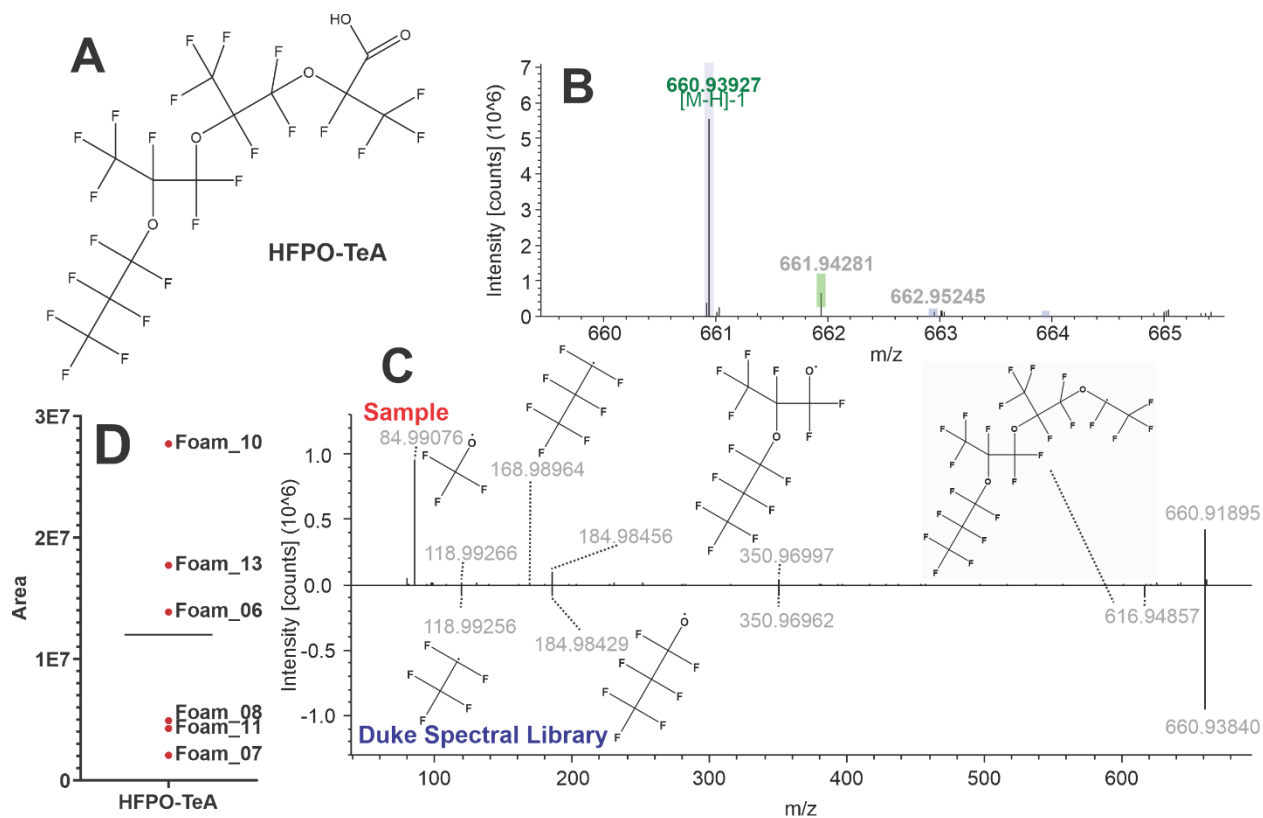


**Figure 4:** Quantitative results for the sea foam samples collected. Results are separated by class: (A) perfluoroalkyl carboxylic acids, (B) perfluoroalkyl sulfonic acids. (C) fluorotelomer sulfonates, (D) Sulfonamides, (E) perfluoroalkyl ether carboxylic acids, (F) perfluoroalkyl ether sulfonic acids, (G) Zwitterions, (H) log<sub>10</sub> total sum of all PFAS quantitated.

