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FINAL REPORT

Assessing and Mitigating Bias in PFAS Levels During Ground and Surface Water Sampling

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The combination of multiple factors has led to regulatory concern and restrictive guidance to prevent false positive per- and polyfluoroalkyl substances (PFAS) sampling results. In response, this project was completed to assess several potential sources of bias. This project assessed whether field sampling materials, equipment, and processes may pose a significant risk of sample contamination (or if current guidelines are unnecessarily restrictive, increasing time and cost in the field). The project also evaluated whether PFAS stratification in groundwater and surface water columns is significant enough to bias results, including the amount of the surface microlayer that is captured in the sample using different sampling methods. The project also reviewed existing studies on PFAS precursor transformation as a function of sample storage temperature and duration.

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LIST OF ACRONYMS AND ABBREVIATIONS

°C	degrees Celsius
AFFF	aqueous film forming foam
CA	California
CV	coefficient of variation
DOC	dissolved organic carbon
DoD	Department of Defense
Ecology	Washington Department of Ecology
EDQW	Environmental Data Quality Workgroup
EF	enrichment factor
EGLE	Michigan Department of Environment, Great Lakes, and Energy
EPA	Environmental Protection Agency
EtFOSAA	Ethylperfluorooctane sulfonic amido acetic acid
FAQs	frequently asked questions
FHxSA	perfluoro-1-hexanesulfonamide
FOSA	perfluoro-1-octanesulfonamide
FTS	fluorotelomer sulfonates
g/L	grams per liter
HDPE	high density polyethylene
ITRC	Interstate Technology and Regulatory Council
LC-MS/MS	liquid chromatography tandem mass spectrometry
LC-QToF	liquid chromatography quadrupole time of flight mass spectrometry
LDPE	low density polyethylene
Maine DEP	Maine Department of Environmental Protection
MeFOSAA	methylperfluorooctane sulfonamido acetic acid
mg/L	milligrams per liter
MIP	membrane interface probe
mL	milliliters
MPCA	Minnesota Pollution Control Agency
NaCl	sodium chloride
NAVFAC	Naval Facilities Engineering Command
ng/cm^2	nanograms per square centimeter
ng/L	nanograms per liter
NGWA	National Ground Water Association
NHDES	New Hampshire Department of Environmental Services
NMR	Nuclear Magnetic Resonance
NYSDEC	New York State Department of Environmental Conservation
OSU	Oregon State University
PFAA	perfluoroalkyl acids
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonate
PFCA	perfluoroalkyl carboxylate
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFDS	perfluorodecane sulfonate
PFEtCHxS	perfloroethylcyclohexane sulfonate
PFHpA	perfluoroheptanoic acid
PFHpS	perfluoroheptane sulfonate
PFHxA	perfluorohexanoic acid

PFHxS	perfluorohexane sulfonate
PFNA	perfluorononaoic acid
PFNS	perfluorononane sulfonate
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
PFPeA	perfluoropentanoic acid
PFPeS	perfluoropentane sulfonate
PFPrS	perfluoropropane sulfonate
PFSA	perfluoroalkyl sulfonates
PFUnDA	perfluoroundecanoic acid
PIGE	particle-induced gamma-ray emission
PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
RPD	relative percent difference
SERDP	Strategic Environmental Research and Development Program
SML	surface microlayer
SOP	standard operating procedures
SPE	solid phase extraction
SPr-FHxSA	N-sulfo propyl perfluorohexane sulfonamide
SWRCB	State Water Resources Control Board
TOC	total organic carbon
U.S.	United States

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- "Surface microlayer sampling for per- and polyfluoroalkyl substances (PFAS) on an AFFF-impacted freshwater lake" by T. Schwichtenberg, D. Bogdan, C.E. Schaefer, and J.A. Field, **2023**. *Environ. Sci. Technol. Water 3(4)*: 1150-1160, DOI:10.1021.acsestwater.2c00618. Copyright 2023, American Chemical Society.

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- "Field sampling materials unlikely source of contamination for perfluoroalkyl and polyfluoroalkyl substances in field samples" by A.E. Rodowa, E. Christie, J. Sedlak, G.F. Peaslee, D. Bogdan, B. DiGuiseppi, and J.A. Field, **2020**. *Environ. Sci. Technol. Lett. 7* (3), 156-163. DOI: 10.1021/acs.estlett.0c00036. Open access (Paid by AECOM and Jacobs). Copyright 2020, American Chemical Society.

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- Roark, S., Fallon, A., Struse, A., Rectenwald, H., Bogdan, D., Heron, C., Field, J. **Accepted**. Comparison of surface freshwater PFAS sampling methods to evaluate potential for bias due to PFAS enrichment in the surface microlayer. *Int. Environ Assess Manage.*

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Views, opinions, and findings contained in this report are those of the author(s) and should not be construed as an official DoD position or decision unless so designated by other official documentation.

ABSTRACT

Introduction and Objectives

Per- and polyfluoroalkyl substances (PFAS) are a large group of anthropogenic compounds that can be detected at sub-nanogram per liter concentrations; widespread detections of various PFAS have been reported in environmental media. Regulatory standards and guidance for individual PFAS are low; some are based on analytical reporting limits. In addition, PFAS have been documented to be present in a variety of commonly used field equipment, materials, and products. The combination of these factors has led to regulatory concern over false positive sampling results and other forms of bias that may affect PFAS sampling results.

In response to a SERDP Statement of Need to develop standardized analytical and sampling methods for PFAS, the project team identified multiple objectives to address via laboratory and field research tasks and technology transfer activities. Project objectives were as follows: (1) determine the factors that impact PFAS stratification in water columns, including both surface water and groundwater wells, (2) systematically evaluate field materials and procedures to eliminate bias when collecting water samples for PFAS analyses, 3) quantify the impact of laboratory sample hold times and storage conditions to eliminate bias in measured PFAS concentrations, and 4) conduct outreach and research translation with state and federal project managers, consultants, and contract laboratories.

Technical Approach

Over a three-year period, each task was addressed via literature review, outreach, controlled laboratory experiments, and field experiments. Task 1 studies were completed at bench-scale to assess the extent to which stratification occurs in monitoring wells and surface water and the impact of stratification on PFAS sampling results. The impact of PFAS concentration and salinity on stratification was evaluated as well as the potential impact of field sampling procedures. Sampling techniques to mitigate sampling bias were evaluated at inland (neutral pH and low salinity) and coastal (higher pH and salinity) field sites. Task 2 studies were completed in the laboratory following a literature review to evaluate the potential effect of surface water and groundwater sampling equipment and other commonly used field sampling materials and products. For Task 3, a literature review was completed to highlight findings from a recent study that quantified the effect of sample hold times and storage conditions on measured PFAS concentrations. Finally, as Task 4, key findings and recommendations were summarized and communicated through an ESTCP technical report (Deeb et al. 2021), multiple peer-reviewed publications (Roark et al., 2023; Schwichtenberg et al., 2023: Schaefer et al., 2022; Rodowa et al., 2020a; and Schwichtenberg et al., 2020), and multiple presentations, webinars, and workshops.

Results

Key project findings regarding potential bias in PFAS concentrations were as follows:

- Task 1. PFAS stratification in the water column is a function of water chemistry and the chemical properties of PFAS. Field and laboratory data indicate that for surface waters, PFAS are enriched in foam to a greater degree than in the surface microlayer (SML). However, data from the surface water sampling (field) study indicate that PFAS enrichment

in the surface microlayer of surface waters does not have substantial effects on measured PFAS bulk water concentrations. With the exception of surface water sampling methods that include foam (in which PFAS are significantly enriched), the effect of the surface water sampling method (specifically inclusion or exclusion of SML) is within the generally accepted range of variability (approximately 30%) for sampling and analyzing duplicate surface water samples.

- Task 2. A literature review and laboratory studies were conducted to evaluate field materials with the potential to cross-contaminate or otherwise bias PFAS sampling results. Field material extraction and analysis indicate that while PFAS may be present on some materials, few pathways exist for PFAS associated with materials to bias PFAS concentrations in water samples. Results indicate that existing guidance documents err on the side of caution and may add unnecessary cost and time to field sampling efforts by restricting materials and practices that do not pose a significant risk of biasing PFAS concentrations.
- Task 3. Laboratory-specified hold times and sample storage temperatures reported in the literature are scientifically founded and adequately prevent bias due to PFAS sorption to sampling containers or partial degradation of some PFAS to form others. Longer hold times have since been incorporated into standard analytical methods.
- Task 4. Key findings and recommendations to improve current PFAS sampling guidance documents were summarized in multiple peer-reviewed publications, technical reports, and webinars. A summary of key recommendations is provided in **Figure ES-1**.

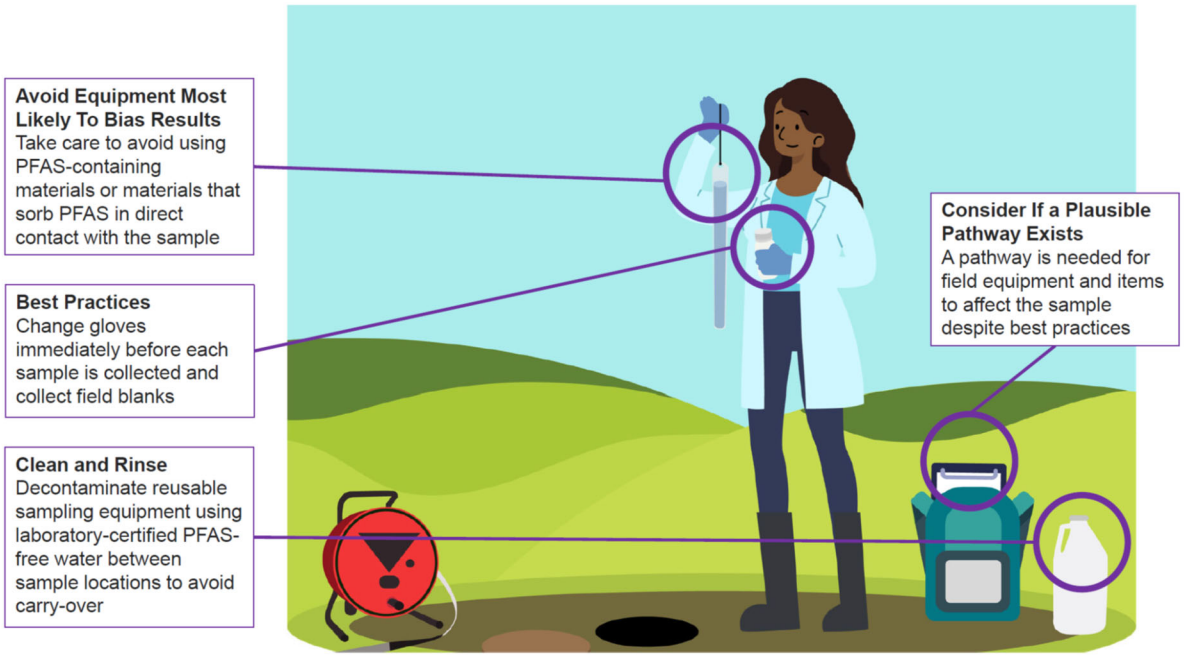

Figure ES-1. Recommended best practices to reduce bias in PFAS sample results (From Deeb et al., 2021)

Benefits

Project activities provide practitioners with a scientific basis for assessing potential bias in PFAS sampling results due to multiple factors, including stratification under realistic groundwater and surface water conditions, presence or contact with commonly used field materials, products, and equipment, and the impact of sample storage hold times and temperatures. Best practices were assembled into technical guidance and communicated directly to stakeholders.

EXECUTIVE SUMMARY

Introduction

Compared to many environmental constituents, sampling and analysis of per- and polyfluoroalkyl substances (PFAS) is complex. Many PFAS, including perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA) and other perfluoroalkyl acids (PFAAs), are amphiphilic—they contain a hydrophobic fluorinated carbon chain attached to a water-soluble, polar head group. PFAS have a propensity to interact with surface and soil components such as organic carbon and minerals. They accumulate at interfaces, including the air-water interface, water-oil interfaces, and liquid-solid interfaces. The propensity of PFAS to accumulate at interfaces, including the air/water interface, has potential implications for PFAS sample collection and measured PFAS concentrations. Understanding the magnitude of PFAS accumulation at the air-water interface is important for gauging the impact on measured PFAS concentrations in water samples. Many PFAS also have surfactant properties that improve their performance in a variety of industrial applications. For example, PFAS were added to aqueous film-forming foams (AFFFs) to facilitate the spread of an aqueous film over the surface of a liquid fuel, and to form a surfactant foam to blanket the liquid fuel, preventing the burning fuel from contacting air and simultaneously cooling the burning fuel and adjacent surfaces. Health-based water quality thresholds for several PFAS are quite low (i.e., sub-part per trillion or nanograms per liter), making these interactions worth considering when assessing whether sampling and analytical conditions may introduce artifacts or bias sample results.

The presence of PFAS has been detected in a variety of commonly used field materials, equipment, and products. Researchers have conducted soak tests to evaluate potential sources of PFAS and assess their ability to contribute detectable concentrations of commonly analyzed PFAS to water samples. Multiple Federal and State agencies and professional organizations have urged practitioners to restrict the use of products that contain or may contain PFAS to avoid inadvertently cross-contaminating samples. Yet it is difficult to prove the negative and because there are limitations to scientific study, the product content may change depending on the manufacturer, batch number, and year. Guidance documents for PFAS sampling therefore typically rely on the precautionary principle rather than on the limited number of scientific studies.

Objectives

This project was conducted to identify and minimize potential bias in PFAS results when sampling groundwater and surface water, and to provide a scientific basis for PFAS sampling and analytical protocols to minimize the potential for bias in measured PFAS concentrations. Several factors were proposed that could result in sampling bias including PFAS stratification due to accumulation at the air/water interface, and the use of field equipment, materials, and products that could contribute PFAS or PFAS precursors (biasing concentrations high) or adsorb PFAS present in the water sample (biasing concentrations low).

Technical Approach and Results

PFAS Stratification in Groundwater

In groundwater, accumulation may be affected by geochemical conditions including salinity, organic matter, and colloidal material. Bench-scale studies were conducted to test the hypothesis that PFAS stratification in the water column is a function of water chemistry as well as the chemical properties of PFAS. Bench-scale studies were designed to elucidate biases associated with PFAS stratification in groundwater (Section 2.2.1) due to accumulation at the air/water interface and a difference in salinity.

The project team constructed model groundwater wells using polyvinyl chloride pipe with sampling ports located close to the top, mid-point, and bottom of the length of the model wells. Columns were filled with freshwater (i.e., groundwater collected from a site where AFFF had been used), brackish water (i.e., groundwater from the same site amended with 11.9 g/L sodium chloride, which resulted in a salinity measurement of 21.8 μS/cm), or PFAS-free deionized water as a control. Columns were sampled immediately after setup and after three months of being allowed to sit undisturbed. Samples from three depths in the model wells were analyzed for PFAS. There were no significant differences in PFAS concentrations among depths during the 3 months of inactivity. Therefore, the project team concluded that there was no need for a field evaluation or modification of groundwater sampling methods.

PFAS Stratification in Laboratory-Synthesized Surface Waters

In surface waters, natural organic matter can accumulate in the surface microlayer (Cunliffe et al., 2013). The presence of natural organic matter, including natural surfactants present in decaying plant matter) can reduce surface tension and cause foam to form on surface water (Wegner et al., 2022). Foam can also be generated as a microlayer on the surface of lakes or streams where air mixes into the water due to waves or turbulent flow.

Multiple experiments were conducted to assess stratification in surface waters and assess the role of the surface microlayer (or foam) as a potential reservoir of PFAS that may impact sample concentrations if included in surface water samples. Initial laboratory experiments used synthetic surface waters and focused on understanding the composition of the surface microlayer and constituents that facilitate the formation of natural foam (Section 2.2.2). A synthetic recipe of organic compounds was used to form stable foam in natural systems, including amino acids, phospholipids, humic acids, plant-based saponins, and xanthan gum (Wegner et al. 2002, Garret 1965, Bittar et al. 2018, Kuznetsova et al. 2004, Napolitano et al. 1995, Penezic et al. 2010, Zancker et al. 2017). These compounds were added to create a generic mixture of total organic carbon (TOC) to approximate surface water systems. Bench-scale microlayer/foam tests of two water conditions were simulated: freshwater and brackish waters. Surface samples were collected using a Garret metal screen (Agogue et al. 2004) and bulk samples were collected using a micropipette. The volume of collected foam was estimated gravimetrically. Small but measurable TOC enrichment was measured in the surface microlayer in the absence of foam. As expected, TOC enrichment was greater in brackish waters compared to freshwaters.

Experiments then shifted to focus on PFAS stratification and PFAS accumulation in the surface microlayer and foam (Section 2.2.3). Results are presented in terms of a surface enrichment factor (EF), which is the PFAS concentration in the microlayer (or foam) divided by the PFAS concentration in bulk water (Schwichtenberg et al., 2020; Ju et al., 2008). As expected, EFs were greatest for the long-chained PFAS, and generally decreased with chain length (e.g., Schwichtenberg et al., 2020). PFAS concentrations in the surface microlayer were greater in brackish water than in freshwater (e.g., Brusseau and van Glubt, 2019). When thin/unstable foams formed using a saponin-free organic mixture, no PFAS surface enrichment (EF ~1) was observed.

PFAS stratification experiments were also evaluated at a larger scale in the laboratory, in an experimental flow cell that was constructed to simulate waves on a lake or bay (Section 2.2.3). PFOS EFs observed in foam in the simulated lake environment were higher than EFs observed in the smaller-scale batch experiments. This trend was consistent with EFs measured in field surface water samples (e.g., Schwichtenberg et al., 2020), i.e., EFs are lower in small-scale laboratory studies than in field-observed measurements. PFAS EF values in the flow cell were not dependent on the concentration of saponin in the TOC mixture, but rather on enhanced mixing at the water surface.

Stratification in Natural Waters

Additional laboratory experiments were conducted using natural waters collected from nine sites and amended with PFAS (Section 2.2.3). The trend and magnitude of measured EF values were consistent with measurements in synthetic waters. The profile of organics present in the natural waters did not enhance PFAS accumulation near the water surface. Additional experiments were conducted; results suggested that foam (with its relatively high air-water surface area) is not the primary cause of PFAS enrichment but rather that organic-rich liquid at the water surface accumulates PFAS (Schwichtenberg et al. 2020).

Stratification and the Effect of Field Sampling Methods

Field sampling of surface waters was conducted (Section 2.2.5). Experiments were conducted to evaluate different surface microlayer sampling techniques including a large glass plate method and microscope slide methods, and to evaluate EFs for various PFAS as a function of hydrophobicity. Surface microlayer and foam PFAS concentrations were significantly greater than bulk water PFAS concentrations, with EF values ranging up to 4000 (Schwichtenberg et al., 2020). General water quality chemistry parameters and concentrations of dissolved organic carbon (DOC) in bulk surface water and the surface microlayer were also analyzed and compared (Schwichtenberg, 2023).

Pilot study sampling at two sites was conducted to assess potential causes of variability, followed by a full-scale field study sampling 11 different surface waters to evaluate whether inclusion of the surface microlayer in bulk surface water sampling methods would result in samples with a high bias in measured PFAS concentrations. A secondary objective was to quantify EFs of PFAS concentrations in the surface microlayer compared to bulk water. Samples were collected from

natural water bodies with varying water chemistry across a large geographic range.

Bulk water samples were obtained using a peristaltic pump with tubing (n=3), fully submerged bottle (n=3), and partially submerged bottle (n=5). During the pilot study, five replicate samples were collected using a partially submerged sampling method were composited in a 3.78-L food-grade high density polyethylene PFAS-free container and then separated into five 250-mL bottles, for a total of five replicates, to capture only analytical variability in PFAS concentrations. Surface microlayer samples (n=5) were also collected at each site using glass plate samplers (Section 2.2.4).

Summary statistics of pilot study results indicated that mean coefficients of variation (CV) for detected PFAS were greater for individual replicates than for composite replicates. The difference between individual and composite replicates indicated that 42% (Site 2) to 75% (Site 9) of the observed variation was due to analytical variation, with the remainder attributable to spatial or other sampling variation. However, the Levene's test for homogeneity of variance did not identify any significant differences in variation for any PFAS/site combination.

Data from both the full-scale study and pilot-scale study were evaluated to assess differences among sampling methods. Although few statistically significant results were observed, results were consistent. Concentrations from partially submerged bottle samples were not biased high; rather, they were lower than peristaltic pump sample concentrations. Findings were generally inconsistent with the expectation and impetus for this study that bulk water PFAS concentrations might be biased high due to enriched PFAS in the SML being captured using the partially submerged sampling method.

Importantly, the observed differences among sampling methods were small enough that they may not be of practical importance. Averaged across PFAS and sites, the magnitude of the difference between partially submerged methods and the fully submerged and peristaltic pump methods was 3% and 5%, respectively. The mean relative percent difference (RPD) of PFAS concentrations among sites and analytes was greatest between the peristaltic pump and partially submerged bottle sampling methods (mean of 13.9%, range was 0 to 151%). For the peristaltic pump and partially submerged bottle sampling methods, 91% of RPDs were less than 30%. Given that an RPD of 30% is generally considered to be an acceptable level of variation for PFAS concentration duplicates, the observed differences among methods may not be of practical importance. It is likely that outside of this controlled study, any of these approaches could be used to sample PFAS from surface water bodies without a significant concern about bias due to the SML enrichment.

Median and maximum enrichment factors (EFs) generally increased with increasing retention time. There were no significant correlations of PFAS EFs with bulk water DOC, pH, specific conductance, or turbidity.

Systematic Evaluation of Field Materials and Procedures

Field equipment, materials, and products can potentially contribute PFAS or PFAS precursors to a water sample (bias concentrations high) or adsorb PFAS present in the water sample (biasing concentrations low). The project team gathered readily available PFAS investigation guidelines,

protocols, and work plans, and developed a comprehensive list of protocols for field sampling. A literature review was conducted to evaluate the scientific basis of recommended PFAS sampling restrictions and recommendations. Readily available scientific studies were summarized to determine which materials or equipment had previously been evaluated for PFAS contribution to samples. Several peer-reviewed studies (e.g., Bartlett and Davis, 2018; Denly et al., 2019; van der Veen et al., 2020) evaluated equipment rinsate blanks or conducted soak tests for various materials to evaluate the presence of PFAS and/or total fluorine. Additionally, field sampling procedures were evaluated to determine the pathway by which PFAS could transfer from materials, not directly in contact with the samples, to the samples. Peer-reviewed literature review findings were evaluated in combination with unpublished data, including large datasets of equipment rinsate blanks, to identify scientific data gaps and guide recommendations for additional laboratory testing. Results are summarized in Section 3, including lists of materials, equipment, and products that have been tested and did or did not contribute PFAS to field samples as well as a closer look at the potential contact and migration pathways for field equipment, products, and materials to come into contact with PFAS samples.

Impact of Laboratory Hold Times and Storage Conditions

Laboratory practices regarding hold times and storage temperatures is another area where initial PFAS practices were initially more conservative. Most laboratories using modified versions of method 537 specified hold times of 14 days for PFAS samples. Longer hold times for samples would be more convenient and result in cost savings. Therefore, the project team had proposed to conduct additional laboratory studies to evaluate the stability of PFAS in samples stored in a freezer or held at refrigerated temperatures for longer periods of time. However, following the submittal of the proposed scope of work for the project, a study conducted by Woudneh et al. (2019) was published. The study was carefully designed to evaluate the effect of hold times and temperatures on PFAS results. Section 4 therefore summarizes key findings from Woudneh et al. (2019), who concluded that hold times and storage practices could be extended without biasing analytical results, provided that samples are frozen upon receipt at the laboratory. (Concentrations of sulfonamido ethanols and 8:2 fluorotelomer sulfonate (FTS) decreased significantly when refrigerated over a 14-day hold time). With the publication of Environmental Protection Agency Method 1633, longer hold times for frozen samples are included as a provision of the method.