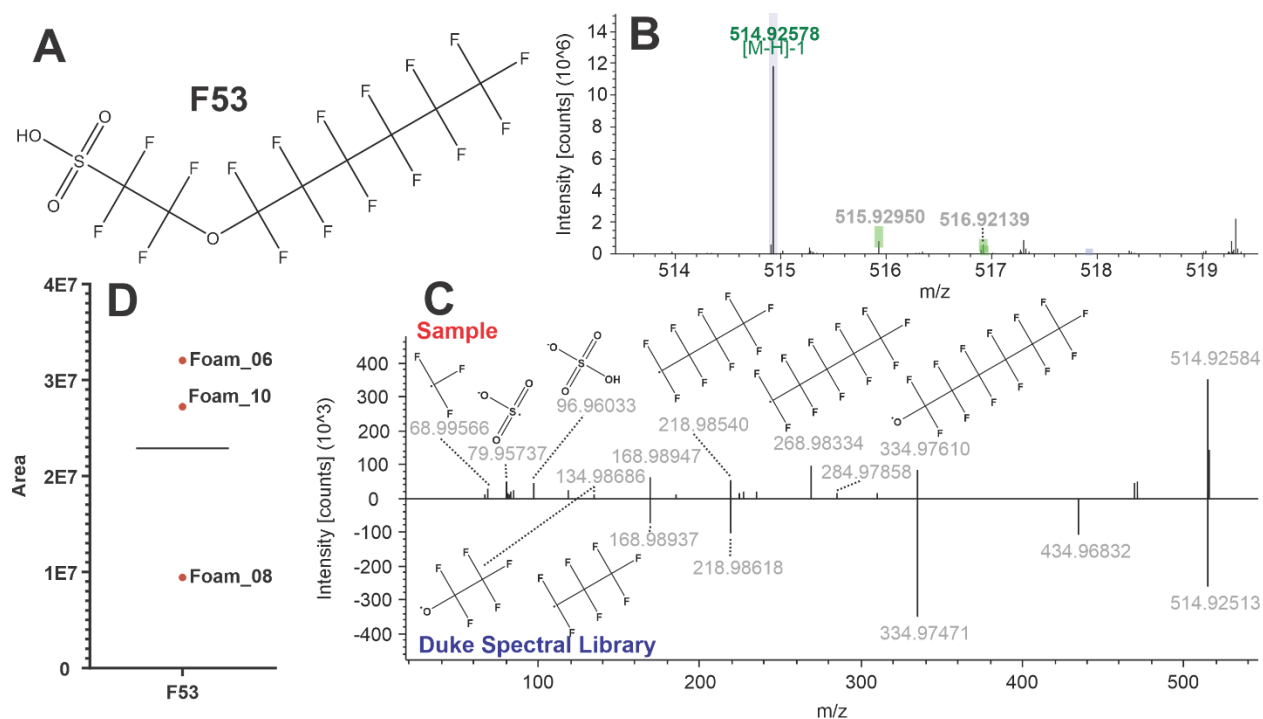




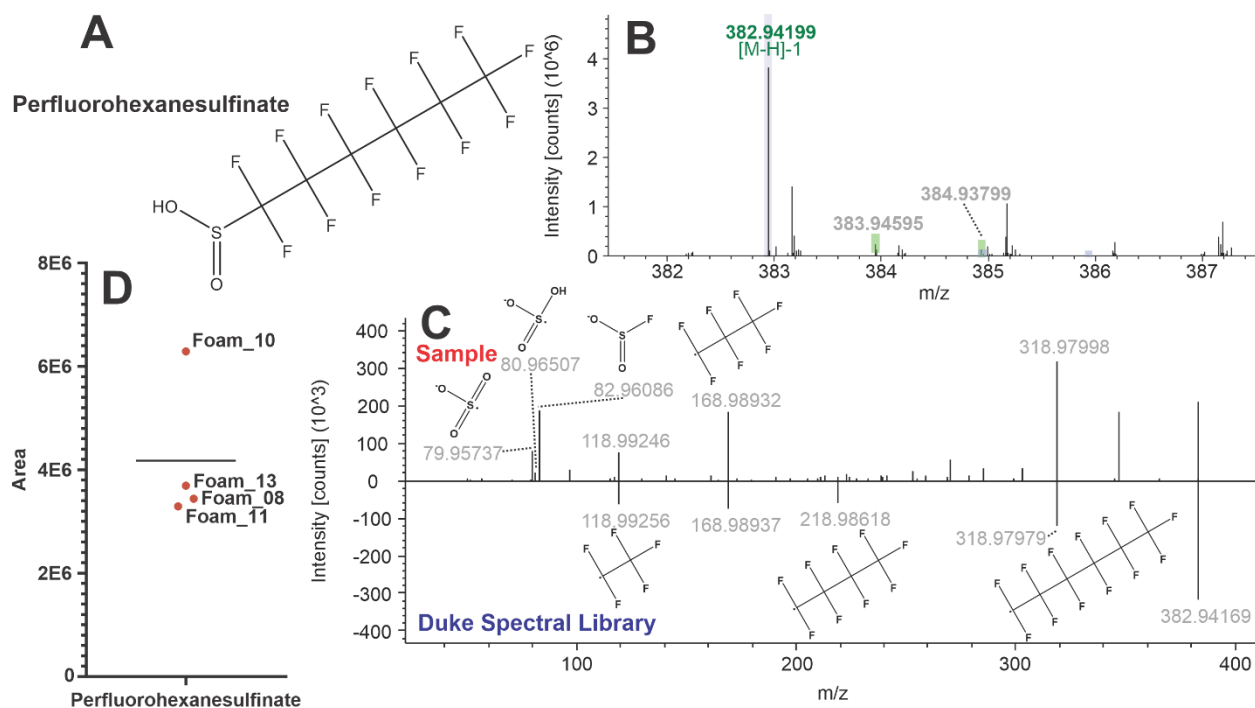
**Figure 5:** NTA results for UPFOS (A) structure, (B) isotopic pattern, (C) product ion match to the database used and (D) relative peak areas for this feature across all samples where this feature was detected.



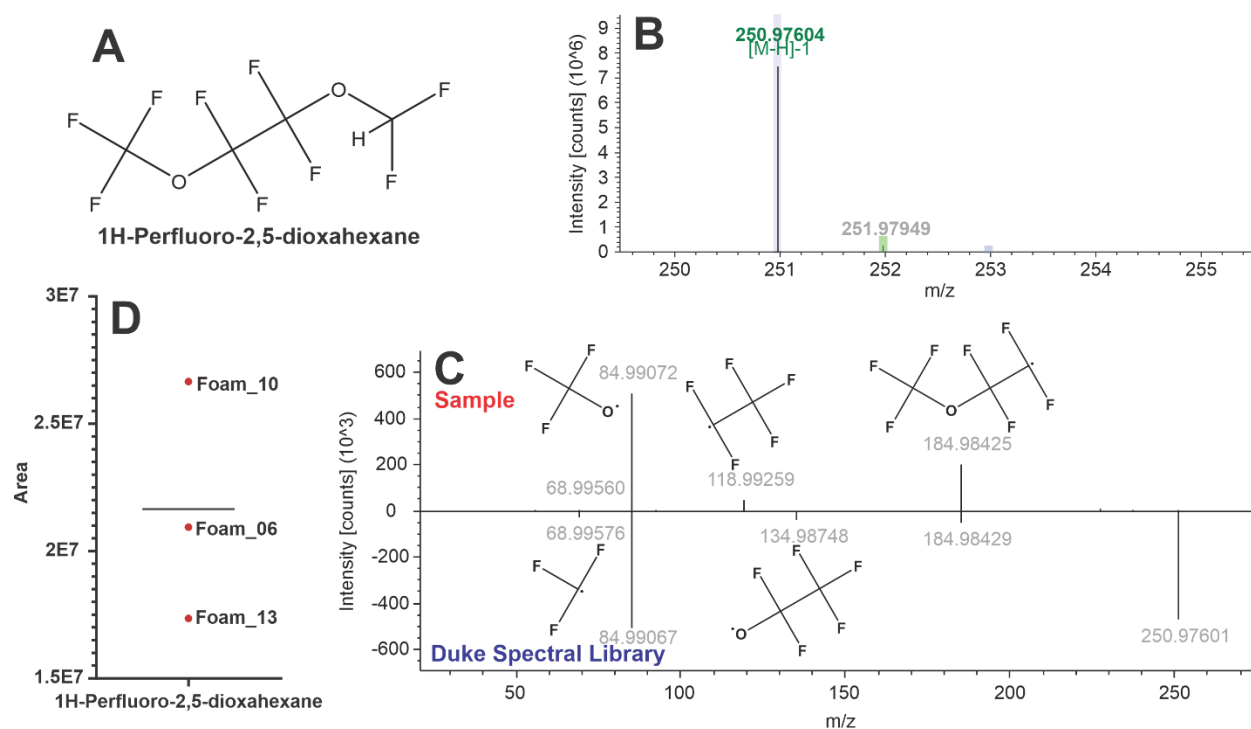
**Figure 6:** NTA results for HFPO-TeA dimer (A) structure, (B) isotopic pattern, (C) product ion match to the database used and (D) relative peak areas for this feature across all samples where this feature was detected.



**Figure 7:** NTA results for F53 (A) structure, (B) isotopic pattern, (C) product ion match to the database used and (D) relative peak areas for this feature across all samples where this feature was detected.



**Figure 8:** NTA results for PFHxSI (A) structure, (B) isotopic pattern, (C) product ion match to the database used and (D) relative peak areas for this feature across all samples where this feature was detected.



**Figure 9:** NTA results for 1H-Perfluoro-2,5-dioxahexane (A) structure, (B) isotopic pattern, (C) product ion match to the database used and (D) relative peak areas for this feature across all samples where this feature was detected.

## ASSOCIATED CONTENT

The following files are available free of charge.

Supplemental Information (.docx)

Quantitative Results (.xlsx)

Compound Discoverer Settings (.xlsx)

Full NTA Results (.xlsx)

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