Translation of Research Findings to Inform Sampling Guidance

Findings from project tasks were summarized and published in a technical report, along with recommendations to improve PFAS sampling guidance documents:

- Based on our review of scientific studies and consideration of potential pathways for sample cross-contamination, many PFAS sampling restrictions in current guidance are based on the precautionary principle rather than on scientific merit. A limited number of restrictions and recommended best practices are substantiated by scientific studies. Some guidance documents unnecessarily restrict the use of materials and equipment in the field that are never in direct contact with water inside sample bottles and have no credible pathway for biasing sample results.

- In general, earlier sampling guidance (produced prior to 2018) was more precautionary and restrictive of materials that could be used during PFAS field sampling. This approach was beneficial because it bolstered confidence in sampling results, reduced the need for discussions regarding the acceptability of data for decision-making, and avoided the potential need to re-sample or to collect additional samples. However, some of the earlier and more restrictive precautions still remain in use.

- Guidance that is highly specific and restrictive increases the time and effort required for field work planning and implementation, likely resulting in higher cost and more waste generated.

- Sampling guidance can be improved by differentiating between the limited field practices and equipment that are scientifically known to result in PFAS detections in laboratory tests (e.g., PTFE bailers or tubing) from those that do not.

- Current sampling protocols already provide an additional layer of sample protection by specifying glove changes prior to the collection of each sample and the collection of field equipment blanks.

1. OBJECTIVES

This project was conducted to identify and minimize potential bias in per- and polyfluoroalkyl substances (PFAS) results when sampling groundwater and surface water, and to provide a scientific basis for PFAS sampling and analytical protocols to minimize the potential for bias in sampling results. Several factors were proposed that could result in sampling bias including PFAS stratification due to accumulation at the air/water interface, and the use of field equipment, materials, and products that could contribute PFAS or PFAS precursors (biasing concentrations high) or adsorb PFAS present in the water sample (biasing concentrations low).

The project team proposed a variety of activities to assess the potential effect of each of these factors on PFAS sample concentrations, including a literature review, review of unpublished datasets of equipment blank results, laboratory bench-scale studies conducted by project participants at Oregon State University (OSU) and CDM Smith laboratories, and field sampling campaigns led by OSU, AECOM and Jacobs. Throughout the project, Geosyntec led the translation and outreach of technical results, including outreach to state and federal project managers, consultants, and contract laboratories and preparation of a technical report inform optimized sampling and analytical practices (Deeb et al. 2021).

The following sections summarize key findings related to each of the factors that have the potential to bias PFAS concentrations:

- **Section 2. PFAS Stratification**. This section summarizes results from the project team's evaluation of factors that could impact PFAS stratification in water columns, including both surface water and groundwater wells. Laboratory and field results were used to assess whether changes in sampling procedures are needed to minimize bias during sampling (Task 1).
- **Section 3. Systematic Evaluation of Field Materials and Procedures**. This section presents results of a systematic evaluation of field equipment, materials, products, and field sampling procedures, with the goal of evaluating their potential impact on measured PFAS water concentrations (Task 2).
- **Section 4. Impact of Laboratory Hold Times and Storage Conditions**. This section describes the impact of literature reports on laboratory sample hold times and storage conditions on artifacts that may affect measured PFAS concentrations (Task 3).
- **Section 5. Translation of Research Results into Best Practices**. This section summarizes best practices and recommendations for PFAS sampling guidance to minimize potential bias in sample concentrations.
- **Section 6. References** provides a list of cited references.

Over the course of the project, the team prepared multiple presentations, publications, and reports submitted to the Strategic Environmental Research and Development Program (SERDP). Supporting data are provided as **Appendix A**. A list of publications is provided as **Appendix B**.

2. PFAS STRATIFICATION

This section summarizes project team activities to assess several factors that could lead to PFAS stratification in water columns (surface water or groundwater wells) and identify whether modifications in sampling approaches are recommended to minimize bias during sampling.

2.1. Background

Many PFAS, including perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA) and other perfluoroalkyl acids (PFAAs), are amphiphilic—they contain a hydrophobic fluorinated carbon chain attached to a water-soluble, polar head group. This structure results in a propensity for PFAS to accumulate at interfaces, including the air-water interface, water-oil interfaces, and liquid-solid interfaces. Many PFAS also have surfactant properties that improve their performance in a variety of industrial applications. For example, PFAS were added to aqueous film-forming foams (AFFFs) to facilitate the spread of an aqueous film over the surface of a liquid fuel, and to form a surfactant foam to blanket the liquid fuel, preventing the burning fuel from contacting air and simultaneously cooling the burning fuel and adjacent surfaces.

The propensity of PFAS to accumulate at interfaces, including the air/water interface, has implications for PFAS sample collection and measured PFAS concentrations. A laboratory study in 2018 measured the surface tension of various PFOS and PFOA solutions with concentrations ranging from 0.1 to >1,000 milligrams per liter (mg/L) and found substantial accumulation of PFOS and PFOA at the air-water interface (Brusseau et al. 2018; Schaefer et al., 2019). Others documented PFAS accumulation at the air-water interface and the effect of salinity on air-water partitioning (Schaefer et al. 2019). Both researchers concluded that PFAS enrichment at the surface could lead to stratification within surface water and groundwater.

Understanding the magnitude of PFAS accumulation at the air-water interface is important for gauging the impact on measured PFAS concentrations in water samples. In groundwater, accumulation may be affected by geochemical conditions including salinity, organic matter, and colloidal material. In surface waters, natural organic matter can also accumulate in the surface microlayer (Schaefer et al. 2019). The presence of natural organic matter, including natural surfactants present in decaying plant matter) can reduce the surface tension and cause the formation of foam on surface water (Wegner et al. 2022). Foam can also be generated as a microlayer on the surface of lakes or streams where air mixes into the water due to waves or turbulent flow. Thus, the surface microlayer serves a potential reservoir of PFAS and thus may impact PFAS concentrations if the surface microlayer (or foam) is captured while sampling surface water.

Bench-scale studies were conducted to test the hypothesis that PFAS stratification in the water column is a function of water chemistry and the chemical properties of the PFAS present. Bench-scale studies were designed to elucidate biases associated with PFAS stratification in groundwater (Section 2.2) or surface water (Section 2.3) due to accumulation at the water-air interface and differences in salinity.

2.2. Technical Approach and Results

2.2.1. PFAS stratification in groundwater monitoring wells (Task 1.1)

Oregon State University researchers constructed seven model groundwater wells using 2.5-inch-diameter polyvinyl chloride (PVC) pipe with sampling ports located close to the top, mid-point, and bottom of the length of the model wells. (Sample ports were located 4, 30, and 54 inches from the top of the model wells).[1] Three of the seven columns were filled with freshwater (i.e., groundwater collected from a site where AFFF had been used), three were filled with brackish water (i.e., groundwater from the same site amended with 11.9 g/L sodium chloride (NaCl), which resulted in a salinity measurement of 21.8 µS/cm), and one was filled with PFAS-free deionized water as a control. The columns were sampled immediately following setup and after three months of being allowed to sit undisturbed. Samples from the three sampling ports were analyzed for PFAS using tandem liquid chromatography quadrupole time of flight mass spectrometry (LC-QToF).

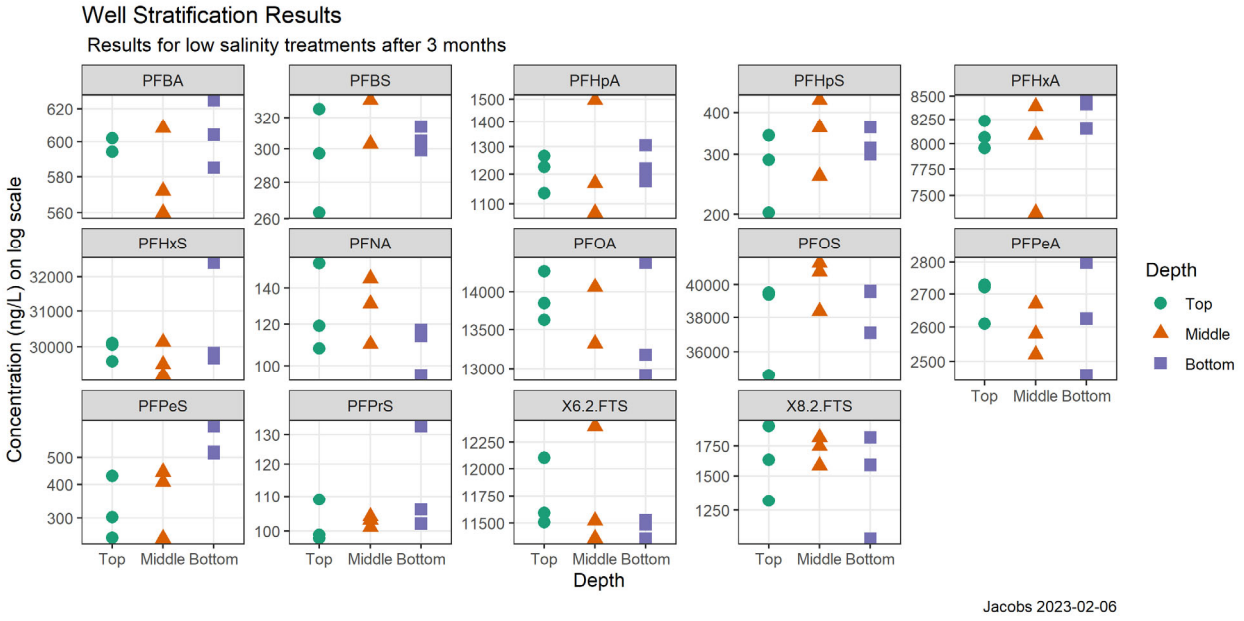

Figure 1. PFAS concentrations measured in different depths in model groundwater monitoring wells

For each PFAS at each salinity level, a two-way analysis of variance was used to assess whether concentrations differed by date or by depth. Initially, the interaction of date and depth was included in the analysis. Because there were no significant interactions between these variables, analyses were rerun with the interaction term removed. There were no significant differences between individual PFAS concentrations in the top, middle, and bottom ports at either freshwater or brackish conditions (with the single possible exception of 8:2 fluorotelomer sulfonate [8:2 FTS]

[1] Note that the top sampling port was not positioned at the interface so sample results do not reflect concentrations at the surface microlayer. However, glass slides were used to sample the microlayer and to support the development of analytical methods for surface microlayer sampling.

in brackish wells which had a p-value for depth of 0.05). For example, average PFOS concentrations in the top, middle, and bottom ports in freshwater wells immediately after filling (**Figure 2a**) and after 3 months (**Figure 2b**) had 95% confidence intervals that overlapped, with a p-value for depth of 0.62. Also, the 95% confidence intervals for PFOS in brackish wells did not differ at time zero (**Figure 2c**) and after 3 months (**Figure 2d**), with a p-value for depth of 0.95. However, in both cases (freshwater and brackish conditions), the concentrations of PFOS at 3 months were significantly less than the concentrations at time zero. This likely reflects losses of PFOS throughout the water column to the well material, not just at the surface due to stratification. In addition, PFNA, which has a similar hydrophobicity as PFOS, displayed the same outcome (**Figure 3**). Some exceptions were noted (e.g., differences in PFBS, PFHpS, and 8:2 FTS concentrations in the bottom port in brackish conditions and differences in PFBA, 6:2 FTS, and PFHxS in one port in freshwater conditions. These exceptions did not align with a trend with hydrophobicity or head group and may be due to the small samples size (n=3) for each port.

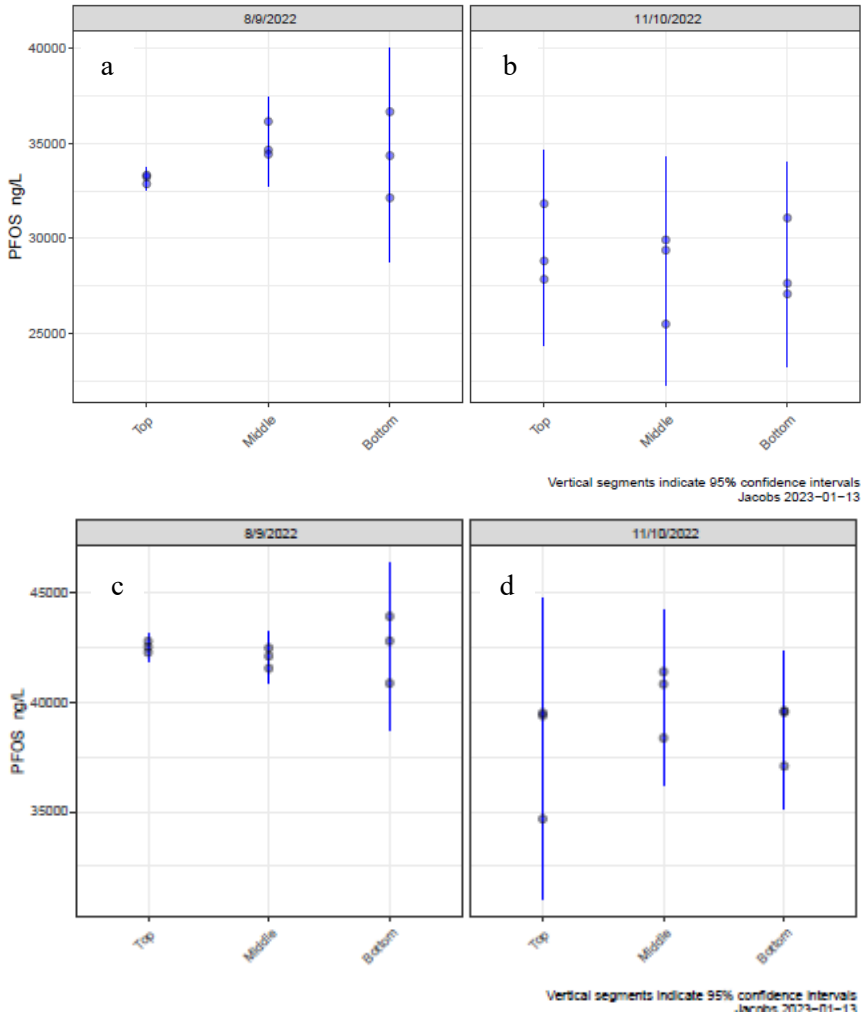

Figure 2. PFOS concentrations in freshwater (a, b) and brackish (c, d) model groundwater wells from top, middle, and bottom ports

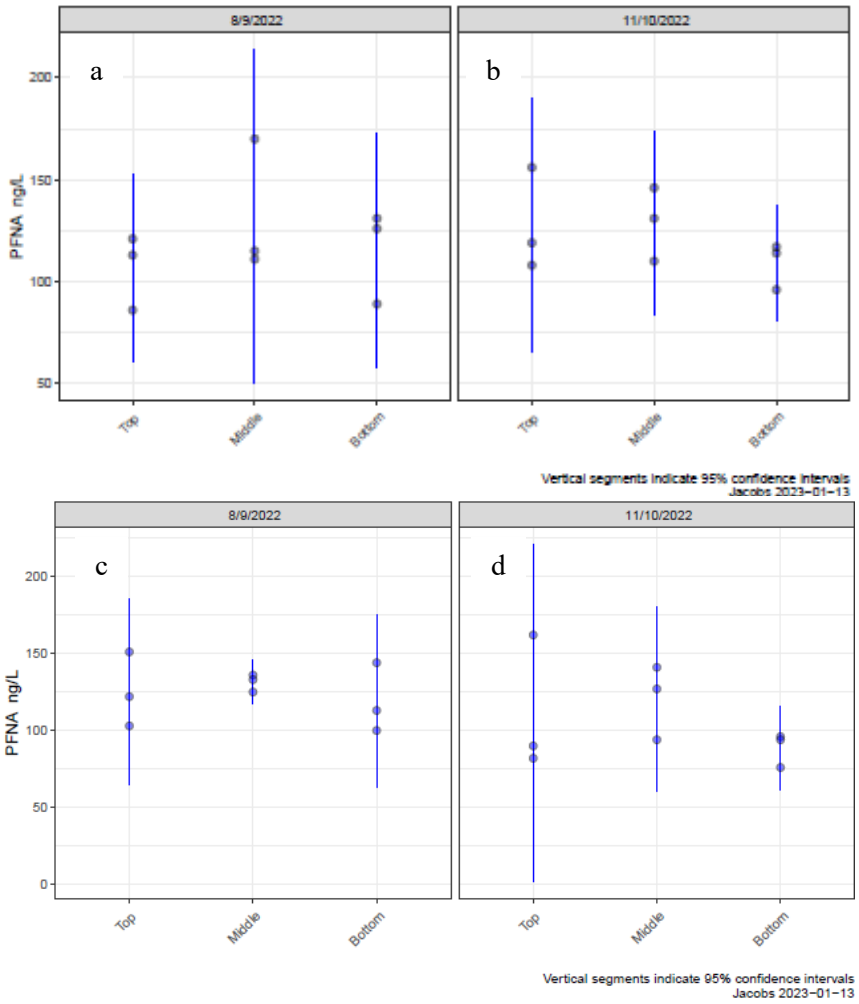

Figure 3. PFNA concentrations in low salinity (a, b) and high salinity (c, d) model groundwater wells from top, middle, and bottom ports

As a follow up, the PVC model wells (low and high salinity) were sampled using microscope slides (Section 2.2.4) to quantify the PFAS in the SML of the model wells. The slides were extracted into methanol, spiked with extracted internal standards, and the PFAS were quantified by LC-QTOF. In addition, the PVC of a low and one high salinity model well were cut into 15 cm^2 sections and the interior was extracted with methanol. The methanol extract was spiked with extracted internal standards and analyzed by LC-QTOF. The mass of each target PFAS associated with the water column, SML, and PVC well were summed and compared to the initial mass initially present in water added to the model well. For the low salinity wells, mass balances ranged from 86% (8:2 FTS) to 103% (PFNA). In the case of the high salinity wells, mass balances ranged from 72% (8:2 FTS) to 99% (PFHpA). The mass associated with the PVC walls of the wells ranged from < 2% (for all PFAS) in the low salinity wells and 0.11% to 20% (PFOS) for high salinity wells, while the percent of PFAS mass associated with the SML in the low salinity wells was <0.01% and <0.3% in high salinity wells. As expected, the PFAS in the SML and associated with the PVC was higher in the high salinity wells compared to the low salinity wells. Likewise, the PFAS mass associated with the PVC well material was greater for higher salinity wells than lower salinity wells. Enrichment factors were only significantly greater than one for longer-chain PFAS in the high salinity wells (PFNA, PFHpS, PFOS, and 8:2 FTS) and for the low salinity wells, only PFNA

and PFOS gave enrichment factors greater than 1.

2.2.2. PFAS stratification in synthetic surface waters (Task 1.2)

During Task 1.2, multiple experiments were conducted to assess PFAS stratification in surface water. Initial experiments focused on understanding the composition of the surface microlayer and constituents that facilitate the formation of natural foams (Section 2.2.2.1). Natural organic microlayers and foams are present in many surface waters, yet their impact on PFAS distribution is poorly understood. Experiments then shifted to focus on PFAS stratification and PFAS accumulation in the surface microlayer and foam (Section 2.2.2.2). See Appendix A Schaefer et al., 2019 for Supplemental Data associated with this section.

2.2.2.1. Experiments to Assess Organic Microlayer, Foam Formation, and Organic Carbon Enrichment Near the Water Surface

Many different organic compounds can be present in the surface microlayer; a review of the available literature suggests that the profile of organic compounds is highly site-specific. CDM Smith conducted laboratory studies to test a synthetic recipe of organic compounds that were found to be responsible for stable foam formation in natural systems: amino acids (glutamic acid, glycine, serine, alanine and hyaluronic acid), phospholipids (azolectin) humic acids, and plant-based saponins (Wegner et al. 2002, Garret 1965, Bittar et al. 2018, Kuznetsova et al. 2004, Napolitano et al. 1995, Penezic et al. 2010, Zancker et al. 2017). These organic compounds were tested in different concentrations to create an appropriate generic mixture of total organic carbon (TOC) that approximated surface water systems. Xanthan gum, which is used to simulate exopolymer particles from marine biota, was added to the recipe to facilitate formation of an organic microlayer and foam. The synthetic water recipe is listed in **Table 1**. The original recipe contained 100 mg/L of saponin; however, excessive foaming was observed. To mitigate excess foaming, the saponin concentration in the water was decreased from 100 mg/L to 20 mg/L in the flow cell experiments. The modified synthetic water recipe yielded a more reasonable foam layer in the flow cell.

Table 1. Organic components of synthetic water used in this study.

Organics	Concentration (ppm)
Humic acid	10
Azolection (phospholipids)	50
Xanthan gum	60
Saponin	20 or 100
Hyaluronic acid	1.0
Glycine	0.037
Serine	0.021
Alanine	0.018
Glutamic acid	0.015

Surface tension is an inherent characteristic of material interfaces, and it plays a fundamental role in many natural and industrial phenomena. Initial tests were performed to better understand the extent to which the organic compounds that were used to make the synthetic water partitioned into the air-water interface. A total of four solutions were tested in triplicate. For this experiment, saponin was removed from original recipe.

1. Low salinity electrolyte (deionized water + 0.2 g/L NaCl)
2. Low salinity electrolyte + organics (no saponin), at a 1x concentration of organics
3. Low salinity electrolyte + organics (no saponin), at a 0.4x concentration of organics
4. Low salinity electrolyte + organics (no saponin), at a 5x concentration of organics

Surface tension was measured using Theta Tensiometer equipment, by Pendant Drop method in the OneAttension software. Surrounding interferences, such as vibrations and airflow, were minimized to ensure accuracy and precision in instrument readings. After each measurement, the pipette tip was changed. Experiment was conducted at room temperature (~25 ^{o}C) in triplicate.

Once the droplet had been dispensed and was stable, surface tension recording was initiated (**Table 2)**. Water has surface tension of 72 mN/m at room temperature due to its strong hydrogen bonds.

Table 2. Surface tension measured by pendant drop method.

	Surface Tension (mN/m)			Average	Stde	CV
	Reading A	Reading B	Reading C			
Low Salinity Electrolyte	71.9	71.1	72.4	71.8	0.6	1%
Low Salinity Electrolyte + organics (NO saponin) - 1X	71.5	70.6	69.5	70.6	1	1%
Low Salinity Electrolyte + organics (NO saponin) - 0.4 X	71.3	69.7	71.2	70.7	0.9	1%
Low Salinity Electrolyte + organics (NO saponin) - 5 X	69.4	71.4	71.6	70.8	1.2	2%

Microlayer/foam testing was initially performed in small pans in a bench-scale study. Two different water conditions were tested: low salinity (NaCl = 0.2 g/L to simulate freshwater) and high salinity (NaCl = 5 g/L to simulate brackish water). Initial experiments were performed in absence of the organics, as well as in the presence of organics with and without surface-active saponins. (Saponin was demonstrated to stabilize foam formation following solution agitation). Organic amendments listed in **Table 1** were added and the solution was mixed gently using a stirbar at approximately 80 revolutions per minute (rpm).

Surface and bulk solution samples were collected for TOC analysis using a Garret metal screen (Agogue et al. 2004) and a micropipette, respectively. The metal screen was stainless steel and had a mesh size of 1.18 millimeters (mm) and a wire diameter of 0.23 mm; the screen dimensions were 15.2 centimeters (cm) by 15.2 cm. The metal screen was used to collect a single sample per time point of the surface microlayer, as shown in **Figure 4**. For samples where a stable foam had formed on the water surface (approximately 3.7 cm thick), the foam was directly collected using a stainless-steel spoon. All results were performed in duplicate. The volume of collected foam was estimated gravimetrically.

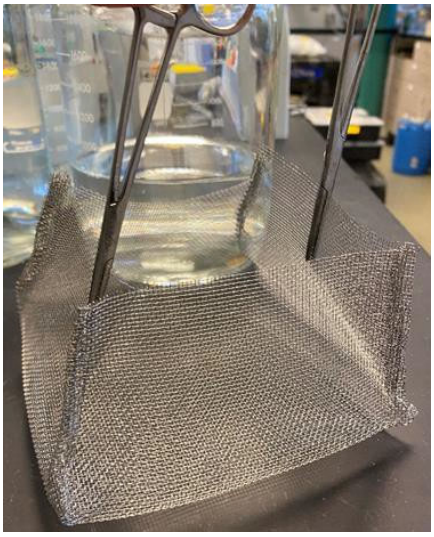

Figure 4. Garret metal screen apparatus

Samples of the bulk water and surface microlayer were collected at time zero (T_0) and after 24 hours (T 24 hr) and 72 hours (T 72 hr) from the initial solution. For experiments amended with the organics (no saponin), vigorous shaking was needed to generate foam. These foams, in the absence of saponin, were not stable and collapsed within 20 minutes; foam thickness was generally less than 0.1 cm on the water surface. To facilitate this vigorous shaking, 2-gallon buckets were used instead of the pans. A picture of the sparse thin foam formed in the 2-gallon buckets is shown in **Figure 5**.

Figure 5. Unstable foam generation observed in the presence of organics, excluding saponin, following vigorous shaking

TOC values for the high-salinity solution, amended with organics excluding saponin, are shown in **Figure 6**. TOC measured in the bulk aqueous solution were compared to TOC measured in the surface microlayer in the absence of foam (i.e., without agitation) and with foam (i.e., with agitation, which resulting in thin sparse foams shown in **Figure 6**). Results showed a small but measurable TOC enrichment in the surface microlayer in the absence of foam, suggesting the existence of organic carbon stratification, as often observed in surface water systems. As expected,

TOC concentrations were higher (by nearly a factor of 2) in the surface layer when foam formed.

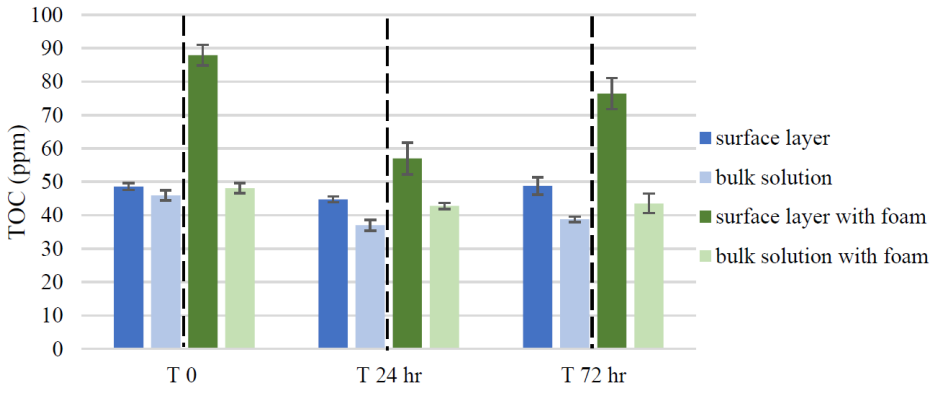

Figure 6. TOC results measured in high-salinity tests in the surface layer and bulk solution

Note: Dark and light blue bars in **Figure 6** represent TOC results from low agitation experiments (surface and bulk water, respectively). Dark and light green bars represent TOC results from high agitation/foam formation experiment (surface and bulk water, respectively). Dashed lines separate TOC results under foaming and no foam conditions for different equilibration times.

TOC surface enhancement tests also were performed using low-salinity water. Elevated ionic strength typically produces a "salting out" effect that enhances the accumulation of surface-active organic compounds in the air-water interface. As expected, TOC concentrations were more enhanced in the surface microlayer in the high-salinity solution compared to the low-salinity solution. Foam also formed more readily in the high-salinity solution compared to the low-salinity solution (**Figure 7**).

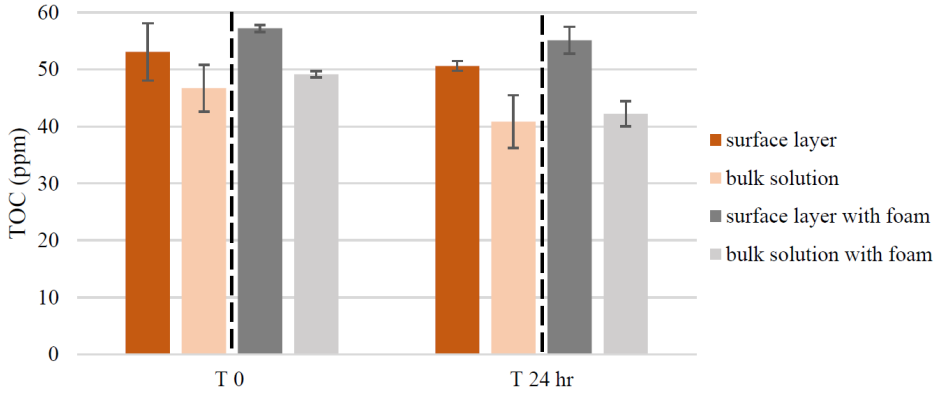

Figure 7. TOC results measured in low-salinity tests in the surface layer and bulk solution

Note: Dark and light orange bars in **Figure 7** represent TOC results from low agitation experiments (surface and bulk water, respectively). Dark and light gray bars represent TOC results from high agitation/foam formation experiment (surface and bulk water, respectively). Dashed lines separate TOC results under foaming and no foam conditions for different equilibration times.

Subsequent foam testing with saponin was performed. Addition of saponin greatly improved foam formation, including foam thickness and stability (**Figure 8** and **Figure 9**). Results appeared to be representative of foam conditions observed in natural waters near the AFFF-impacted groundwater sample collection location.

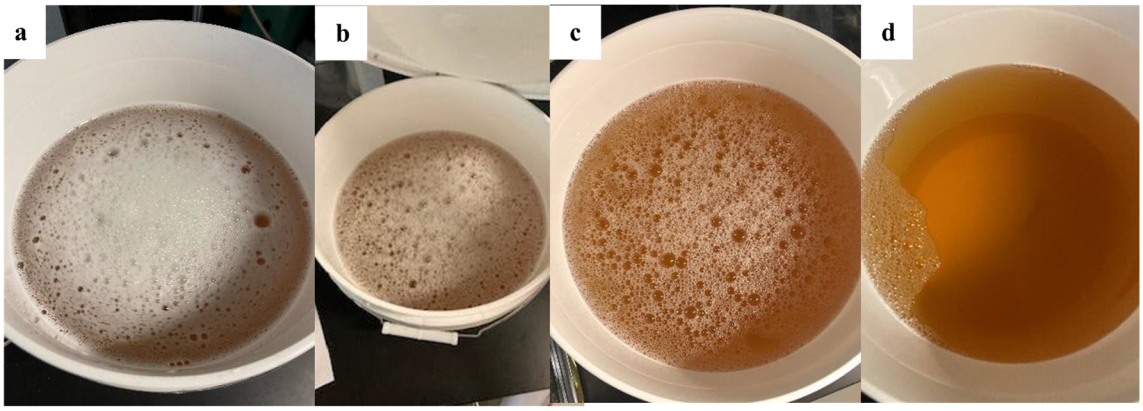

Figure 8. High-salinity synthetic water with 100 mg/L of saponin (a) immediately after agitation, (b) 1 hour after agitation, (c) 6 hours after agitation, and (d) 15 hours after agitation

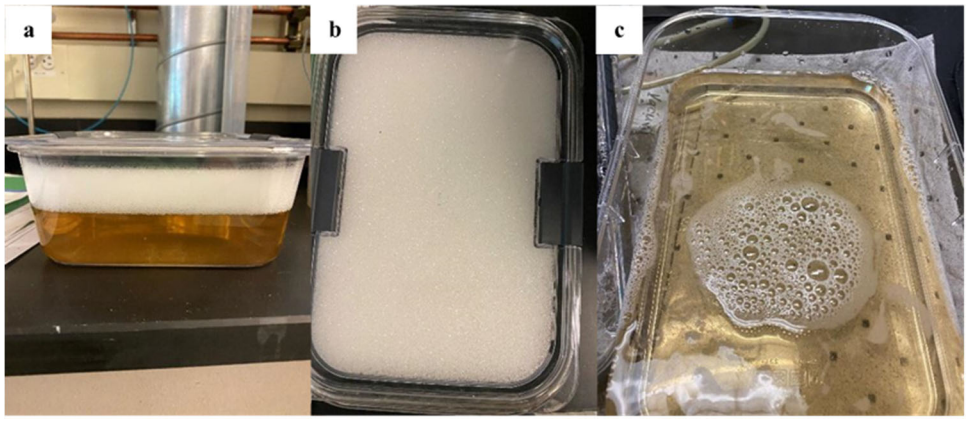

Figure 9. High-salinity synthetic water with saponin after 24 hours on the shaker table at 80 rpm (a) lateral and (b) top views immediately after removal from the shaker table. Foam thickness was approximately 4 cm, and (c) top view of water without saponin immediately after removal from the shaker table

TOC levels in the foam versus bulk water phases in saponin-amended high-salinity water are provided in **Figure 10**.

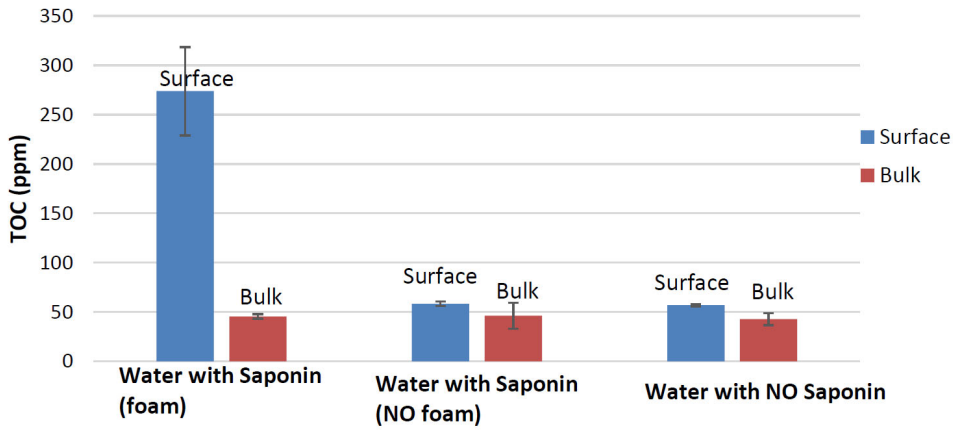

Figure 10. Comparison of TOC results measured in high-salinity tests in the surface layer and bulk solution when saponin and foaming is present or absent

Note: Blue bars in **Figure 10** represent surface TOC results from water with and without foam and with and without saponin, respectively. Orange bars represent bulk TOC results from water with and without foam and with and without saponin, respectively. Results of the saponin-free solution are also provided for comparison. After 24 hours of agitation, results clearly showed that addition of the surface-active saponin enhanced TOC accumulation in the foam relative to the bulk water, as TOC levels were approximately six times greater in the foam than in the bulk water. However, in the absence of foam, TOC enhancement (with saponin) was negligible, indicating the importance of the foam for accumulating TOC at the water surface for the synthetic system studied. Saponin-amended water may be more indicative of waters where thick and relatively stable (greater than 1 hour) foams are formed, whereas the organic mixture without saponin may be more representative of surface waters where sparse, unstable, and thin foams are observed.

A modified synthetic water recipe with 20 mg/L saponin was also tested under low salinity (0.2 g/L NaCl) conditions. **Figure 11** shows differences in the amount of foam generated with the modified and original recipe.

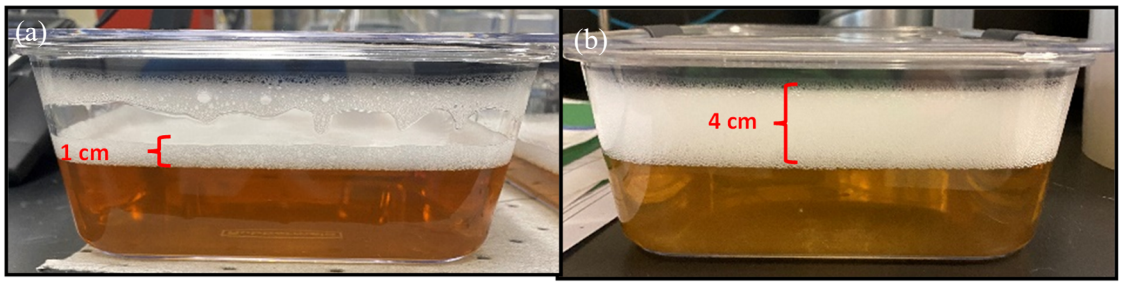

Figure 11. Small-scale batch system showing foam generated after 24 hours on the shaker table at 80 rpm using (a) modified synthetic water with 20 mg/L saponin and (b) synthetic water with 100 mg/L saponin

2.2.2.2. Assessment of PFAS Accumulation in the Microlayer and Foam

Experiments were performed to assess the impacts of TOC accumulation and foam formation on PFAS distribution between the bulk water and the water surface. Depending on the system tested, PFAS surface accumulation can be due to monolayer adsorption at the air-water interface, partitioning into an organic surface microlayer, and/or partitioning into a foam phase. Synthetic freshwater and brackish water were prepared as described in Section 2.1, with and without the organics listed in **Table 1**, and with and without saponin. Each experimental system was also amended with a PFAS mixture to attain the bulk water solution characteristics shown in **Table 3**. All experiments were performed in duplicate.

Table 3. Concentrations of individual PFAS in bulk water solution

Name	Acronym	CAS	Target Bulk Water Concentration (nM)
Perfluorobutanoic acid	PFBA	375-22-4	3
Perfluoropentanoic acid	PFPeA	2706-90-3	3
Perfluorobutane sulfonate	PFBS	375-73-5	3
Perfluorohexanoic acid	PFHxA	307-24-4	3
Perfluoroheptanoic acid	PFHpA	375-85-9	3
Perfluorohexane sulfonate	PFHxS	432-50-8	3
6:2 fluorotelomer sulfonate	6:2 FTS	27619-97-2	3
Perfluorooctanoic acid	PFOA	335-67-1	3
Perfluorononanoic acid	PFNA	375-95-1	3
Perfluorooctane sulfonate	PFOS	1763-23-1	3

PFAS samples were collected using a similar method as TOC samples, as described in Section 2.2.2.1. The Garret metal screen apparatus was used to sample the water surface and thin foam layers, and bulk water was sampled using a 5-mL pipette. A stainless-steel spoon was used to sample the thick foam that formed in the presence of saponin. PFAS sampling was performed at 24 hours and 28 hours to assess equilibrium.

While performing TOC testing, small amounts of foam would often remain on the metal screen. To mitigate concerns that this foam would retain PFAS on the screen (biasing sample results low), a modified approach was used to collect a sample from the metal screen. The modified method was employed for all PFAS samples collected using the screen, not just when foam was present. The approach is summarized in **Figure 12**. Initial sampling of the surface microlayer/foam (step 1) was identical to that performed for TOC sampling. During step 2, the screen was submerged in 40 mL of methanol to remove any foam that would have otherwise persisted on the screen and dissolve individual PFAS that could potentially collect on the screen; typically, 4 mL of water were recovered from the screen. The solution was then transferred to a pre-rinsed (using methanol and deionized water) high density polyethylene (HDPE) bottle for PFAS analysis. For consistency in sample dilution and matrix, a similar methanol dilution was performed with the bulk water sampling (step 4). As with TOC sampling, only one screen sample was collected per sampling event so as not to dilute the surface. Experiments were conducted at room temperature (21°C).

PFAS analyses were performed by SGS AXYS Analytical Services Ltd., Sidney, BC, Canada.

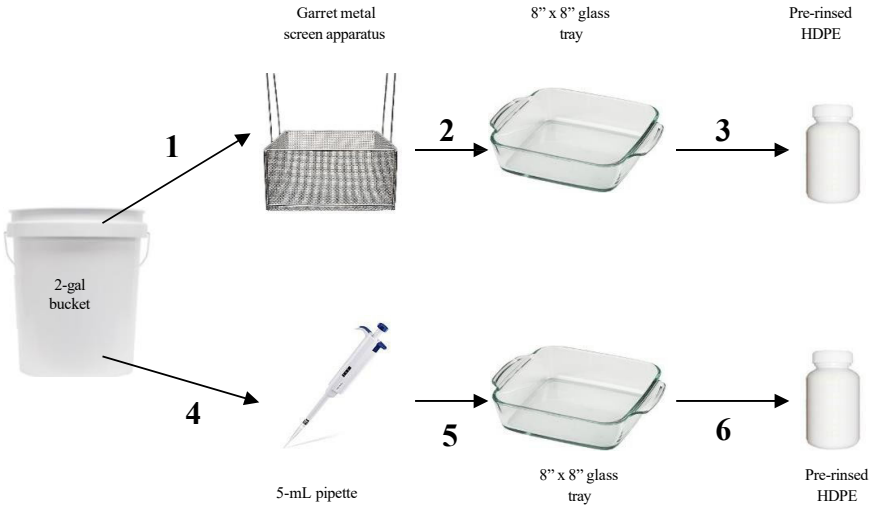

Figure 12. Sampling procedure to assess PFAS present in surface water (steps 1 through 3) and bulk solution (steps 4 through 6)

Results of the measured PFAS distribution between the surface microlayer and bulk water phases, in the absence of added organics, for both the freshwater and brackish water systems, are summarized in **Figure 13**.

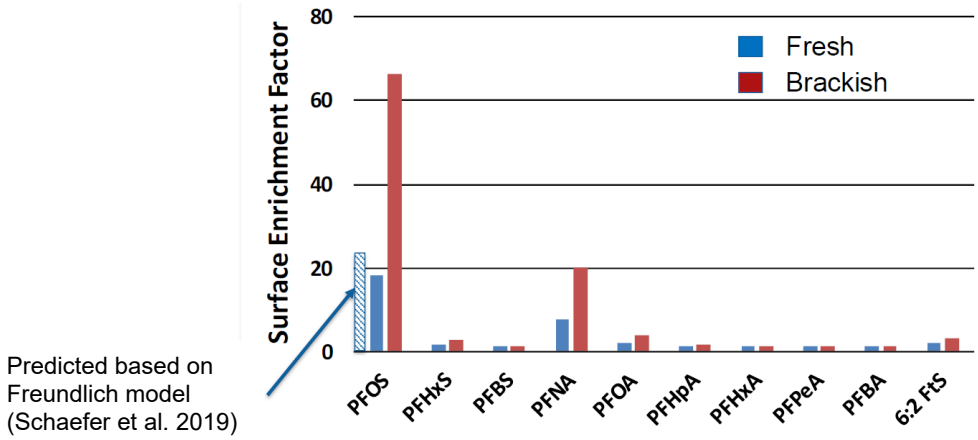

Figure 13. PFAS surface enrichment factor in freshwater and brackish water systems, without amended organics

Results are presented in terms of a surface enrichment factor (EF), which is the PFAS concentration in the microlayer (or foam) divided by the PFAS concentration in bulk water (Schwichtenberg et al. 2020, Ju et al, 2008). The microlayer concentration was estimated assuming that the interrogated surface layer was 250 microns (µm) thick (estimated based on the screen area

and water volume collected in the mesh). EFs were greatest for the long-chained PFAS, and generally decreased with chain length. This is consistent with the expected trend for PFAA adsorption at the air-water interface and EFs reported by Schwichtenberg et al. (2020). Results were also consistent with predictions from a previously developed model of PFOS accumulation at the air-water interface. This indicates that PFAS accumulation near the water surface is governed by air-water interfacial adsorption, as described by the Gibbs adsorption equation (Schwichtenberg et al. 2020), in the absence of an organic surface microlayer or foam (Schaefer et al. 2019). As expected, based on studies of PFAS adsorption at the air-water interface, PFAS concentrations within the surface microlayer were greater in brackish water than in freshwater due to the increased ionic strength (e.g., Brusseau and van Glubt, 2019).

Note that **Figure 13** data were collected after 24 hours. The system was not agitated, so no foam formation was observed. The Freundlich-predicted value for PFOS was based on a freshwater system. In the presence of the saponin-free organic material, and without vigorous agitation to cause foam formation, no PFAS surface enrichment (EF ~1) was observed, based on equilibration times of 24 and 48 hours. Results suggest that the synthetic organics (without saponin) competed with PFAS for air-water interface adsorption.

When thin/unstable foams formed using the saponin-free organic mixture, no PFAS surface enrichment (EF ~1) was observed, indicating that foam was not a sink for PFAS (**Figure 14**) in the absence of saponin, after 48 hours equilibration. The system was agitated, and a thicker and more stable foam was observed. Larger enrichment factors were observed for some of the long-chained PFAS (e.g., PFOS and PFNA) for organic addition experiments with saponin (**Figure 15**).

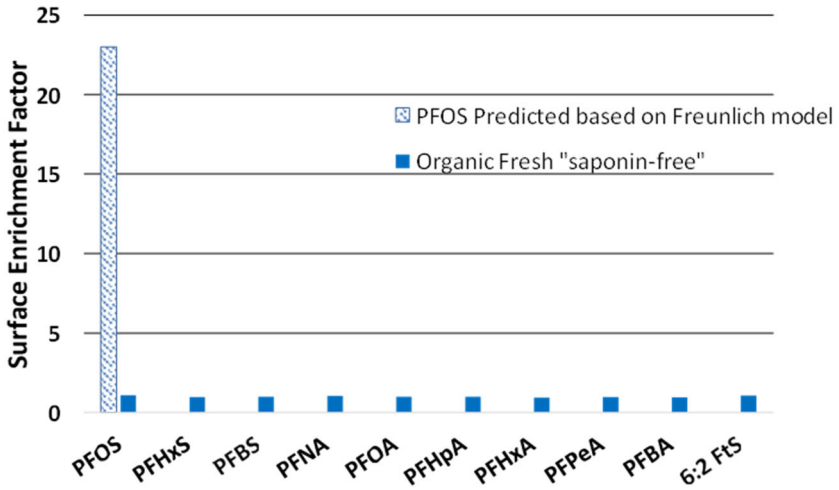

Figure 14. PFAS surface enrichment factors in a freshwater system in the presence of saponin-free foam

Note that equilibrium was attained in 24 hours. The system was agitated and unstable foam formation was observed. The Freundlich-predicted value for PFOS shown in Figure 14 is based on a freshwater system.

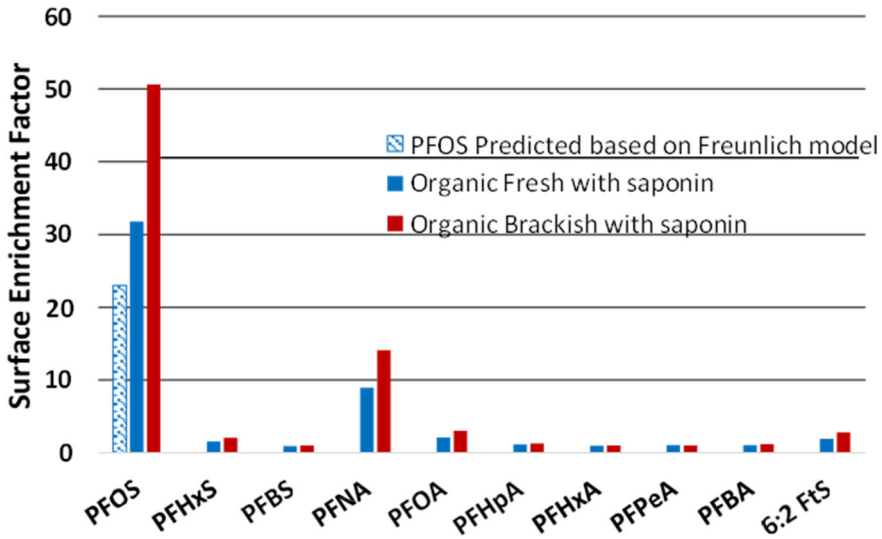

Figure 15. PFAS surface enrichment factors in freshwater and brackish water systems with amended organics

2.2.2.3. Assessment of PFAS Distribution in Synthetic Water in Flow Cell Lake Simulator

Additional experiments were conducted to assess PFAS distribution in surface waters and bulk waters at a larger scale in the laboratory, in an environment that simulated waves on a lake or bay.

An experimental flow cell system was constructed to simulate surface water flow, surface agitation, and formation of a surface microlayer, with the capacity for depth-discrete sampling. Specifically, a 24 x 12 x 36 inch (L x W x H) rectangular tank was constructed using ¼-inch thick polycarbonate sheets. The sheets were first held together using 90-degree corner clamps and subsequently fused using methylene chloride. A waterproof silicone caulk was applied to the inside corners of the tank and allowed to cure for 24 hours. The outside of the polycarbonate tank was reinforced with an aluminum frame to provide additional structural rigidity (**Figure 16a**).

Eight holes were drilled on each side of the tank to facilitate installation of injection or extraction ports constructed of PVC bulkhead fittings with rubber gaskets. Each threaded fitting was sealed using a non-hardening, polyisobutylene, thread sealant. Slightly larger ports were installed near the bottom of the tank to account for the higher head pressures and achieve similar flow dynamics at each port. The injection and extraction ports were strategically placed in a staggered configuration to minimize dead volume during recirculation. A multi-port manifold was constructed of PVC fittings and neoprene tubing, in conjunction with a 44 gallon per minute (gpm) variable flow inline pump to facilitate recirculation inside the tank. The target flow velocity was 0.2 centimeters per second (cm/sec), which is typical of surface water systems. Following installation, a 24-hour leak test was performed. In addition, a dye tracer test was conducted using a food coloring reagent to verify sufficient mixing during recirculation within the cell system. The polycarbonate tank was placed over a plastic secondary containment bin to prevent accidental leakage (**Figure 16b**). The top of the flow cell was covered with an HDPE lid to prevent any aerosols or foam containing PFAS from escaping (**Figure 17**).

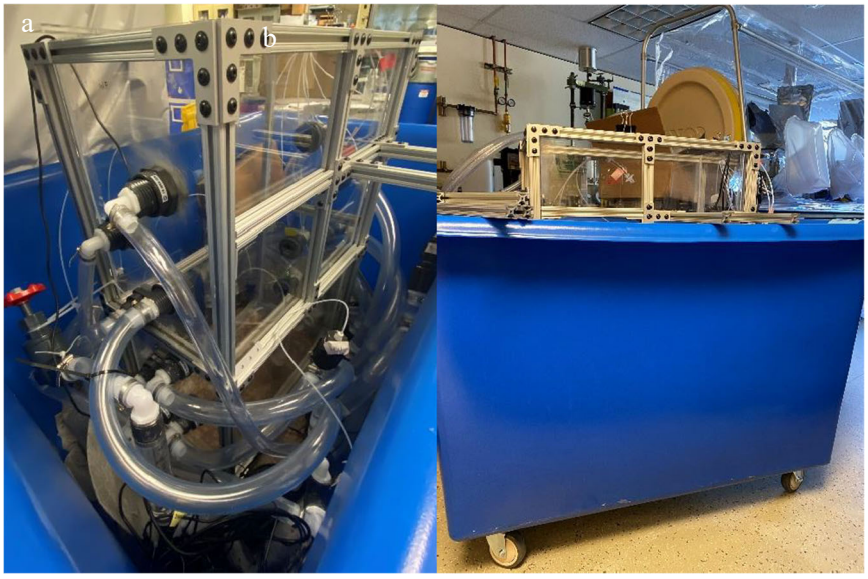

Figure 16. Experimental lake simulator flow cell showing (a) polycarbonate sheets with aluminum frame to provide structural rigidity and (b) secondary plastic containment

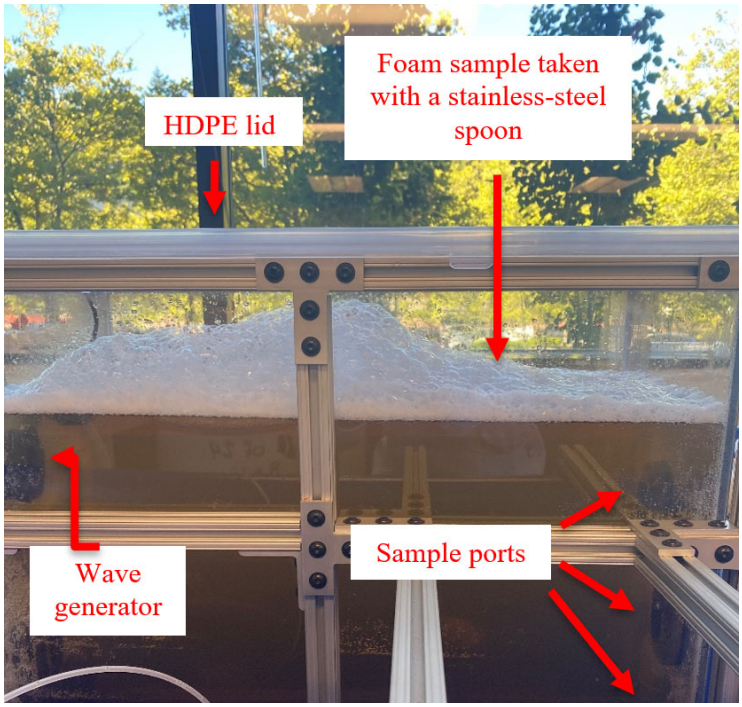

Figure 17. Foam generation in flow cell using modified synthetic surface water with saponin

Using the modified synthetic surface water recipe with saponin, an experiment was set up in the flow cell reactor. After one hour of mixing, the wave generator was turned off, and a foam sample was collected using a stainless-steel spoon. Bulk water samples were collected via the side ports and a Snap Sampler®. Results of the measured PFAS distribution between the surface foam and

bulk water samples (i.e., EFs) are summarized in **Figure 18**.

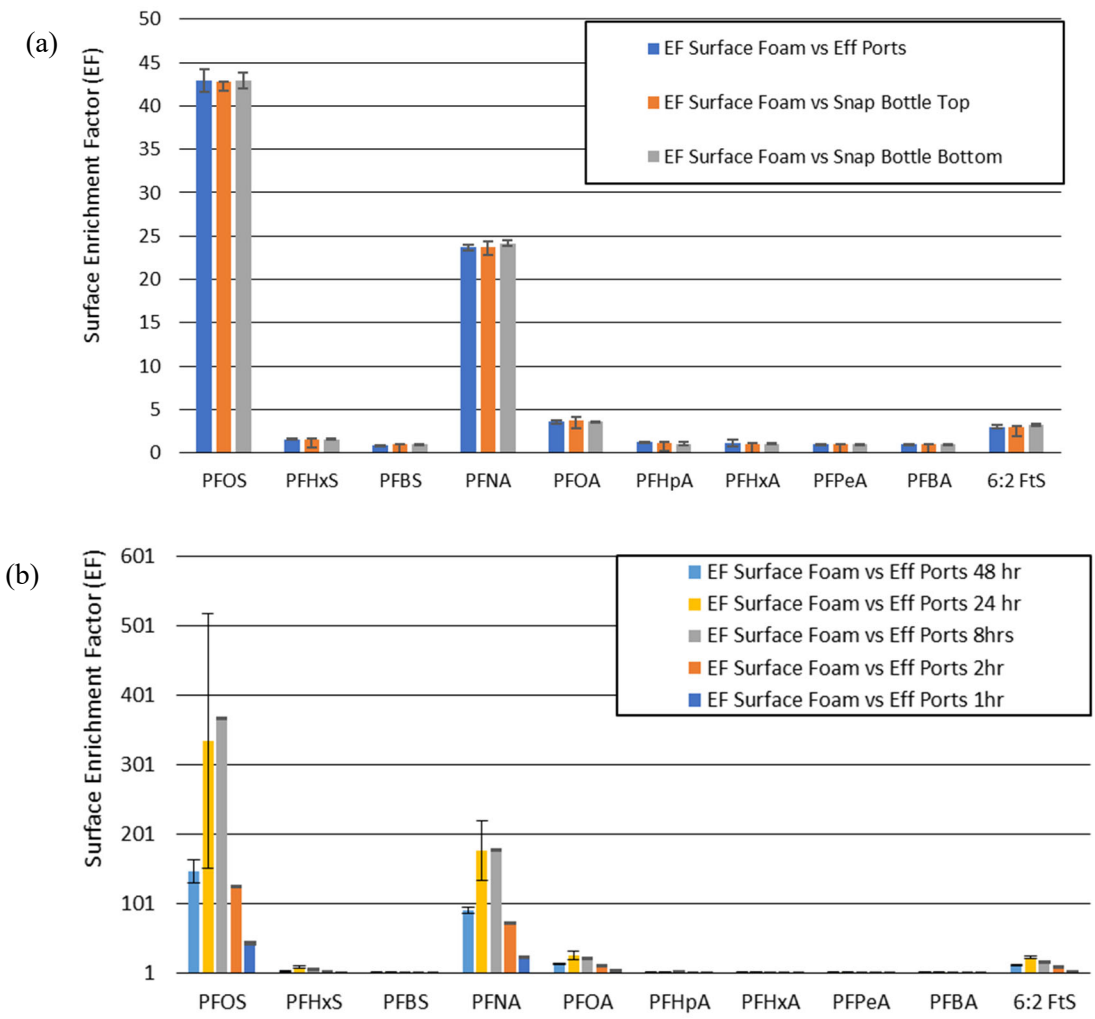

Figure 18. PFAS surface enrichment factors in modified synthetic freshwater (0.2 g/L NaCl) amended with a PFAS mixture using (a) Snap Sampler® and (b) grab samples

Note that sample results shown in **Figure 18a** were collected after 1 hour of mixing. Blue bars show the EFs observed between surface microlayer sample and average sampled ports; orange and gray bars show EFs between the surface microlayer sample and Snap Sampler® results from sampling the top and bottom sampling ports of the tank, respectively. **Figure 18b** shows results after 1, 2, 8, 24 and 48 hours of mixing. Duplicate samples were collected at each sampling event.

PFOS EFs observed in the simulated lake environment were approximately 43, compared to an EF value of 23 observed in the small-scale batch experiments. This trend is consistent with EFs measured in van Etten Lake water (Schwichtenberg et al. 2020), i.e., EFs are lower than the field-observed EF values (ranging from 285 to 2,260) (Schwichtenberg et al. 2020). Results from pan experiments suggest that the relatively high PFAS EF values measured in the flow cell with the wave generator were not dependent on saponin concentration, but rather the enhanced mixing at

the water surface. Experimental results also indicated that the Snap Sampler® showed good agreement with direct sampling of effluent ports and therefore did not introduce artifacts such as sample carryover or cross contamination, even when foam was present.

2.2.3. PFAS stratification in natural waters (Task 1.3)

Information is needed on the concentration of per- and polyfluoroalkyl substances (PFAS) in foams on surface waters impacted by AFFF (Schwichtenberg et al., 2020; See Appendix A Schwichtenberg et al., 2020 for Supplementary Data). Foam and bulk water were collected from nine locations around Van Etten Lake in northeastern Michigan. Foams 1-5 were located on the western short near areas of known PFAS plumes, likely originating from AFFF sources, while Foams 6-8 were located on the eastern shore. Foam 6 was collected near the mouth of a stream impacted by a former municipal dump (e.g., unlined landfill) and Foam 7 and Foam 8 were collected in a location with residential septic tanks. In a separate sampling trip, a foam was collected west of the lake of interest as a possible background sample (Figure 1). Bulk water at this location was collected in a smaller amount, allowing for only PFAS analysis. Samples were analyzed for PFAS by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF) and for total dissolved organic carbon (DOC). The DOC of two foam:bulk water pairs were characterized by ^{1}H NMR.

Of the 50 target analytes, 16 analytes encompassing eight PFAS classes were found above the LOQ in foams, while only five analytes were quantified above the LOQ in underlying bulk water (**Table 4**). Foams 1-5 and Background Foam had long-chained PFCAs ranging from PFOA – PFUnDA at concentrations above the EPA HAL for PFOA, while only one foam (Foam 2) had PFHxA above its LOQ (**Table 4**). Foam 6, Foam 8, and Background had PFUndA. Background Foam was comprised of PFNA, PFDA, and PFUndA, but the source of these PFCAs is unknown. Only PFHxA and PFOA were found in three of the underlying bulk waters (Bulk Water 1, 2 and 4; **Table 4**). Background Bulk Water had no PFCAs > LOQ (**Table 4**). Foams 1-5 all contained PFHxS, PFHpS, and PFOS, while two foams also contained PFNS (Foam 2 and 3; Table 1). The concentrations of PFOS ranged from 2,300 - 97,000 ng/L. In the case of Foam 2, the combined PFOS and PFOA concentration (98,200 ng/L) is 1,400 times greater than the EPA HAL for PFOS and PFOA combined (70 ng/L) in drinking water. Foams 6-8 and Background Foam all had PFOS > LOQ (Table 1) but at concentrations an order of magnitude lower than Foams 1-5. Bulk Water 1-4 and Background Bulk Water all had PFHxS and PFOS at comparable concentrations, while only Bulk Water 8 across from the AFFF-impacted side of the lake only had PFOS (**Table 4**).

Table 4. Concentrations of dissolved organic carbon (DOC; mg/L) and individual PFAS (ng/L) in foams and underlying bulk water (BW), branched: linear isomer ratio, and chromatographic retention time (Rt)[a-c]

Sample ID	DOC	PFHxA	PFOA	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS	PFNS	PFEtCHxS	FHxSA	EtFOSAA	SPr-FHxSA[c]	5:3 FTCA	6:2 FTS	8:2 FTS
Foam 1	**250**	<LOQ	**840 (8:92)**	**340 (9:91)**	**260 (5:95)**	**250 (8:92)**	**1,200 (7:93)**	**610 (13: 87)**	**32,000 (41:59)**	<LOQ	**340**	**950 (30:70)**	<LOQ	ND	ND	**830**	<LOQ
Foam 2	**240**	**140 (0:100)**	**1200 (7:93)**	**850 (7:93)**	**630 (5:95)**	**510 (9:91)**	**2,000 (6:94)**	**2,300 (12:88)**	**97,000 (49:51)**	**130 (70:30)**	**730**	**1,000 (29:71)**	<LOQ	ND	ND	**1300**	**100**
Foam 3	**330**	<LOQ	**1300 (7:93)**	**1500 (7:93)**	**960 (6:94)**	**660 (10:90)**	**1,700 (6:94)**	**2,800 (10:90)**	**68,000 (46:54)**	**130 (72:28)**	**560**	**1,100 (28:72)**	**130 (16:84)**	ND	ND	**1000**	<LOQ
Foam 4	**240**	<LOQ	**530 (6:94)**	**320 (6:94)**	**290 (5:95)**	**260 (8:92)**	**890 (7:93)**	**690 (12:88)**	**49,000 (41:59)**	<LOQ	**220**	**690 (36:64)**	<LOQ	**140**	ND	**770**	**130**
Foam 5	**260**	ND	**280 (10:90)**	**380 (11:89)**	**420 (12:88)**	**410 (9:91)**	**330 (14:86)**	**160 (13:87)**	**32,000 (49:51)**	<LOQ	<LOQ	<LOQ	**100 (21:79)**	<LOQ	ND	<LOQ	<LOQ
Foam 6	**250**	ND	ND	<LOQ	<LOQ	**110 (6:94)**	<LOQ	ND	**2,300 (43: 57)**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**190**	ND	ND
Foam 7	**230**	ND	ND	ND	<LOQ	<LOQ	<LOQ	ND	**2,300 (40:60)**	<LOQ	<LOQ	<LOQ	<LOQ	ND	ND	ND	ND
Foam 8	**210**	ND	ND	<LOQ	<LOQ	**190 (7:93)**	<LOQ	ND	**3,700 (40:60)**	<LOQ	<LOQ	<LOQ	<LOQ	ND	ND	ND	ND
Background Foam	**260**	ND	<LOQ	**130 (0:100)**	**420 (4:96)**	**340 (16:84)**	ND	ND	**1500 (34:66)**	ND	ND	ND	ND	ND	ND	ND	ND
BW 1	**12**	**13 (0:100)**	**15 (5:95)**	ND	ND	ND	**46 (15:85)**	<LOQ	**36 (56:44)**	ND	<LOQ	<LOQ	ND	ND	ND	**24**	ND
BW 2	**16**	**14 (0:100)**	**13 (7:93)**	ND	ND	ND	**52 (16:84)**	<LOQ	**43 (59:41)**	ND	<LOQ	<LOQ	ND	ND	ND	**15**	ND
BW 3	**15**	<LOQ	<LOQ	ND	ND	ND	**27 (27:73)**	<LOQ	**24 (65:35)**	ND	ND	ND	ND	ND	ND	<LOQ	ND
BW 4	**15**	**15 (0:100)**	**18 (6:94)**	ND	ND	ND	**59 (13:87)**	<LOQ	**51 (60:40)**	ND	<LOQ	<LOQ	ND	ND	ND	**45**	ND
BW 5	**21**	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND
BW 6	**14**	ND	<LOQ	ND	ND	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BW 7	**14**	ND	<LOQ	ND	ND	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BW 8	**15**	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	**13 (55:45)**	ND	ND	ND	ND	ND	ND	ND	ND
Background BW	--[d]	ND	<LOQ	ND	ND	ND	**17 (0:100)**	ND	**28 (67:33)**	ND	ND	ND	ND	ND	ND	ND	ND

[a]LOQ for each analyte and matrix is found in Table S7. [b]ND was defined as below the limit of detection (LOD) which was defined as 1/3 LOQ. [c]Analyte found in suspect list at confidence level of 2. [d]Insufficient sample was available, so no DOC analysis.

Other ECF-derived PFAS quantified in Foams 1-5 included perfloroethylcyclohexane sulfonate (PFEtCHxS), perfluoro-1-hexanesulfonamide (FHxSA), Ethylperfluorooctane sulfonic amido acetic acid (EtFOSAA), and N-sulfo propyl perfluorohexane sulfonamide (SPr-FHxSA) (**Table 4**). PFEtCHxS was originally reported in all five Great Lakes and associated with hydraulic fluids and wastewater treatment plant effluents (De Silva et al., 2011). The C6 sulfonamide, FHxSA, was first reported in AFFF-impacted groundwater (McGuire et al., 2014) and in urban waters impacted by AFFF, although at much lower concentrations (D'Agostino et al,. 2017). The sulfonamide-based precursor (SPr-FHxSA), a suspect PFAS, was found only in Foam 4. While this precursor was first reported in 3M AFFF (Barzen-Hanson et al., 2017) (and recently in AFFF-impacted soil (Nickerson et al., 2020), to the best of our knowledge it is the first report of this precursor associated with foam. In the case of EtFOSAA, it is typically associated with soil and sediment (Benskin et al., 2013; Houtz et al., 2013; Sepulvado et al., 2011) and not surface waters. Of the ECF-based PFAS in foam, only PFHxS and PFOS were detected in underlying bulk water (Bulk Water 1-4; **Table 4**). Each of these ECF-based PFAS were only observed in foam and not in surface water. Thus, foam appears to enrich in hydrophobic PFAS and its analysis provides for a sensitive detection of PFAS that may be at or below detection in bulk water, allowing for a more comprehensive site assessment. Although foam is being considered as a remedial tool for recovering PFAS from water, (Ebersbach et al., 2016; Lee et al., 2017; Meng et al., 2018) it is unclear if collection of foam is a practical, cost-effective means for removing PFAS from large freshwater lakes.

The 6:2 fluorotelomer sulfonate (FTS) was detected in Foams 1-5, while the 8:2 FTS was found above the LOQ in only Foams 2 and 4 (Table 1). The 6:2 FTS was detected in three underlying bulk waters but not the more hydrophobic 8:2 FTS. The detection of FTSAs and predominantly linear PFCAs in foams near known PFAS plumes (Figure 1) is consistent with groundwater data that indicates fluorotelomer-based AFFFs were used at the site (Rodowa et al,. 2020b). Only one observation of the fluorotelomer-derived 5:3 FTCA was recorded for Foam 6.

Enrichment factors (foam:bulk water) ranged from 10 (PFHxA) up to 2,830 (PFOS). Surface water foams impacted by AFFF gave the greatest concentrations and number of PFAS classes with PFOS concentrations exceeding the EPA health advisory level (70 ng/L). PFAS concentrations were significantly below published critical micelle concentrations and constituted <0.1% of overall DOC concentrations in foam, indicating that PFAS are a minor fraction of DOC and that DOC likely plays a central role in foam formation. Estimates indicate that foam ingestion is a potentially important route of exposure for children and adults to longer chain PFAS when they are in surface waters where foam is present (**Table 5**).

Table 5. Preliminary estimates of exposure (ng/k-day) and risk (hazard quotient, unitless) to PFAS from daily incidental ingestion of foam and bulk water, geometric mean (max)

	Exposure		Hazard Quotient	
Age	Foam	Bulk Water	Foam	Bulk Water
1 to <2	4.9 (70)	0.42 (3.0)	**2.4 (35)**	0.21 (**1.5**)
2 to <3	4.7 (92)	0.41 (4.0)	**2.4 (46)**	0.21 (**2.0**)
3 to <6	2.6 (47)	0.23 (2.0)	**1.3 (23)**	0.11 (**1.0**)
6 to <11	1.8 (25)	0.15 (1.1)	0.88 (**13**)	0.08 (0.54)
11 to <16	1.1 (22)	0.10 (0.96)	0.57 (**11**)	0.05 (0.48)
16 to <21	0.68 (12)	0.06 (0.53)	0.34 (**6.1**)	0.03 (0.26)
21+	0.51 (9.5)	0.04 (0.41)	0.26 (**4.7**)	0.02 (0.21)

Additional experiments were conducted using natural waters to assess TOC and PFAS accumulation near the water surface. Nine natural waters were collected and studied from two regions outside of Seattle, Washington and Wurtsmith Air Force Base, Michigan and amended with PFAS. Systems were agitated – however, foams were unstable and collapsed within 2 minutes. Thus, samples did not include the collection of any foam. Results of the measured PFAS distribution between the surface microlayer and bulk water phases are summarized in **Figure 19**. Note: equilibrium was attained in 24 hours. The trend and magnitude of measured EF values are consistent with measurements in electrolyte solutions. Thus, for the natural waters used in this study, the profile of organics present in the natural waters did not enhance PFAS accumulation near the water surface.

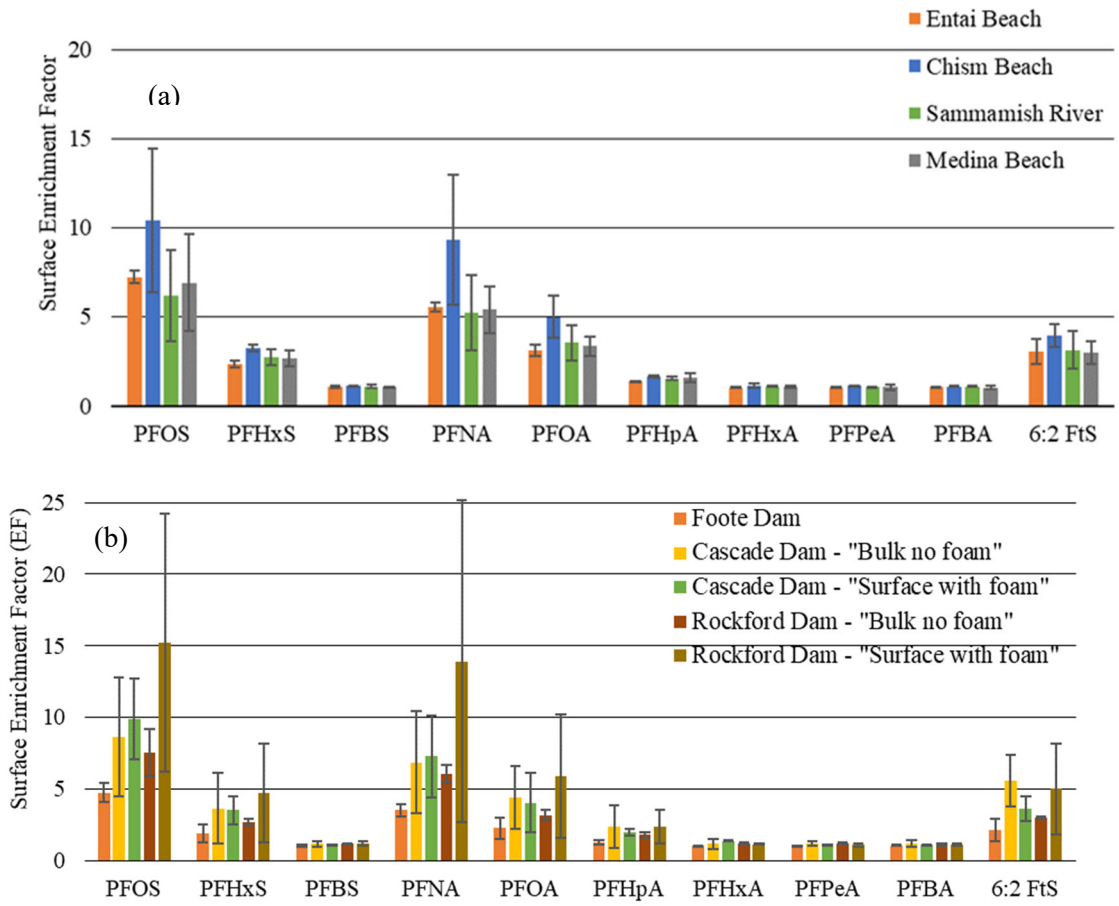

Figure 19. PFAS surface enrichment factor in PFAS-amended natural waters collected from (a) the outskirts of Seattle, Washington and (b) area around Wurtsmith Air Force Base, Michigan

AFFF-impacted natural foams, previously collected by Oregon State University from lakes near Wurtsmith Air Force Base, were shipped (as collapsed liquids) to CDM Smith's laboratory to further assess PFAS stratification. Upon receipt, condensed foam was apportioned into two glass bottles (i.e., duplicate experiments), filled with 15 mL each of the condensed foam samples (**Figure 20**). Bottle contents were stirred (using a stir bar) at constant rotation for four days such that the condensed foam was partially "re-foamed". Foam was separated from the liquid and a sample of the bulk liquid was collected using a glass pipette, placed into a 15- mL conical tube, and centrifuged for 5 minutes at 10,000 g. The supernatant was collected from each of the two replicate samples and shipped to Oregon State University for PFAS analysis.

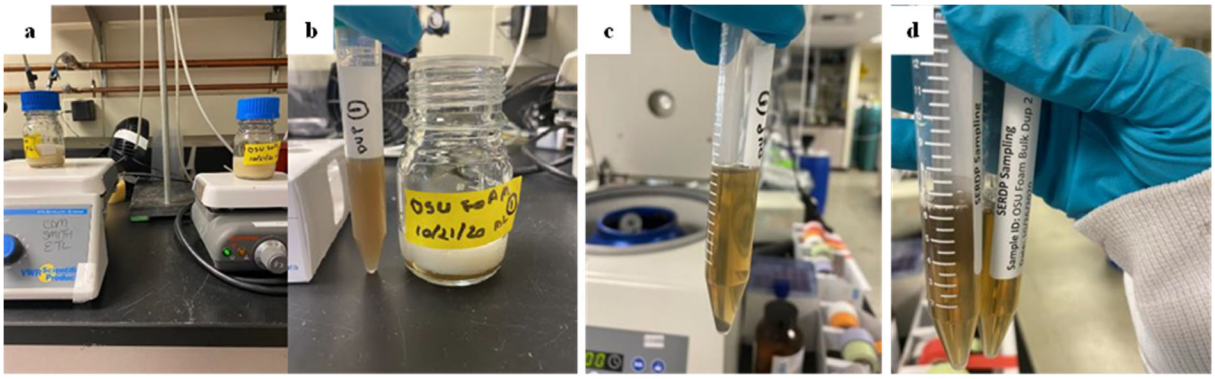

Figure 20. Natural waters foam experiment (a) original foam sample with no dilution on a stir plate, (b) separation of bulk liquid from foam, and (c) separation of solids from liquid after centrifuging for 5 min at 10,000 g, and (d) supernatant shipped to OSU for PFAS analysis

PFAS concentrations in foam and condensed foam are summarized in **Table 4**. PFAS partitioning between the condensed phase and the foam phase was minimal. This suggests that the foam itself (with its relatively high air-water surface area) is not the primary cause for the elevated PFAS surface enrichment factors (Schwichtenberg et al. 2020) but rather that organic-rich liquid at the water surface likely serves as a sink for PFAS. PFAS concentrations in this study were similar to (i.e., within a factor of 4 of) PFAS concentrations measured at Oregon State University in other bulk condensed foams.

Table 6. PFAA results in Wurtsmith Air Force Base foam and bulk solution—no dilution[1]

Sample ID	PFOA	PFNA	PFDA	PFUdA	PFHxS	PFHpS	PFOS	PFEtCHxS	FHxSA	6:2 FTS
Foam composite	421	338	337	292	592	518	23,700	163	417	385
Condensed Foam[2]	410	160	95	73	840	165	24,000	79.5	245	275
Surface Enrichment Factor	1.03	2.11	3.55	3.99	0.7	3.14	0.99	2.05	1.7	1.4

[1] PFAS below laboratory quantification limits were not presented in this table

[2] Average result from the duplicate experiment performed by CDM Smith

The same experiment was repeated after diluting the AFFF-impacted natural foam condensate by 50% using low-salinity (NaCl = 0.2 g/L) water. This was done to assess whether natural waters would readily form foam. Both bulk and foam samples were shipped to OSU for PFAS analysis.

Results are shown in **Table 7**, along with calculated EFs. EFs increased substantially from those listed in **Table 6**, indicating that dilution of the organic-rich layer facilitated PFAS enrichment in the foam phase. This finding was consistent with a mechanism for surface PFAS enrichment that is dependent upon an organic-rich microlayer, whether the microlayer is agitated to form an actual foam or not. As the organic-rich layer becomes diluted (as it would with depth in the water column), PFAS enrichment within the organic-rich foam and the underlying condensed phase would increase. This would result in observed stratification in PFAS concentrations between the foam and underlying liquid phase.

Table 7. PFAAs results in Wurtsmith Air Force Base foam and bulk solution—50% low salinity water dilution[1]

Sample ID	PFOA	PFHxS	PFOS	FHxSA
Foam	595 ± 93	750 ± 35	89,500 ± 27,217	1,050 ± 88
Condensed Foam	330 ± 70	590 ± 17	12,300 ± 6,497	435 ± 132
Surface Enrichment Factor	1.8	1.3	7.3	2.4

[1] PFAS below laboratory quantification limits were not presented in this table. Results include 95 percent confidence based on duplicate sample results.

TOC content was evaluated in Wurtsmith Air Force Base samples for both the condensed phase and foam (aka surface samples). As expected, TOC concentrations were enriched in surface samples relative to bulk water samples (**Figure 21**). TOC concentrations in natural lake waters were similar to those measured in synthetic waters (using saponin to simulate a surface microlayer), after accounting for the 50% dilution of the lake water.

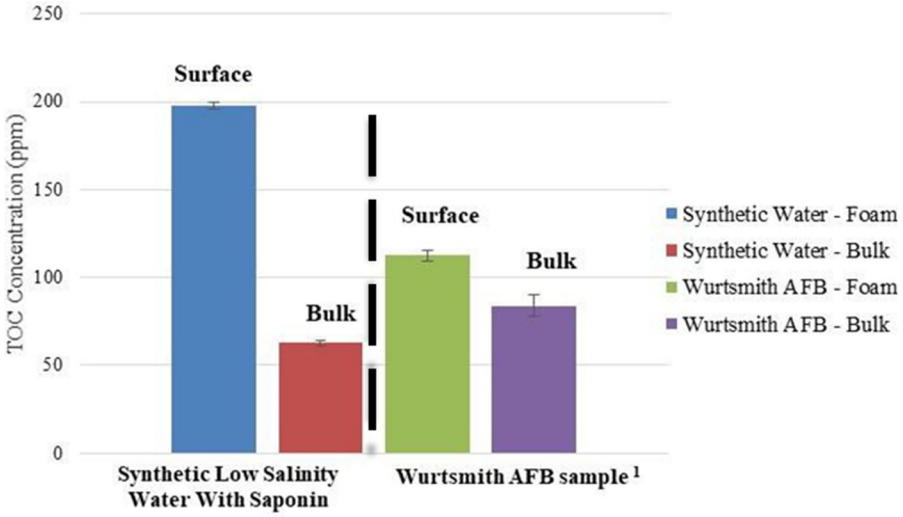

Figure 21. TOC results from synthetic freshwater experiments and Wurtsmith Air Force Base natural lake samples diluted 50% with low-salinity water. Dashed lines separate distinct test experiments

2.2.4. Surface microlayer sampling techniques (Task 1.4)

The screen sampling method described in Section 2.2.2.1 was not suitable for sampling a dynamic surface under field conditions (e.g., waves), as also seen in the laboratory experiments under Task 1.2. Therefore, additional experiments were conducted to evaluate surface microlayer sampling techniques based on a glass plate or microscope slide method (See Appendix A Schwichtenberg et al., 2023 for Supplemental Data associated with this section).

Surface microlayer sampling was conducted at a field site to generate data that could be used to compare results from the large glass plate method and the microscope slide approaches. Samples were analyzed by LC-QTOF and analyzed for 50 target PFAS. One objective was to determine if the two surface microlayer sampling approaches provided significantly different concentrations

for the target PFAS. Both methods yielded similar surface microlayer PFAS concentrations (**Table 9**). The precision of the surface microlayer (SML) sampling methods, which included spatial variability, ranged from 3-23% for PFOA and 24-31% for PFOS. Collection using a single microscope slide sampled less SML and thus collected less PFAS mass, such that this sampling technique offered limited sensitivity. In contrast, using three glass slides to collect a greater volume of SML offered greater sensitivity, yet similar precision as a single slide. Glass slides and glass plates both collected a SML thickness of 100 mM (Cunliffe et al., 2009). Sample results for bulk water at 3 and 30 cm below the surface water were not statistically different, which suggested that bulk water was well mixed.

Table 8. Average PFAS concentrations in SML by large glass plate, single microscope slide, and three microscope slides

	PFOA (ng/L)	PFOS (ng/L)
Glass Plate	220	9,900
Microscope Slide (1)	280	15,000
Microscope Slide (3)	200	14,800
Bulk Water (3 cm)	130	1,000
Bulk Water (30 cm)	120	930

Although the SML PFAS concentrations were significantly greater than bulk water in the case of PFOS (**Table 8**), EFs calculated from the SML and underlying bulk water data yielded values that were significantly lower than foam EFs, which ranged up to 4,000 (**Table 7**; Schwichtenberg et al., 2020). The EFs determined for PFOS were in good agreement with the laboratory lake model (EF for PFOS of 8). The EF for PFNA was greater in the model system compared to the measured value of 2.3 (**Table 9**). Most PFAS gave EF values < 2 which agreed well with laboratory data (**Table 1**). In general, EFs increased with retention time, which is a proxy for hydrophobicity.

Table 9. EFs calculated from measured SML and bulk PFAS concentrations using a large glass plate and three microscope slides

Increasing Hydrophobicity

Enrichment Factors	Foam	SML (glass plate)	SML (3 slides)
PFBA	~	1.1	~
PFPeA	~	0.9	~
PFHxA	~	1.3	~
FBSA	~	0.9	~
PFHpA	~	0.7	~
PFHxS	27	1.4	1.5
5:3 FTCA	~	1.4	~
6:2 FTS	46	1.4	~
PFOA	59	1.6	2.1
PFHpS	~	2.7	~
FHxSA	~	1.3	~
PFOS	1558	9.3	14.8
PFNA	~	2.3	~
8:2 FTS	~	13.8	~
FOSA	~	13.5	~

~ Indicates bulk and/or SML concentrations were <LOD

The SML concentration was used together with bulk water concentrations, assuming a SML thickness of 100 mM, to examine the agreement with published air-water partition coefficients (K_i) (Cuniff et al., 2013). Using the PFOS concentration in the SML (9,900 ng/L) and a bulk water concentration of 1,000 ng/L, an estimate K_i of 0.099 cm is obtained which compares well with the reported value of 0.1 cm (Cuniff et al., 2013). The good agreement indicates that the SML is governed by air-water partitioning and can be described as a 2-D interface and not a 3-D interface. The SML has a much lower interfacial area compared to foam and results in a much lower measured concentration than in foam (Schwichtenberg et al., 2020). Three SML sampling techniques were deployed at 10 sites around a freshwater system with some locations significantly impacted by AFFF (Section 2.2.5). Techniques included a large glass plate, a single glass microscope slide, and a combination of three glass microscope slides. Bulk water was also obtained with a pipette, at two separate depths of 3 and 30 cm. Data from two underlying depths offers insight into the distribution of PFAS below the SML. All samples were analyzed by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF) for 50 target PFAS. Accuracy, precision, limits of detection (LOD) and quantification (LOQ) were determined to support recommendation of optimal field SML sampling techniques. The PFAS concentrations in the SML were ratioed to the underlying concentration of PFAS in bulk water to obtain EFs. The average EFs obtained by the three SML sampling methods were compared to EFs obtained from open marine water, sea spray aerosols, and freshwater foam. Finally, PFAS concentrations in the SML and bulk water were used to estimate K_i values, with comparison to Ki values predicted based on the Gibbs Adsorption Equation.

EFs were calculated by dividing the SML PFAS concentration obtained from the glass plate by the bulk water concentrations from a depth of 3 cm. Average EFs were calculated for 15 PFAS for Sites 8-10 (**Figure 22**) and ranged from 1±12 with individual EFs up to 20 for FOSA.

Enrichment factors increased with increasing chromatographic retention time, a proxy for hydrophobicity, with the highest EFs for 8:2 FTS and FOSA (**Figure 22**).

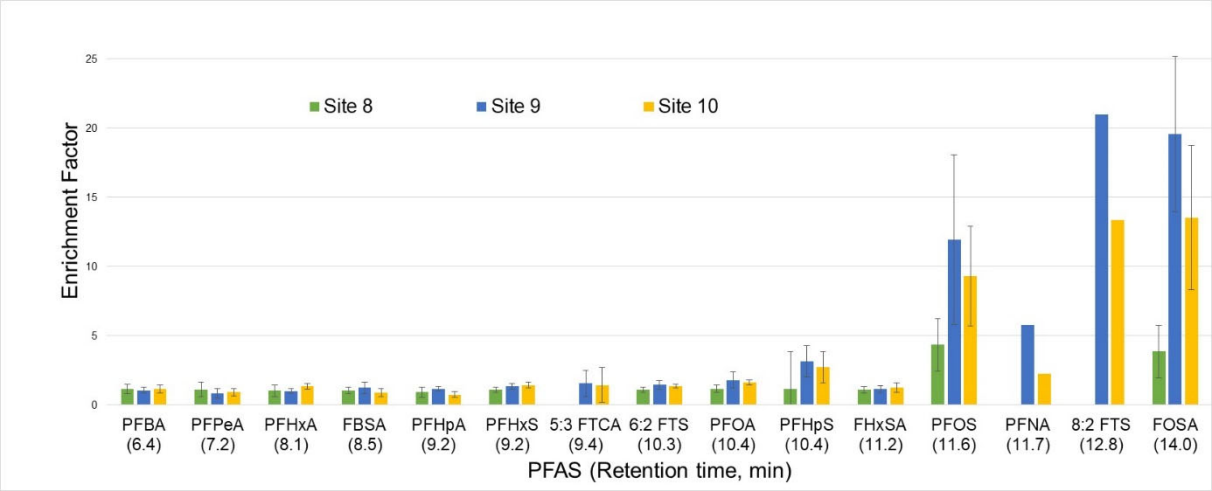

Figure 22. Average EFs for PFAS at Sites 8-10 (Error bars = propagated error of 95% confidence interval)

Note in **Figure 22**, various PFAS on x-axis are ordered by increasing hydrophobicity (see Table S5 for EF values and 95% CIs). The 95CI was not computed for PFNA or 8:2 FTS since each only had two EF values Sites 9 and 10, which were co-located, gave higher EFs for PFHxS and more hydrophobic PFAS than at Site 8 (**Figure 22**). The lower EFs for Site 8 are due to lower SML concentrations and not bulk water concentrations. Sites 9 and 10 are located in a marsh and are impacted by anoxic groundwater discharging from a landfill an AFFF plume. Lower dissolved oxygen concentrations at Sites 9 and 10 (5 mg/L) compared to Site 8 (6 mg/L) potentially indicates that aeration and mixing may be occurring as water flows from Sites 9 and 10 into Site 8.

The field-derived concentrations of PFAS in SML and bulk water provide an opportunity to calculate air-water partitioning coefficients for PFOS, PFNA, 8:2 FTS, and FOSA, which gave EFs greater than 1 (**Table 9**).

Table 10. Ki values (m) generated from field data for an average SML thickness of 75 µm. FOSA was <LOD in the bulk water for Site 7 so no Ki value was calculated

PFAS	Retention time (min)	Site				
		7	8	9	10	Avg Ki (m)
PFOS	11.6	$4.7x10^{-4}$	$3.3 x10^{-4}$	$8.9 x10^{-4}$	$6.75 x10^{-4}$	$5.9 x10^{-4}$
PFNA	11.7	ND	ND	$4.3x10^{-4}$	$1.7x10^{-4}$	$3.0x10^{-4}$
8:2 FTS	12.8	ND	ND	$1.6x10^{-3}$	$1.0x10^{-3}$	$1.3x10^{-3}$
FOSA	14.0	ND	$2.9 x10^{-4}$	$1.5 x10^{-3}$	$1.0 x10^{-3}$	$9.2 x10^{-4}$

The field-measured Ki values for PFOS (**Table 10**) were the only values that could be compared to laboratory Ki values fitted with a Freundlich and Langmuir models and the Ki values for PFOS were within an order of magnitude of model predictions (**Figure 23**).

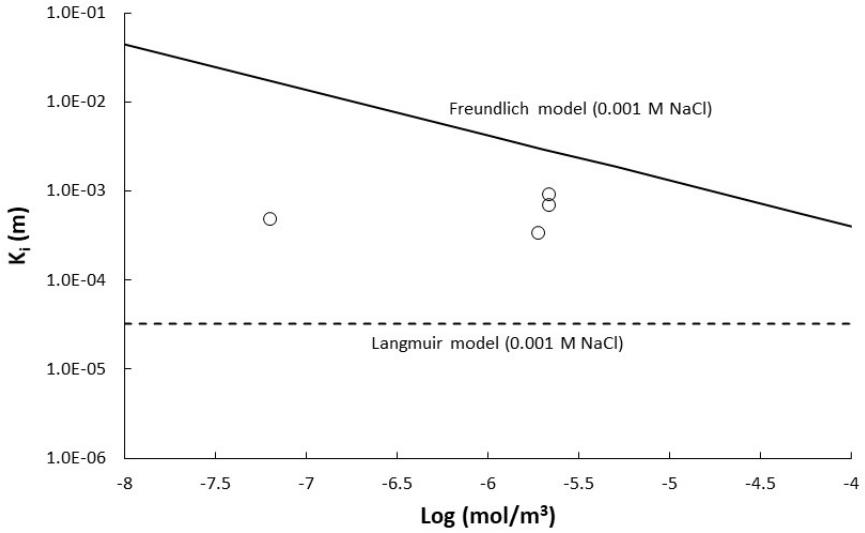

Figure 23. Air-water partition coefficients (Ki) for PFOS calculated from measured PFOS concentrations in surface microlayer and underlying bulk water at Sites 7-10 and plotted on Freundlich and Langmuir fits of laboratory-based film measurements

2.2.5. Field evaluation of surface water sampling techniques (Task 1.5)

This section presents a summary of field study objectives, design, and findings. The field study of surface water sampling techniques is separately described in a manuscript that has been accepted for peer-reviewed publication (Roark et al., 2024). Supplemental tables and figures not included in this final report (Appendix A, Roark et al., 2024) are provided in the publication to provide additional detail on statistical analyses (Roark et al., 2024).

2.2.5.1. Study Objectives and Design

The primary objective of this task was to determine if inclusion of the SML in bulk surface water samples resulted in a high bias in measured PFAS concentrations. The project team first performed

a pilot study at two field sites to assess field sampling and analytical variability and inform the design of the full field study of sampling methods at 11 different surface water bodies (**Table 11**). A secondary objective of this task was to determine EFs from the measured PFAS concentrations in the SML and bulk water. Samples were collected from 11 natural water bodies with varying water chemistry across a large geographic range. General water quality parameters were measured in the field and samples were also collected for dissolved organic carbon (DOC). Samples were then analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) and the data were evaluated with a linear mixed effects model.

Table 11. Field site geographies

Site	Geography
1	Northeast Michigan
2	Northeast Michigan
3	Cape Cod Peninsula Massachusetts
4	Cape Cod Peninsula Massachusetts
5	Cape Cod Peninsula Massachusetts
6	Cape Cod Peninsula Massachusetts
7	Central Maryland, West Shore of Chesapeake Bay
8	Orange County California
9	Northeast Michigan [a]
10	Northeast Michigan [a]
11	Northern Alaska Peninsula

Note
a - Separate locations on a single large water body

The pilot study was conducted at two sites (Sites 2 and 9) with low and high PFAS concentrations, approximately 12 ng/L and 1200 ng/L PFOS, respectively. Replicate bulk water samples were obtained using the peristaltic pump with tubing (n=3) (**Table 12**), fully submerged bottle (n=3), and partially submerged bottle (n=5) sampling methods, approximately 1 meter apart to capture the small-scale spatial variation in PFAS concentrations. In addition, a composite sample was created by obtaining five samples using the partially submerged bottle method, combining them in a 3.78-L food-grade PFAS-free HDPE container, and then separating the composite sample into five 250-mL bottles, for a total of five replicates, in order to capture only analytical variability in PFAS concentration. In addition, SML samples (n=5) were also collected at each site using glass plate samplers. Given the replication inherent to the sampling, no additional quality control samples were collected.

Table 12. Peristaltic pump equipment and materials used for bulk surface water sampling

Site	Peristaltic Pump	Water Quality Meter	Turbidimeter	Tubing
1	Geotech Peristaltic Pump	YSI ProDSS Multiparameter	YSI ProDSS Multiparameter	Low density polyethylene (LDPE) tubing and Masterflex®
2	Geotech Peristaltic Pump	YSI ProDSS Multiparameter	YSI ProDSS Multiparameter	LDPE tubing and Masterflex®
3	Master Flex 12 VDC Powered Drive	YSI Professional Plus	Scientific Inc MicroPi	ARGOS HDPE
4	Master Flex 12 VDC Powered Drive	YSI Professional Plus	Scientific Inc MicroPi	ARGOS HDPE
5	Master Flex 12 VDC Powered Drive	YSI Professional Plus	Scientific Inc MicroPi	ARGOS HDPE
6	Master Flex 12 VDC Powered Drive	YSI Professional Plus	Scientific Inc MicroPi	ARGOS HDPE
7	Geotech Peristaltic Pump	Horiba U-52	Horiba U-52	C-Flex Tubing Unlined HDPE tubing
8	Geotech Peristaltic Pump	YSI 556 Multi Probe System	YSI 556 Multi Probe System	C-Flex Tubing Unlined HDPE tubing
9	Geotech Peristaltic Pump	YSI ProDSS Multiparameter	YSI ProDSS Multiparameter	LDPE tubing and Masterflex®
10	Geotech Peristaltic Pump	YSI ProDSS Multiparameter	YSI ProDSS Multiparameter	LDPE tubing and Masterflex®
11	Alexis Peristaltic Pump	YSI 556 Multi Probe System	YSI 556 Multi Probe System	Masterflex L/S High-Performance Precision Pump Tubing

For the full investigation, 11 water bodies (including the two used in the pilot study) across the United States (U.S.) were selected based on the following criteria: historical data demonstrating presence of PFAS contamination, the absence of significant flow, water at least 30 cm deep, geographic differences, varying water chemistry, and accessibility (**Table 13**). Single samples of bulk water samples were collected at the 11 sites using the peristaltic pump with tubing and the fully submerged and partially submerged bottle methods along with a single SML sample obtained using a glass plate. General water quality parameters were measured using the peristaltic pump prior to PFAS sample collection.

At Sites 1, 2, 9, and 10, a field blank was collected by transferring laboratory-issued, PFAS-free water from one 250-mL HDPE bottle to another. In addition, a trip blank consisting of a laboratory-issued 250-mL HDPE bottle containing PFAS-free water that remained unopened the entire time. In all cases, PFAS concentrations in field and trip blanks were less than the method detection limit (MDL).

Sampling by peristaltic pump with tubing (**Table 13**) was accomplished using LDPE tubing (Sites 1, 2, 9, and 10) or HDPE tubing (Sites 3 through 8 and 11) except for a section of silicone or C-Flex® tubing that was inserted in the peristaltic pump head (**Table 13**). No tubing was reused between sampling sites. Although LDPE tubing is not recommended for use during PFAS sampling (Deeb et al., 2021), equipment blanks showed no evidence of contamination. A water quality meter and a turbidity meter (**Table 13**) were placed in line for initial water quality parameter recording but were removed prior to collecting the bulk surface water sample. Water

quality parameters included dissolved oxygen, turbidity, specific conductance, temperature, and pH. The field equipment blank for the peristaltic pump with tubing method consisted of pumping laboratory-issued, PFAS-free water from one 250-mL HDPE bottle to another. All equipment blanks gave target PFAS concentrations <MDL.

Table 13. Site general water quality chemistry parameters, and DOC concentrations in surface (bulk) water and surface microlayer

Site	Bulk Surface Water						Bulk Surface Water DOC			Surface Microlayer DOC		
	pH	Dissolved Oxygen (mg/L)	Turbidity (NTU)	Temperature (°C)	ORP (mV)	Specific Conductivity (mS/cm)	Mean (mg/L)	Std Dev (mg/L)	Sample Size	Mean (mg/L)	Std Dev (mg/L)	Sample Size
1	8.84	12.43	2.63	2.3	202.2	0.297	10.07		1	NA	NA	0
2	7.07	12.65	30.99	4.8	230.5	0.345	9.56	3.05	6	6.90 [a]	1.02	3
3	7.19	11.73	13.65	7.1	160.3	153.1	0.363	0.122	3	0.176	NA	1
4	7.82	9.20	15.67	8.60	86.6	145.3	0.758	0.082	3	1.11	NA	1
5	8.4	8.2	9.46	13.6	245.6	63.4	4.64	0.579	3	4.74	NA	1
6	5.79	10.97	57.77	12.7	197.1	82.7	4.12	3.01	3	7.16	NA	1
7	5.78	8.62	0.90	11.92	306.0	0.87	17.2	14.5	3	11.5	NA	1
8	8.77	11.44	--	21.9	109.3	569.0	4.45	0.422	3	2.64	NA	1
9	8.01	14.21	4.76	2.8	307.3	0.26	12 [b]	2.6 [b]	1	NA	NA	0
10	7.98	13.75	4.74	2.8	305.3	0.262	12 [b]	2.6 [b]	1	NA	NA	0
11	6.21	1.53	4.82	4.70	203.1	0.13	6.635	0.983	2	8.28	NA	1

Notes
a - n=4 replicates
b - mean and standard deviation of 9 samples around lake shoreline as reported by (Schwichtenberg 2023a)
NA – not analyzed

The fully submerged bottle method consisted of submerging a capped 250-mL HDPE bottle so the opening was 10 to 15 cm below the water surface. The cap was then removed and the bottle was allowed to fill with water. The cap was replaced and the sample bottle was removed from the water. The partially submerged bottle method was conducted by opening a 250-mL HDPE sample bottle, submerging the bottle at an angle so that approximately 25 to 50 percent of the bottle mouth was below the water surface, allowing the bottle to fill with water that included the SML. Equipment blanks for the fully and partially submerged bottle method were collected by passing PFAS-free water from one 250 mL bottle to another in the field; all gave target PFAS concentrations <MDL.

SML samples were collected using 10 cm x 10 cm x 10 cm x 5 mm glass plates fabricated at Smith Glass (Corvallis, Oregon). Prior to use, each glass plate was rinsed using Alconox or Liquinox soap followed by certified PFAS-free water. New glass plates were used at Sites 1, 2, 9, and 10, and two sets of glass plates were reused among the remaining sites. When plates were reused, they were cleaned by rinsing with Alconox or Liquinox followed by triple rinsing with PFAS-free water. To collect an SML sample, a spring clamp was attached to the top of the glass plate and the plate dipped into the water three times to rinse and wet the surface of the plate. The SML sample was then collected by dipping the plate vertically into the water, leaving approximately 2.5 cm of the plate above the water to avoid wetting the clamp. The plate was then withdrawn from the water at approximately 10 cm/min and the bulk water was allowed to drain from the plate. A silicone scraper was then used to scrape water adhered to the plate into a 15-mL polyethylene centrifuge tube. The sample collection steps were then repeated as many times as necessary to fill two 15-mL HDPE centrifuge tubes. The equipment blank consisted of pouring PFAS-free water from a 250 mL HDPE bottle into a 15-ml HDPE centrifuge tube; all gave target PFAS <MDL. To verify that the rinsing of plates did not result in PFAS detections, a decontaminated glass plate was soaked in PFAS-free water for 24 hours and the water was then tested for PFAS; all target PFAS were <MDL.

All samples were placed immediately upon ice, shipped to the laboratory on ice, refrigerated at 4 degrees Celsius until analysis, and analyzed within two weeks of receipt. All samples were analyzed for a select list of target PFAS (Roark et al., 2024 Table S-1) using extracted stable-isotope labeled standards by isotope dilution and liquid chromatography tandem mass spectrometry by a laboratory (Vista Laboratory, now Enthaply Analytical EDH). The laboratory performed a matrix spike and matrix spike duplicate on a sample to check for the presence of matrix interference by adding known concentrations of native PFAS standards. By verifying the increase in concentration is proportional to the concentration spiked, the sample was verified as being free of matrix interference. Additionally, the spiked sample was performed in duplicate to establish a relative percent difference (RPD) to verify the lab's precision. Dissolved organic carbon analyses were performed after filtering first through a Whatman no. 1 paper filter followed by analysis on a Schimadzu TOC-Vcph/cpn (Kyoto, Japan) total organic carbon analyzer.

For the pilot study, PFAS included in the statistical analyses for each site (Sites 2 and 9) were limited to those with 100% detection frequency (>MDL). For the full investigation, sites included for each PFAS were limited to those with 100% detection frequency in each of the three bulk water sampling methods. For EF calculations for each PFAS, sites included were limited to those with detected results for the respective PFAS in the SML and at least one bulk water method sample.

The arithmetic mean of bulk water samples was used in the EF calculations, and included results for each sampling method with non-detects, if present, represented by the MDL.

For the pilot study, full investigation, and EF evaluation, general statistical analyses (median, mean, standard deviation, coefficient of variation, correlations, ratios, etc.) were performed using base R (R Core Team 2022). Data manipulation, graphical analyses, and creation of figures were performed using the *tidyverse* package (Wickham H et al., 2019). Hypothesis tests, Shapiro-Wilk normality test, analysis of variance, and the Kruskal Wallis rank sum test used for comparisons of concentrations among sampling methods were also performed using base R. Levene's test, used for comparisons of variance of concentrations among sampling methods, was performed using the *car* package (J. Fox & S. Weisberg, 2019). Dunn's test of multiple comparisons from the *rstatix* package (Kassambra, 2023) was used as a nonparametric post-hoc test following the test.

For the repeated measures design for the full investigation, sites represent the unit of replication, and the bulk water sampling methods represent the treatments. Differences among sampling methods were evaluated for each PFAS compound across sites using a linear mixed effects model, with sampling method as a fixed effect and site as a random effect. The model partitions variance among fixed and random effects and determines whether levels of random effects are significant (with $\alpha = 0.05$ for each PFAS) predictors of PFAS concentration. The mixed effects model was implemented using the *lme4* package (Bates et al., 2015) with each method having a random intercept and fixed slope, with the mean and standard deviation of the intercepts as parameters estimated in the model. The developers of *lme4* opted not to include p-values in the output of the linear mixed effects models because estimating p-values is complex and controversial (Bates et al., 2015). Thus, the package *lmerTest* (Kuznetsova et al., 2017) was used here to generate p-values for the overall model and the fixed effects, but per Bates et al. (2015) potential limitations of the p-values should be recognized. Additional information on the statistical models is provided in the Supporting Information.

2.2.5.2. Results and Discussion

Results from the pilot study indicated that concentrations of 14 PFAS at Site 2 and 8 PFAS at Site 9 (**Table 14**, Roark et al., 2024 Table S-2) were detected in each bulk water sample using all three methods. Results were used to calculate summary statistics (mean, standard deviation, and coefficients of variation) (**Table 14**, Roark et al., 2024 Table S-2). Abbreviations, CAS numbers, and median MDL, limit of quantitation, and limit of detection for all target PFAS are presented in Roark et al., 2024 Table S-1.

Table 14. Concentrations of 14 PFAS in bulk surface water and the surface microlayer at 11 sites measured with multiple sampling methods

Site	Type	n	PFBA	PFPeA	PFBS	PFHxA	PFPeS	PFHpA	PFHxS	6:2 FTS	PFOA	PFHpS	PFOS	PFNA	8:2 FTS	PFOSA
1	PP	1	23.1	77.3	4.14	73.5	5.89	33.5	248	24.2	97.1	8.89	706	5.25	<1.02	49.6
	FS	1	22.2	74.5	3.87	69.9	5.67	32.2	243	19.7	94.8	9.07	686	4.80	<1.00	44.0
	PS	1	21.9	74.8	3.78	68	5.68	32.2	245	20.2	92.9	8.57	692	4.97	<1.15	48.4
	SML	1	28.2	79.4	<12.4	74.5	<12.4	36.7	273	22.8	111	<12.4	1070	<12.4	<12.4	77.0
2	PS	3	22.4	74.9	5.14	79.5	7.67	29.5	319	47.1	117	15.1	1037	8.98	5.23	15.8
	FS	3	22.4	73.7	5.20	77.8	7.12	30.6	328	46.5	113	14.8	1040	9.14	5.59	13.2
	PS	5	20.6	71.4	5.00	73.02	6.72	30.08	273	43.7	108	14.9	1294	8.62	6.20	9.14
	HPS	5	20.3	70.8	5.04	73.5	6.97	30.1	283	42.7	108	14.2	1162	8.43	5.59	7.64
	SML	3	24.9	73.4	<8.15	81.03	<8.24	32.4	363	58.3	155	27.9	5690	22.1	39.2	141
3	PP	1	6.54	14.4	3.75	16.5	6.92	11	73.3	2.01	28.4	1.93	59.7	5.21	<0.993	2.49
	FS	1	5.18	14.0	3.97	19.3	6.12	10.2	68.5	1.84	26.3	2.00	65.1	5.2	<0.961	2.41
	PS	1	5.17	14.0	3.01	18.7	5.5	9.95	65.0	1.53	26.4	2.00	66.8	4.73	<0.983	3.00
	SML	1	<17.4	19.2	<17.4	22.1	<17.4	<17.4	72.6	<17.4	27.6	<17.4	72.2	<17.4	<17.4	<17.4
4	PP	1	5.00	12.7	4.59	16.6	5.1	9.06	46.7	2.33	17.5	1.66	98.2	27.7	<0.993	1.28
	FS	1	5.10	14.6	5.26	14.9	4.36	9.40	55.7	3.64	17.6	1.86	88.8	24.8	<1.02	1.85
	PS	1	6.10	14.0	3.69	17.8	3.31	9.09	55.8	3.53	17.9	1.48	99.2	25.1	<1.02	1.56
	SML	1	<18.5	25.4	<18.5	19.3	18.5	<18.5	56.7	<18.5	<18.5	<18.5	121	32.8	<18.5	<18.5
5	PP	1	9.46	25.8	3.88	51.1	8.76	16.0	230	<0.979	28.7	1.68	77.9	7.66	<0.979	<0.979
	FS	1	8.21	19.2	4.09	61.1	5.12	14.0	223	<0.993	29.4	1.99	94.0	8.67	<0.993	<0.993
	PS	2	8.85	19.5	4.08	60.1	6.61	15.3	244	<0.984	31.1	1.83	87.4	8.86	<0.984	<0.984
	SML	1	<16.5	26.2	<16.5	71.1	16.5	<16.5	327	<16.5	57.9	<16.5	3320	67.7	16.5	264
6	PP	1	3.00	4.93	9.46	10.1	17.4	3.63	173	<0.964	6.8	3.45	505	1.32	<0.964	1.18
	FS	1	2.96	7.00	7.00	10.7	12.4	3.44	170	<0.999	6.82	3.33	687	1.87	<0.999	1.75
	PS	1	2.46	4.72	7.59	8.37	12.6	2.81	142	<0.994	5.18	3.39	585	1.39	<0.994	8.43

Site	Type	n	PFBA	PFPeA	PFBS	PFHxA	PFPeS	PFHpA	PFHxS	6:2 FTS	PFOA	PFHpS	PFOS	PFNA	8:2 FTS	PFOSA
	SML	1	<18.3	<18.3	<18.3	22.8	18.3	<18.3	253	<18.3	<18.3	<18.3	485	<18.3	<18.3	18.8
7	PP	1	76.1	219	31.0	291	41.0	192	1390	486	377	32.7	2270	413	307	30.2
	FS	1	67.8	213	32.0	269	43.9	162	1370	448	388	32.2	2550	405	342	37.5
	PS	1	66.2	196	27.6	243	32.9	145	998	415	333	26.9	2310	366	349	35.4
	SML	1	76.5	241	37.3	330	45.7	170	1200	536	452	23.8	6250	564	1180	68.5
8	PP	2	6.13	12.1	5.28	12.8	<1.06	4.07	4.20	<1.00	13.8	<1.00	52.1	3.69	<1.00	4.14
	FS	1	6.38	12.9	6.55	13.5	<1.01	3.88	4.28	<1.01	12.6	<1.01	24.4	1.96	<1.01	4.13
	PS	1	7.10	11.6	5.09	12.2	<0.983	3.66	4.07	<0.983	13.3	<0.983	32.2	2.37	<0.983	4.83
	SML	1	<17.1	<17.1	<17.1	<17.1	<17.1	<17.1	<17.1	<17.1	<17.1	<17.1	36.5	<17.1	<17.1	<17.1
9	PP	3	2.23	2.77	<0.999	1.91	<0.999	1.36	8.19	<0.999	3.32	<0.999	13.6	<0.999	<0.999	12.9
	FS	3	2.33	2.41	<1.00	1.94	<1.00	1.30	8.57	<1.00	2.96	<1.00	11.7	<1.00	<1.00	8.50
	PS	5	1.84	1.94	<1.03	1.83	<1.03	1.28	7.77	<1.05	2.93	<1.04	13.5	<1.03	<1.03	11.1
	HPS	5	1.79	1.82	<1.03	1.88	<1.03	1.3	7.3	<1.03	2.90	<1.03	11.8	<1.03	<1.03	10.4
	SML	3	<9.69	<9.69	<9.69	<9.69	<9.69	<9.69	<9.69	<9.69	<9.69	<9.69	55.3	<9.69	<9.69	35.1
10	PP	1	1.89	2.20	<1.01	1.82	<1.01	1.11	6.53	1.06	2.49	<1.01	13.1	<1.01	<1.01	6.43
	FS	1	1.95	1.98	<1.04	1.72	<1.04	1.09	6.21	1.12	2.15	<1.04	14.1	<1.04	<1.04	6.55
	PS	1	1.79	2.19	<1.02	1.9	<1.02	1.07	6.54	1.25	2.49	<1.02	14.7	<1.02	<1.02	5.83
	SML	1	<7.48	<7.48	<7.48	<7.48	<7.48	<7.48	<7.48	<7.48	<7.48	<7.48	26	<7.48	<7.48	22.7
11	PP	1	4.79	16.3	3.01	22.0	5.61	9.75	81.5	45.5	16.8	16.0	1090	2.52	2.14	3.72
	FS	1	6.51	24.2	5.61	35.5	9.40	15.2	129	70.8	26.0	25.2	1980	3.61	3.56	5.21
	SML	1	<17.8	41.9	<17.8	72.9	26.6	39.1	586	365	154	359	53300	58.3	152	47.9

Notes:
All concentrations are shown in ng PFAS/L. PFAS abbreviations are provided in Table S-1 and standard deviations are presented in Table S-2.
"<" symbols indicated non-detected results reported at the method detection limit; if more than one sample, mean of MDLs or mean of MDLs and detected result(s)
Sample types include PP - Peristaltic pump, FS - Fully submerged sample bottle, PS - Partially submerged sample bottle, HPS - Homogenized partially submerged sample bottles (subsampled), and SML - Surface microlayer collected with glass plate sampler.

To evaluate the relative contribution of small-scale spatial variation and other potential sources of variation relative to analytical variation, replicate PFAS concentrations from the individual partially submerged bottle (spatial and analytical variation) and replicate composite partially submerged bottle (analytical variation only) methods were compared. The mean CVs among all PFAS for the individual replicates (12.3% and 11.3% for Sites 2 and 9) were greater than those for the composite replicates (4.75% and 8.41% for Site 2 and 9) (Roark et al., 2024 Table S-3 and Figure S-1). Similarly, most CVs for each PFAS at each site were greater for individual replicates than for composite replicates. Specifically, at Site 2, 13 of 14 PFAS had greater CVs for individual replicates than the composite replicates, and at Site 9, 7 of 8 PFAS had greater CVs for individual replicates than composite replicates (Roark et al., 2024 Table S-3). The difference between individual and composite replicates indicates that 42% (Site 2) to 75% (Site 9) of the observed variation was due to analytical variation, with the remainder attributable to spatial or other sampling variation. However, despite the evidence of consistently greater CVs for individual replicates than composite replicates (Roark et al., 2024 Table S-3), the Levene's test for homogeneity of variance (Roark et al., 2024 Table S-4) did not identify any significant differences in variation for any PFAS/site combination.

Across the 11 sites for the three bulk water sampling methods (peristaltic pump, fully submerged bottle, and partially submerged bottle), 26 PFAS were detected in at least one sample. Of those 26 PFAS, 14 were detected using each bulk water sampling method and were used in the analysis for the full investigation to address potential sampling bias (**Table 8**, Roark et al., 2024 Table S-2). At Site 11, the partially submerged method sample bottle leaked during transport, losing nearly half its volume and therefore was excluded from the sampling bias investigation.

PFAS concentrations in bulk water, on average across sites, were significantly related to sampling method for seven of 14 PFAS: PFBA, PFPeA, PFBS, PFPeS, PFHpA, PFHxS, and PFOA (α = 0.05) (**Figure 24**, Roark et al., 2024 Table S-5). For all seven PFAS with models indicating statistically significant differences in concentrations among methods, the partially submerged bottle method gave the lowest concentration. For five of these seven PFAS, the peristaltic pump method gave the greatest concentration. Six of the seven statistically significant results occur among the seven PFAS with the lowest chromatographic retention time (e.g., lower hydrophobicity) (**Figure 24**, Roark et al., 2024 Table S-5). Specific comparisons to evaluate differences among fully submerged bottle, partially submerged bottle, and peristaltic pump methods indicated that for six of 14 PFAS (PFBA, PFPeA, PFPeS, PFHpA, PFHxS, and PFOA) the partially submerged bottle concentrations were significantly less than peristaltic pump concentrations, and in only one case (PFPeS) was the fully submerged bottle concentration also significantly less than the peristaltic pump concentration (Roark et al., 2024 Table S-5). Setting aside the emphasis on statistical significance for each PFAS (recall the uncertainty associated with p-values on linear mixed effects models, Bates et al., 2015), we note that for 13 of the 14 PFAS (i.e., all except PFOS) the concentrations from the partially submerged method were less than that of the peristaltic pump method, and for 10 of 14 PFAS, concentrations from the fully submerged method were less than that of the peristaltic pump method (**Table 11**). These findings are generally inconsistent with the expectation and impetus for this study that bulk water PFAS concentrations might be biased high due to enriched PFAS in the SML being captured using the partially submerged sampling method.

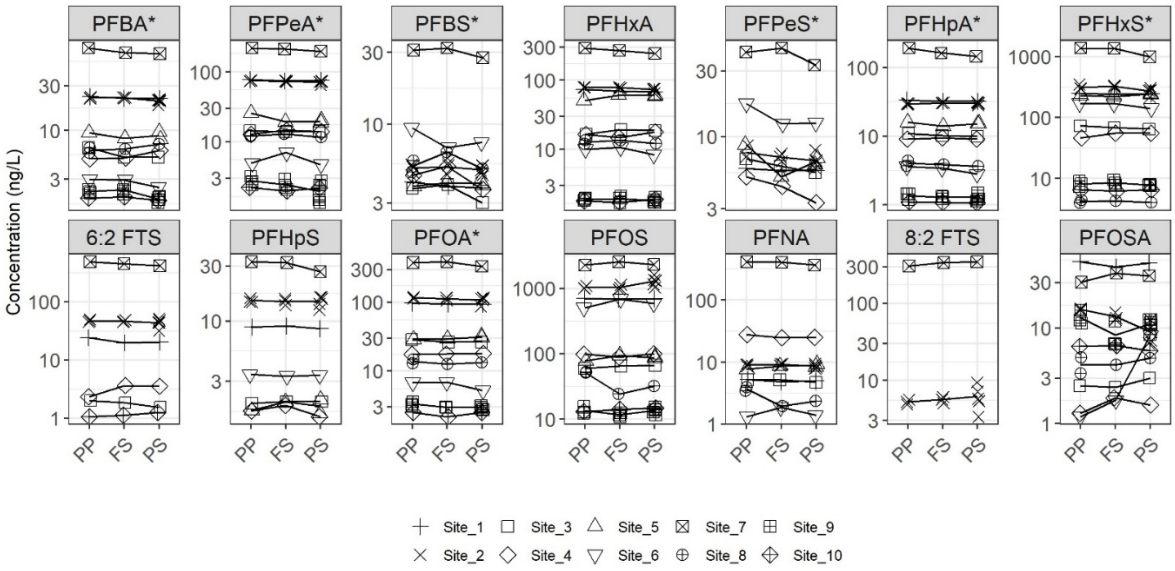

Figure 24. PFAS concentrations in bulk water collected using three sampling methods at 10 sites. PFAS are arranged in increasing order of hydrophobicity, based on retention time, from left-to-right, first top row then bottom row

Note in **Figure 24**, lines connect the three methods at each site; lines connect the arithmetic mean of method replicates where applicable. Analytes with asterisks had statistically significant differences among methods (linear mixed effects model, $p < 0.05$). PP, peristaltic pump; FS, fully submerged bottle; PS, partially submerged bottle.

To provide a simpler illustration of the unexpected result that the partially submerged bottle had lower concentrations than the other bulk water sampling methods, a simplified analysis was performed using mean-normalized PFAS concentrations. Specifically, for each PFAS, the concentration from each sampling method (or mean of concentrations if there was more than one result) was normalized to the arithmetic mean of the three methods at each site, and then the normalized concentrations for all PFAS were combined. The results of this simplified analysis were generally consistent with that of the mixed effects model: the three sampling methods differed significantly (Kruskal Wallis test, $p < 0.05$). The Dunn's multiple comparisons indicated PFAS concentrations in samples collected by peristaltic pump and fully submerged bottle samples were not statistically different, but both were significantly greater than concentrations measured on samples collected using the partially submerged bottle method (**Figure 25**). The median of the mean-normalized concentration of partially submerged methods was 97% and 95% of the fully submerged and peristaltic pump methods, respectively.

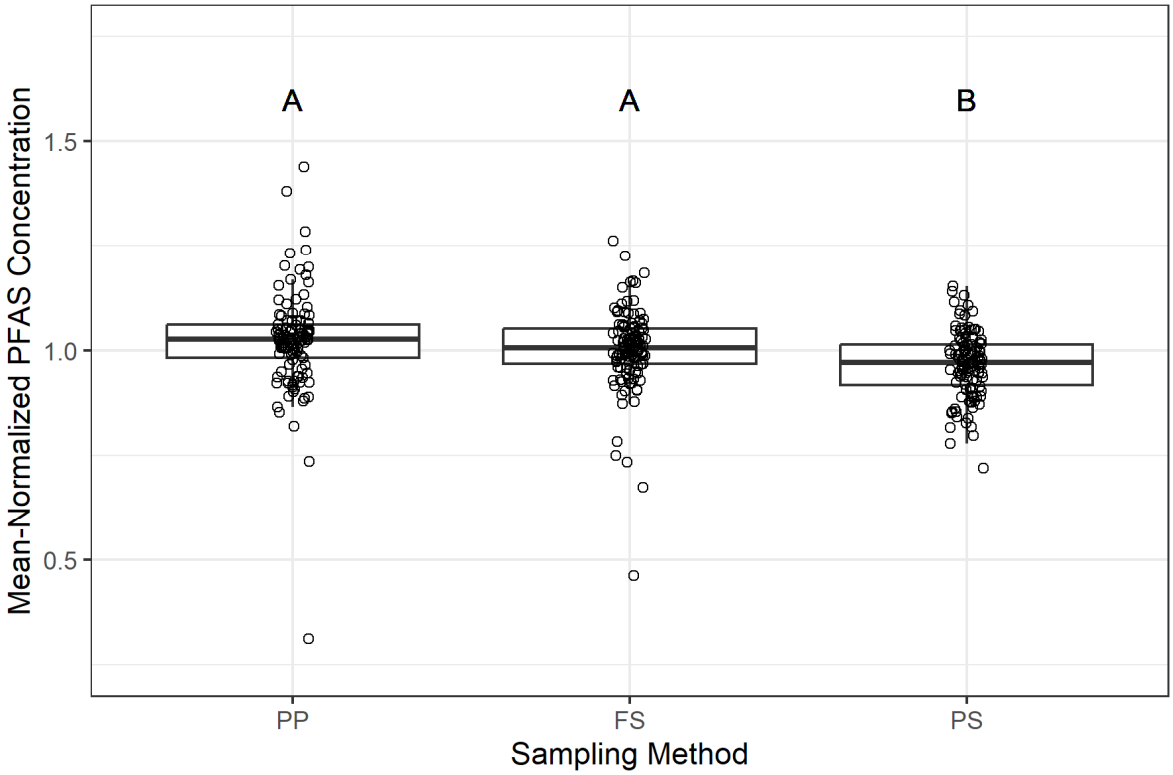

Figure 25. Boxplots of mean-normalized PFAS concentrations in bulk water collected using three sampling methods at 10 sites

Note in Figure 23, the PFAS concentration in each sample was normalized to overall mean (mean of means for each sampling method) at each site. Sampling methods with different letters (A and B) have statistically different PFAS concentrations (Dunn's non-parametric pairwise multiple comparison test, adjusted $p < 0.05$). Dark black lines represent medians; box bounds 1st and 3rd quartiles; whiskers extend to ±1.5x the height of the box. PP=peristaltic pump, FS=fully submerged bottle, PS=partially submerged bottle.

Data from the pilot study (replicate analyses at Sites 2 and 9), while originally collected for the purpose of assessing sampling and analytical variation, were also useful as an additional line of evidence for assessing the difference among sampling methods. For Site 9, only two PFAS had sufficient detection for comparisons, and Kruskal Wallis test results indicated no significant differences (Roark et al., 2024 Table S-7). For Site 2, four of nine PFAS (PFBA, PFHxA, PFHxS, and PFOSA) had significant differences in concentration among methods, and in each case the partially submerged method had the lowest concentration. Dunn's post-hoc test conducted to evaluate pairwise differences indicated only two significant post-hoc results (PFHxA and PFOSA) and in both cases the peristaltic pump method was greater than the partially submerged bottle method (Roark et al., 2024 Table S-8). While few statistically significant results were observed, in each case the results were consistent with that of the full investigation: Concentrations from partially submerged bottle samples were not biased high; rather, they were lower than the peristaltic pump sample concentrations. In the pilot study, three of the PFAS (PFBA, PFHxA, and PFHxS) with lesser concentrations in the partially submerged bottle samples were at the lower end

of the hydrophobicity range and one (PFOSA) was at the higher end.

A substantial body of literature exists, particularly for seawater, about the physicochemical and biological characteristics of the SML (Cunliffe et al 2013). EFs for many classes of contaminants, including chlorinated hydrocarbons, petroleum hydrocarbons, polycyclic aromatic hydrocarbons, and metals (reviewed in Wurl and Obbard, 2004) are similar to or greater than those reported here and in Schwichtenberg et al. (2023). However, these studies, including Schwichtenberg et al. (2023) have compared the biology and chemistry of samples from the 1 to 1,000 µm SML with that of samples at various depths from the upper meter of underlying bulk water. We identified no studies that explicitly evaluated chemistry in the upper 1 to 2 cm of near-surface water, including SML, in comparison to bulk water samples from 15 cm below surface.

The results of this study indicate that lower hydrophobicity PFAS that exhibit little or minimal enrichment in the SML have lower concentrations in the upper 1 to 2 cm of the surface water than found at 15 cm below the surface. Although differences among methods for more hydrophobic PFAS were non-significant, and concentrations among methods relatively more variable, it is difficult to evaluate whether the observed trend (partially submerged bottle concentrations less than the fully submerged and peristaltic pump) actually differs or was only obscured. While we do not have a mechanistic explanation for this observation, it presumably results from physicochemical or potentially biological conditions or processes in the upper few centimeters of bulk surface water. Further investigation would be needed to provide a complete explanation of the results of the present study.

Importantly, the observed differences among sampling methods are small enough that they may not be of practical importance. Averaged across PFAS and sites, the magnitude of the difference between partially submerged methods and the fully submerged and peristaltic pump methods was 3% and 5%, respectively. The mean relative percent difference (RPD) of PFAS concentrations among sites and analytes was greatest between the peristaltic pump and partially submerged bottle sampling methods (mean of 13.9%, range was 0 to 151%). For the peristaltic pump and partially submerged bottle sampling methods, 91% of RPDs were less than 30%. Given that an RPD of 30% is generally considered an acceptable level of variation for PFAS concentration duplicates, the observed differences among methods may not be of practical importance. It is likely that outside of this controlled study, any of these approaches could be used to sample PFAS from surface water bodies without a significant concern about bias due to the SML enrichment.

Enrichment Factors

Median and maximum EFs generally increased with increasing retention time (Roark et al., 2024 Table S-9, **Figure 26)**. However, as evident in Figure 3, Site 11 has the greatest or nearly greatest EF in all cases. If Site 11 were not included, there would be little or no evidence of increasing EF with increasing retention time until retention time exceeded approximately 10 (6:2 FTS has a retention time of 10.3). With the exception of PFOS, the greatest EFs all occur at the same location (Site 11). Comparison to published EFs (Schwichtenberg et al., 2023) indicates a generally similar pattern for PFAS EFs reported in each study, although in the present study Site 11 EFs are greater than those reported in fresh water (Schwichtenberg et al., 2023) (**Figure 26**). The previously reported EFs are variable, but are not inconsistent with EFs reported here (**Figure 26** and Roark et al., 2024 Table S-9).

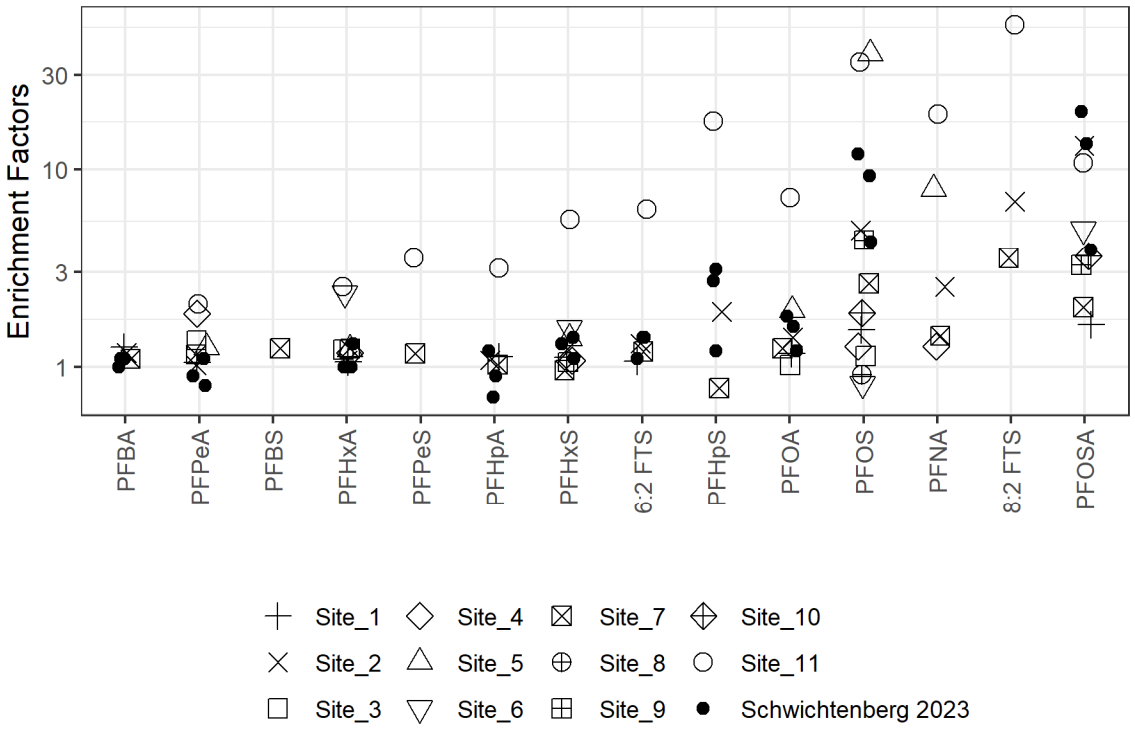

Figure 26. Plot of enrichment factors for 14 PFAS at 11 sites. Sites are indicated by shape; closed black circles present data from Schwichtenberg et al. (2023)

Associations of PFAS enrichment in the SML with bulk surface water quality parameters, DOC, pH, specific conductance, and turbidity were explored. There were no significant correlations of PFAS EF with bulk water DOC, pH, specific conductance, or turbidity (Roark et al., 2024 Table S-10). If PFAS associate with DOC in the bulk water and in the SML in proportion to DOC concentration, then DOC enrichment could be correlated with PFAS enrichment. DOC EFs were calculated as the ratio of mean SML DOC to mean bulk water DOC (**Table 10**). The correlation of DOC EF with PFAS EF was evaluated (Roark et al., 2024 Table S-10), and scatterplots with fitted linear regression lines and 95% confidence bands for PFAS EF versus DOC EF were assessed (Roark et al., 2024 Figure S-2). While there is some evidence of a positive correlation of DOC EF and PFAS EF in that most of slopes are positive (Roark et al., 2024 Figure S-2), sample sizes of 2 or 3 are inadequate to provide confidence in the relationship, and there is considerable variation among locations for PFAS with larger sample sizes. This study was not explicitly designed to support the evaluation of such relationships, and the sample size relative to variation is inadequate to provide confidence in drawing conclusions regarding DOC EFs and PFAS EFs.

2.3. Summary

Laboratory study results indicated that although PFAS may accumulate at the air-water interface in groundwater wells, the magnitude of the effect is unlikely to negatively bias measured PFAS concentrations in groundwater, especially considering that samples are typically collected using low-flow sampling procedures prior to sampling.

In surface water, the presence of natural organic material was shown to enhance PFAS accumulation near the air-water interface. Foaming was shown to occur in the absence of PFAS due to the presence of natural organic surfactants. Natural organic microlayers may enhance PFAS accumulation near the surface, with or without the presence of foams. When sampling surface water, foam collection should be avoided to prevent a positive bias in bulk surface water PFAS concentrations. On average, PFAS EFs increased with increasing retention time. However, the increase was not evident at all sampling sites for all PFAS. At field sites, there was not consistent evidence of a correlation with PFAS EF with bulk water DOC, pH, specific conductance, turbidity, or with DOC EF.

Prior to field testing, experiments were conducted to refine SML sampling techniques using a glass plate and/or microscope slides. Better PFAS recovery was obtained from single microscope slides and screens and may be just a function of less handling. When three microscope slides were used to obtain adequate volume for PFAS analytical measurements, additional optimization was conducted to achieve higher and less variable PFAS recovery.

Field findings indicated that there was no evidence of elevated PFAS concentrations in bulk surface water resulting from inclusion of SML using the partially submerged bottle sampling method. Unexpectedly, for many individual PFAS and for all PFAS combined, samples collected with the partially submerged bottle had slightly lower PFAS concentrations. This was particularly notable in PFAS with lower hydrophobicity (e.g., chromatographic retention time). No explanation for this has been determined. Although this finding warrants further investigation, the differences are generally not large and may not be important.

Although there was some evidence of differences among bulk surface water sampling methods was detected in the field study, the difference was not consistent with potentially expected high bias to the partially submerged bottle due to the enriched SML. The magnitude of difference in PFAS concentration among methods is relatively small; on average, the partially submerged bottle method was 3% and 5% less than peristaltic pump and fully submerged bottle, respectively, 91% of RPDs between the peristaltic pump and partially submerged bottle concentrations were less than 30%. These differences are not large in comparison to generally accepted analytical variation. Therefore, the choice of any one of these sampling methods for PFAS in surface water is acceptable and is unlikely to cause meaningful bias in a PFAS sampling program.

3. SYSTEMATIC EVALUATION OF FIELD MATERIALS AND PROCEDURES TO ELIMINATE BIAS DURING SAMPLING

3.1. Background

Multiple guidance documents have been published by Federal and state agencies regarding the collection of PFAS samples. These documents typically specify do's and don'ts for PFAS sample collection and recommend precautions to prevent false positive results. Some exclude a wide variety of materials from field sites or the sampling area during PFAS sampling events. For example, PFAS guidance documents may prohibit or discourage the use of chemical (blue) ice packs in coolers, certain types of pens and markers used to label sample containers, and field staff clothing laundered using fabric softener. However, there is little scientific evidence that these precautions are necessary to prevent false positive PFAS results. These requirements can increase the time and cost to prepare for sample collection. In order to inform recommendations for improving existing guidance (Section 5), during Task 2 of this project, we conducted a review of scientific literature as well as unpublished large data sets of field equipment blank samples. Results were evaluated to identify whether several sampling restrictions are needed to prevent sample contamination. Results were also evaluated to identify if additional equipment, materials, or products should be tested and if so, conduct testing to fill these data gaps.

3.2. Technical Approach and Results

3.2.1. Data Gathering and Literature Review (Task 2.1)

The project team harvested readily available PFAS investigation guidelines, protocols, and work plans, and developed a comprehensive list of protocols for field sampling. Team members leveraged knowledge from within their firms and also queried well-known PFAS researchers and members of the Interstate Technology and Regulatory Council (ITRC) PFAS team via emails, conference calls and an online survey. Collective knowledge and experiences were therefore leveraged to summarize recommended PFAS sampling protocols and unpublished industry data.

Next, a literature review was conducted to evaluate the scientific basis of recommended PFAS sampling restrictions and recommendations. Readily available scientific studies were summarized to determine which materials or equipment had previously been evaluated for PFAS contribution to samples. Several peer-reviewed studies (e.g., Bartlett and Davis, 2018; van der Veen et al., 2020) evaluated equipment rinsate blanks or conducted soak tests for various materials to evaluate the presence of PFAS and/or total fluorine. Additionally, field sampling procedures were evaluated to determine the pathway by which PFAS could transfer from materials, not directly in contact with the samples, to the samples.

Peer-reviewed literature review findings were evaluated in combination with unpublished industry data (Section 3.2.2) to identify scientific data gaps and guide recommendations for additional laboratory testing at Oregon State University (Section 3.2.3). Findings from the literature review and follow-on studies are summarized in Sections 3.2.4 and 3.2.5.

3.2.2. Equipment Blank Datasets (Task 2.2)

The project team also completed a review of unpublished field equipment datasets (e.g., empirical data) to determine if field equipment and procedures systematically result in positive PFAS detections in groundwater samples. Equipment rinsate blanks and/or equipment soak blanks were collected during PFAS site inspections at over 30 large U.S. Navy installations and 13 PFAS remedial investigations from Michigan. Data were used to assess whether any commonly used, yet untested, groundwater sampling equipment should be considered for PFAS analysis under this SERDP project.

The equipment blank datasets were filtered using a number of criteria as summarized in **Figure 27**. PFAS were detected above the reporting limit in 3 out of 105 equipment blanks collected from 30 U.S. Navy installations. In Michigan, 13 out of 121 equipment blanks collected over a three-year timeframe had detections of PFOS and/or PFOA.

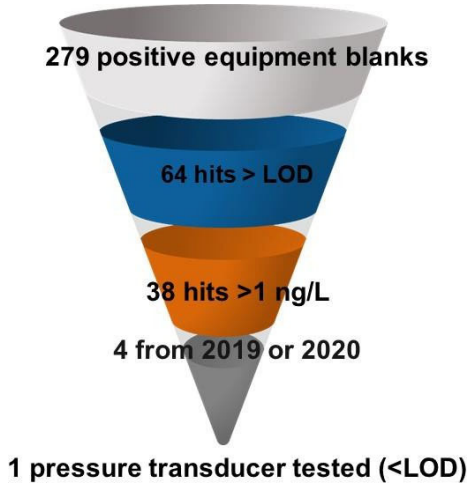

Figure 27. U.S. Navy installation equipment blank dataset characteristics

The majority of the equipment blank samples collected from the U.S. Navy installations were collected after the equipment had been used for sampling and had been decontaminated before moving to the next sample location. In this manner, equipment blanks offer data to assess the adequacy of the decontamination process and a detection does not imply that the equipment contained PFAS materials. Rather, carryover from one sample location to the next may explain positive PFAS detections. Most of the reported detections were qualified as approximate values (J flag) or uncertain due to detections below method detection limits, detection in the associated field blank, trip blank, or laboratory blank (B flag). The data review resulted in identification of three pumps with Teflon or PTFE components as candidates for additional testing, including Grundfos Redi-Flo2 (electric impeller pump), Geotech (bladder pump), and the Monsoon Proactive Stainless-Steel (electric impeller pump). However, these pumps, or their components, were not tested by this research group because prior testing had been completed. The Grundfos Redi-Flo2 impeller pump and Geotech bladder pump (with PTFE and polyethylene bladders) were tested

during earlier phases of a sampling program and did not result in any detectable PFAS concentrations (DiGuiseppi et al., 2014). The Monsoon pump was previously tested by the equipment manufacturer. A vendor-led conservative soak test (15 months of soaking in deionized water) was completed to represent a valid worst-case assessment of the pump and PFAS results with a maximum concentration at 9.4 ng/L of PFOA and lower concentrations of other PFAS.

Equipment blanks from Michigan were typically collected in the field by pouring PFAS-free water over large sampling equipment, pumping PFAS-free water through a pump/tubing setup similar to a sample, or soaking smaller sampling materials and tools in PFAS-free water in a Ziploc® or other self-sealing polypropylene bag for 24 to 48 h. A Level Troll and Rugged Troll from In-Situ, Inc. was identified for further testing. The project team received a loan of a new identical Level Troll pressure transducer from In-Situ, Inc to conduct a soak test. The pressure transducer (surface area 0.01120 square meters) was soaked in 40 mL of methanol for 24 h and analyzed for PFAS by LC- MS/MS. No PFAS were detected. The preponderance of non-detect results in the data sets reviewed indicates that typical equipment used to collect environmental samples did not contaminate field samples with PFAS. Blanks that yielded non-detect results were taken from sampling equipment including hand augers, steam cleaners, truck water tanks and water totes, sampler screens, submersible pumps, trash bags, hand tools, water level meters, and more. These data support the conclusion that there is a low likelihood of systemic cross-contamination from PFAS-containing field equipment and materials. Michigan equipment blank data indicate a greater potential for cross-contamination may come from source water used for decontamination or during drilling.

3.2.3. Testing of Common Field Supplies (Task 2.3)

Readily available scientific studies were summarized to determine which materials or equipment had previously been evaluated for PFAS contribution to samples. Additionally, field sampling procedures were evaluated to determine the pathway by which PFAS could transfer from materials, not directly in contact with the samples, to the samples. See Appendix A Rodowa et al., 2020 for Supplemental Data associated with this section.

Following completion of the literature review and evaluation of equipment blank datasets, project team members at Oregon State University conducted methanol extraction tests for a variety of different field sampling materials for PFAS to determine the potential for sample bias (Rodowa et al., 2020a). Rodowa et al. analyzed 66 materials for 52 PFAS using liquid chromatography tandem mass spectrometry (LC-MS/MS) following sample extraction according to a previous publication (Robel et al., 2017). Particle-induced gamma-ray emission (PIGE) spectroscopy was performed by the University of Notre Dame to quantify total fluorine. Items were categorized into materials and products used for pre-staging, staging, sampling, and transport. Results indicated that none of the 22 materials that gave quantifiable concentrations of individual PFAS had the potential to come into direct contact with sample media. Detailed results from the project laboratory study and other published studies are summarized in the following subsections. Studies yielded relevant information about materials, field equipment, various consumer products, and personal protective equipment. Results were extrapolated to assess the extent of potential bias in PFAS samples when standard field procedures are followed.

3.2.4. Materials, equipment, and products that do *not* contribute PFAS

The following studies used a variety of techniques to test consumer products and found that many commonly used field materials, equipment, and products did not contribute PFAS to analytical samples.

Denly et al. (2019) tested various materials by soaking them in a sample of PFAS-free water for 24 h and then measuring the resulting PFAS concentrations in the water. Samples were then extracted using solid-phase extraction (SPE) and analyzed for 24 individual PFAS using LC-MS/MS analysis. Results indicate the following products and equipment will not contribute detectable PFAS to analytical samples: aluminum foil (Rodowa et al., 2020a); adhesive notes; bubble wrap; most bentonite plugs, bentonite chips, time-release pellets, and granular bentonite (Denly et al., 2019); protein bar wrapper; passive diffusion bags; and, polyvinylidene fluoride (PVDF) water level tape (Denly et al. 2019).

Bartlett and Davis (2018) collected equipment blanks from testing various fabrics sprayed with insect repellants and analyzed the samples for 17 different PFAS using an ultra- performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) method with isotope dilution method. Three insect repellants were tested and none contributed detectable PFAS to equipment blanks: Insect Shield Insect® pretreated clothing, OFF! Deep Woods® Spray for clothing or skin, and Sawyer® do-it-yourself permethrin treatment for clothing.

Rodowa et al. (2020a) tested a variety of items that had no detections of individual PFAS or total fluorine including the following: dryer sheets, aluminum foil (other than non-stick), paper towel adhesive, adhesive notepads, binder plastic cover, nitrile gloves, putty caulk, clear resin, white glue, polyethylene bladder, core bag, elastic sealing film, and plastic bags.

A summary of compiled study results is provided in **Table 15**.

Table 15. Materials, equipment, and products that were tested and found to be PFAS-free

Material, Equipment or Product	Equipment Preparation and Analytical Method	Reference(s)
Adhesive notepad	LC-MS/MS and PIGE spectroscopy; 24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	1, 2
Aluminum foil (not treated for nonstick)	LC-MS/MS and PIGE spectroscopy; 24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	1, 2
Bentonite 3/8-inch chips	Leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Bentonite granular	Leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Bentonite medium chips	Leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Bentonite plugs	Leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Bentonite time-release pellets	Leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Binder plastic cover	LC-MS/MS and PIGE spectroscopy	1

Material, Equipment or Product	Equipment Preparation and Analytical Method	Reference(s)
Bubble wrap	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Clear resin	LC-MS/MS and PIGE spectroscopy	1
Core bag	LC-MS/MS and PIGE spectroscopy	1
Dryer sheets	LC-MS/MS and PIGE spectroscopy	1
Elastic sealing film	LC-MS/MS and PIGE spectroscopy	1
Insect repellant(Insect Shield Insect® pretreated clothing)	Equipment blank from fabric sprayed with product analyzed for 17 PFAS with U.S. Environmental Protection Agency (EPA) 537 modified method	3
Insect repellant (OFF! Deep Woods® Spray for clothing/skin)	Equipment blank from fabric sprayed with product analyzed for 17 PFAS with U.S. EPA 537 modified method	3
Insect repellant (Sawyer® do-it-yourself permethrin treatment for clothing)	Equipment blank from fabric sprayed with product analyzed for 17 PFAS with U.S. EPA 537 modified method	3
Lab tissue packaging	LC-MS/MS and PIGE spectroscopy	1
Paper towel adhesive	LC-MS/MS and PIGE spectroscopy	1
Passive diffusion bag	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Plastic bags	LC-MS/MS and PIGE spectroscopy	1
Polyethylene bladder	LC-MS/MS and PIGE spectroscopy; 24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	1, 2
Protein bar wrapper	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Putty caulk	LC-MS/MS and PIGE spectroscopy	1
Polyvinyl chloride (PVC) pipe	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Resealable plastic storage bags	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Silicone tubing	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
White glue	LC-MS/MS and PIGE spectroscopy	1

Notes: (1) Rodowa et al., 2020a; (2) Denly et al., 2019; (3) Bartlett and Davis, 2018.

Typical conditions during PFAS sampling are more conservative than the methods used for sample preparation (24-h soak of material directly in water [**Table 15**, Note 2] or extraction with methanol [**3**, Note 1]). Therefore, these studies effectively establish that many materials prohibited by current sampling guidance are unlikely to cause bias. In some cases, the method may not be conservative enough—for example, if passive samplers had Teflon components. In addition, it is difficult to generalize these results to similar products, as manufacturing methods may vary.

3.2.5. Materials, equipment, and products that *could* contribute PFAS

Certain materials are known to contain fluorinated compounds and have been considered likely sources of PFAS bias in field samples. These include polytetrafluoroethylene (PTFE) materials such as Teflon™ and Hostaflon®, fluoroelastomers such as Viton™, and fluoropolymer-based stain- or water-resistant materials. Because these materials are known to contain fluorinated compounds, they have not been an area of particular focus for some laboratory researchers. However, the extent to which these fluoropolymers contribute soluble PFAS to water samples

under realistic field conditions over time and as a function of equipment age has not been fully studied.

Several peer-reviewed scientific studies have identified common sampling materials comprised of fluorinated materials that did contribute detectable concentrations of PFAS to equipment blanks after 24 h of soaking in water. For example, Denly et al. detected PFAS in soak tests with PTFE tubing, a PTFE bladder, some water level tape meters, one type of bentonite (time-release pellets), bailer line/twine, nitrile gloves, sample labels, and a waterproof field book cover. Denly et al. also identified perfluorobutanoate (PFBA) concentrations in 1 out of 3 types of HDPE tubing and PFBA, perfluoropentanoate (PFPeA), and other PFAS in 2 types of new low-density polyethylene (LDPE) tubing. Rodowa et al. (2020a) identified one or more individual PFAS were detected in first aid packaging, first aid adhesive wrapper, PTFE tape, nonstick aluminum foil, laboratory tissue, paper towel, laboratory notebook, marker ink, and duct tape samples. In other studies, PFAS were not directly measured in samples, but total fluorine was, indicating the presence of undetected PFAS (Rodowa et al., 2020a) in label backing, waterproof notepaper, plastic shovel packaging, nitrile glove packaging, PVC liner, PVC screen, core catcher, core catcher liner, vinyl end caps, membrane interface probe (MIP) membrane, electrical tape, and cold packs. The materials, equipment, and products that were tested in these studies are summarized in **Table 16**.

Table 16. Materials, equipment, and products that yielded PFAS and/or total fluorine detections

Material, Equipment or Product	Equipment Preparation and Analytical Method	Reference(s)
Bailer line	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Cold pack (outside)	PIGE spectroscopy	1
Core catcher	PIGE spectroscopy	1
Core catcher liner	PIGE spectroscopy	1
Duct tape	LC-MS/MS and PIGE spectroscopy	1
Electrical tape	PIGE spectroscopy	1
Field book cover	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Field book pages	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
First aid adhesive wrapper	LC-MS/MS and PIGE spectroscopy	1
First aid packaging (box)	LC-MS/MS and PIGE spectroscopy	1
HDPE tubing	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Lab notebook	LC-MS/MS and PIGE spectroscopy	1
Lab tissue	LC-MS/MS and PIGE spectroscopy	1

Material, Equipment or Product	Equipment Preparation and Analytical Method	Reference(s)
Label backing	LC-MS/MS and PIGE spectroscopy	1
Labels	PIGE spectroscopy; 24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	1, 2
LDPE tubing	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
MIP membrane	PIGE spectroscopy	1
Nitrile glove packaging	PIGE spectroscopy	1
Nitrile gloves	LC-MS/MS and PIGE spectroscopy; 24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	1, 2
Nonstick aluminum foil	LC-MS/MS and PIGE spectroscopy	1
Paper towels	LC-MS/MS and PIGE spectroscopy	1
Permanent marker ink	LC-MS/MS and PIGE spectroscopy	1
Pizza box	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Plastic shovel packaging	PIGE spectroscopy	1
PTFE bladder	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
PTFE tape	LC-MS/MS and PIGE spectroscopy	1
PTFE tubing	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
PTFE-lined tubing	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
PVC liner	PIGE spectroscopy	1
PVC screen	PIGE spectroscopy	1
Silastic tubing	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Time-release bentonite pellets	Leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Vinyl end caps	PIGE spectroscopy	1
Water level tape	24-h leaching on shaker table, SPE and analysis of 24 PFAS by LC-MS/MS	2
Waterproof notepaper	PIGE spectroscopy	1
Waterproof outdoor clothing	Sequential organic solvent extraction and LC-MS/MS analysis	4

Notes: (1) Rodowa et al., 2020a; (2) Denly et al., 2019; (3) Bartlett and Davis, 2018; (4) Van der Veen et al., 2020.

In addition, manufacturers may change their practices over time. Therefore, particular products that are tested and found to contain PFAS may no longer need to be avoided if the manufacturer changes their practice to avoid PFAS.

3.2.6. Evaluation of pathways for field sampling procedures

Although the literature search confirmed that PFAS are present in several items used in the overall process for collecting environmental samples and can leach to water after 24 hours of soaking, it does not conclude that use of these materials in the field, consistent with industry standard sampling procedures, will result in PFAS in field samples. Therefore, the research team also evaluated the pathways by which PFAS could reach be introduced to the environmental samples.

There are several pathways for field materials and equipment to bias PFAS sampling results:

- Direct sample contact with sampling equipment – Sampling equipment that comes into direct contact with the sample media, such as laboratory sample containers, tubing, pump components (i.e., O-rings), bailers, sleeves and liners, samplers, and filters is the most likely pathway to impact PFAS concentrations in the sample. Some materials and equipment are in prolonged contact with the sampled water (e.g., passive diffusion bags), while other contact may be brief (e.g., momentary contact with a pump O-ring). Some materials are in direct contact with specific types of samples only (e.g., aluminum foil may be used when collecting fish tissue samples, but not groundwater or surface water samples). The quantity of material potentially in contact with a sample is also important to consider. For example, aluminum foil contains 2.7 nanograms per square centimeter (ng/cm^2) of PFOS and PFOA and approximately 3.7 square centimeters of foil would need to be in contact with a water sample to account for up to 10 ng/L of PFOS and PFOA (Rodowa et al., 2020a).
- Incidental contamination while sample bottle is open – Cross-contamination could occur during the brief time that field staff have opened a sample container and are filling it prior to capping the bottle. Cross contamination could theoretically come from personal care products the sampler has used, dust or soil particles that enter the sample, or volatile PFAS entering the sample container.
    - To avoid the transfer of PFAS or introduction of unintended particles, PFAS guidance typically specifies that the field personnel change to a clean pair of gloves immediately prior to sample collection. Also, to be detectable (e.g., >2 ng/L) in a 250-milliliter (mL) sample, approximately 0.5 ng of PFAS would need to be present. This equates to at least 0.2 mg of makeup, 1 to 2 drops of sunscreen product, a peak rainfall rate for 20 min or more into the sample bottle, or peak rainfall near an active PFAS manufacturing facility into the sample container for over 3 min.
    - Cross-contamination due to volatile PFAS is unlikely to occur at most sites because PFAA concentrations measured in outdoor air are far too low (picograms per cubic meter)[23] to result in detectable PFAA concentrations in a sample bottle. PFAA concentrations are higher in indoor air but are still approximately 2 to 3 orders of

magnitude lower than needed to result in detectable PFAA concentrations.

- **Contamination during shipping** – Guidance documents for PFAS sampling typically provide recommendations or restrictions for field staff regarding sample packaging for transport to the laboratory. For example, blue ice and other freezer packs are commonly listed as items to avoid. However, there are not plausible pathways for non-volatile PFAS from these materials to enter into a sample bottle. Sample bottles are capped, making it extremely unlikely that PFAS could diffuse into a sample bottle even if the outside of a blue ice pack was contaminated from the breakage of highly concentrated PFAS samples during or prior to cooler shipment. The suggestion that blue ice packs should be avoided during PFAS sample shipment is not consistent with any other non-PFAS related environmental guidance on sample shipping, or with laboratory results that demonstrate blue ice packs do not contain PFAS. For example, standard operating procedures (SOP)s do not suggest that samples that share a cooler are compromised if a highly concentrated groundwater or surface water sample breaks during transport. Field blanks and trip blanks provide data that support assumptions regarding the integrity of shipping containers.

Many of the materials identified in the literature review as having potential to contribute PFAS to water samples are not in direct contact with environmental samples and other pathways seem unlikely sources of detectable quantities of PFAS in samples. The potential for direct contact for some sampling equipment and materials known to contain PFAS are summarized in **Table 15**. Other materials have PFAS at concentrations that are so low that even if the material were in direct contact with the sample, there would not be enough PFAS mass to bias sample results in low ng/L. Therefore, materials that have been tested or are known to contain PFAS may be acceptable to use.

Table 17. Potential exposure pathways for materials, equipment, and products that contain PFAS to affect PFAS samples when following standard field procedures

Material, Equipment, or Product	Potential for Direct Contact with Sample	No Potential Pathway to Affect PFAS Sample when Following Standard Field Protocols
Bailer line	X	
Cold pack (outside)		X
Core catcher*	X	
Core catcher liner*	X	
Duct tape		X
Electrical tape		X
Field book cover		X
Field book pages		X
First aid adhesive wrapper		X
First aid packaging (box)		X
HDPE tubing	X	
Laboratory notebook		X
Laboratory tissue		X
Label backing		X

Material, Equipment, or Product	Potential for Direct Contact with Sample	No Potential Pathway to Affect PFAS Sample when Following Standard Field Protocols
Labels		X
LDPE tubing	X	
MIP membrane*		X
Nitrile glove packaging		X
Nitrile gloves	X	
Nonstick aluminum foil*	X	
Paper towels		X
Permanent marker ink		X
Plastic shovel packaging		X
PTFE bladder	X	
PTFE tape		X
PTFE tubing	X	
PTFE-lined tubing	X	
PVC liner	X	
PVC screen	X	
Silastic tubing	X	
Time-release bentonite pellets	X	
Vinyl end caps	X	
Water level tape	X	
Waterproof notepaper		X
Waterproof outdoor clothing		X

*Not applicable for groundwater and surface water sampling

Current PFAS sampling guidance does not adequately recognize if a plausible pathway exists for materials to affect PFAS concentrations in environmental samples. More careful consideration and communication of these aspects of guidance can begin to shift industry perceptions from a "contamination" mindset to a more scientifically based perspective of PFAS transport pathways and relative concentrations. When thinking about PFAS sampling from a contamination mindset, any material which contains trace amounts of PFAS is suspect and should not be allowed in proximity to the sample. From a scientifically informed perspective, some of the restrictions and recommendations provided in current PFAS guidance are unnecessary. PFAS sampling guidance can also be improved by describing standard protective measures that apply to all environmental sampling activities and highlighting areas where additional measures are needed to ensure the representativeness of surface water and groundwater samples. Lastly, industry standard sampling procedures that have been used for the past 40 years are specifically designed to avoid having any extraneous material enter a sample bottle.

3.3. Summary

The project team reviewed existing guidance documents, peer-reviewed literature, unpublished data including equipment blank datasets, and supplemental laboratory testing of materials. Lists were compiled to guide practitioners in quickly identifying results from scientific testing of commonly used field materials, equipment, and products and their potential to contribute PFAS

to sample results, or total fluorine. In addition, a distinction was made in the summary tables (see e.g., **Table 14**) between materials, equipment, and products that may have the potential to directly contact a sample and those that do not, i.e., no plausible pathway exists for materials to affect PFAS concentrations in environmental samples. During Task 4, a technical report and other outreach activities summarized key results and recommendations to improve existing PFAS sampling guidance (Section 5).

4. IMPACT OF LABORATORY HOLD TIMES AND STORAGE CONDITIONS

4.1. Background

Many PFAS, particularly PFOS, PFOA, and their carbon-chain length homologs are typically long-lived and difficult to degrade. However, due to sorption and degradation of some precursor PFAS to form others, at the time that the project began, there were questions regarding potential bias in reported PFAS concentrations in groundwater or surface water due to sample storage conditions and duration. PFAS interactions with the sample container material could bias sample results low; transformation of some PFAS precursors to form others during storage could bias sample results high or low. However, at the time of the original proposal there were few data to answer these questions.

The extent to which these processes occur within a sample may vary depending on the type of storage container, hold time, storage temperature, mixture of PFAS originally present in the sample, and other water quality parameters. At the start of the project, current guidance for groundwater and surface water PFAS sampling recommended sample collection into wide-mouth 250-mL or 500-mL HDPE bottles fitted with HDPE screw caps and storage at 4 degrees Celsius (ºC) or less for a hold time of up to 14 days prior to extraction, using a modified U.S. EPA method 537 analysis. No preservative is needed for non-drinking water samples. Drinking water samples require the addition of 5 grams per liter (g/L) Trizma® as a dechlorinating agent, i.e., to remove free chlorine from chlorinated drinking water. The presence of free chlorine was found to affect the recovery of PFAS during Method 537 development. Trizma® also serves as a pH buffer. Trizma® itself does not appear to have an effect on PFAS in non-chlorinated water samples. Ammonium acetate can also be added to drinking water sample bottles to serve as a sample buffer.

At the start of the project, questions arose as to the scientific basis for these restrictions, particularly the 14-day hold time. Note that U.S. EPA Method 1633 specifies a longer hold time of 28 days for water and soil samples and sample extracts kept at 4 ºC and a hold time up to 90 days for soils, tissues, and water sample extracts (U.S. EPA, 2004). These longer hold times for PFAS samples can reduce the need for resampling if shipping delays occur and can enable some samples to be placed on hold following receipt by a commercial laboratory, allowing application of cost-saving sample strategies where the analysis of samples could be contingent on results of a subset of the samples. Another benefit of longer hold times is for researchers who wish to store samples or subsamples for future evaluation.

Several researchers have evaluated the effect of sample storage times and containers on PFAS analytical results. In early research (2011), Berger et al. (2011) evaluated the recovery of perfluoroalkyl carboxylates (PFCAs), perfluoroalkyl sulfonates (PFSAs), and fluorotelomer alcohols over a period of three months of storage in a polypropylene container. Of those PFAS, the percent recovery rates steadily decreased and were unacceptably low (<70%) after 90 days for two longer chained PFAS: perfluoroundecanoate (PFUnDA) and perfluorododecanoate (PFDoDA). Additional PFAS mass was recovered upon rinsing the containers with methanol, indicating significant PFAS losses to the container that could be mitigated by a methanol rinse or other measures. HDPE containers were then suggested as preferable and have since become

common practice.

In 2018, the U.S. EPA Method 537.1 demonstrated stability of PFSAs, PFCAs, ether carboxylates, sulfonates, N-methylperfluorooctane sulfonamidoacetic acid (N-MeFOSAA), and N-ethyl-perfluorooctane sulfonamido acetic acid (EtFOSAA) in drinking water samples over a 14-day period. Data were not provided to demonstrate the validity of hold times specified by other analytical methods (ISO 25101 or ASTM International D7979-17). In addition, as part of the process for the development of PFAS analytical method 8327, U.S. EPA ran time-based studies on PFAS degradation and loss during sample storage over a 45-day timeframe (U.S. EPA, 2019). U.S. EPA also assessed the effects of different container types including plastic and glass on analyte recovery.

U.S. EPA recommended the use of HDPE containers, 'whole bottle' preparation (i.e., rinsing the sample bottle with methanol), and a hold time of up to 28 days prior to sample extraction. The written method 8327 summary also references freezing to prevent loss and degradation of some target PFAS. U.S. EPA plans to develop guidelines for field sampling and currently references the Interstate Technology and Regulatory Council (ITRC) team fact sheets for use as sampling guidelines (ITRC, 2020). For U.S. EPA Method 533, a maximum hold time of 28 days is recommended for samples prior to sample extraction. Extracts should be analyzed within 30 days after sample extraction (Shoemaker et al., 2009). These recommendations are based on a preliminary holding time study.

4.2. Technical Approach and Results

4.2.1. Storage/stability (Task 3.1)

Since the acceptance of the project proposal, Woudneh et al. (2019) published a paper on PFAS storage stability. In 2019, Woudneh et al. performed experiments to assess the effect of temperature, sample matrix, and storage time on PFAS analytical results. Sample matrices consisted of spiked bottled water, surface water, and two types of wastewater treatment plant effluent. HDPE or amber glass containers were used for sample storage. Samples were stored at -20, 4, and +20 ºC. Twenty- nine PFAS were tested, including 11 PFCAs, eight PFSAs, three fluorotelomer sulfonates (FTS), three perfluorooctane sulfonamides, two perfluorooctane sulfonamide ethanols, and two perfluorooctane sulfonamide acetic acids. Key findings of the study were as follows:

- All 29 of the PFAS that were tested were stable over a period of 180 days at -20°C regardless of the sample matrix.
- Other PFAS showed decreasing or increasing trends when stored at 4°C or 20°C. Changes in concentrations of other PFAS were observed within 7 days, in both surface water and wastewater matrices. Increasing the storage temperature led to greater concentration differences, as expected. PFAS that decreased over time included N-Methylperfluorooctanesulfonamide (N-MeFOSA), N-ethyl perfluorooctane sulfonamide (N-EtFOSA), N-methyl perfluorooctanesulfonamidoethanol (N-MeFOSE) and N-ethyl perfluorooctane sulfonamido ethanol (N-EtFOSE), likely due to volatility of these compounds. 8:2 FTS concentrations decreased over time as this compound biodegraded.

Formation of PFOA, perfluorononoate (PFNA), N-methylperfluorooctane sulfonamidoacetic acid (N-MeFOSAA), and N-ethyl perfluorooctane sulfonamido acetic acid (N-EtFOSAA) were observed over time, likely from the degradation of N-MeFOSE and N-EtFOSE precursors also present in the sample.

- Amber glass bottles and HDPE containers showed comparable recovery of analytes.
- The most significant losses occurred for PFUnDA and PFDoDA in the polypropylene container; however, these losses were demonstrated to be reversible by rinsing the container with methanol.

Woudneh et al. (2019) stored their samples with headspace, which is the conditions under which oxidation could occur, thus they captured the potential for oxygen to impact the analytical results. Reduced species such as the sulfinates are not stable under aerobic conditions; however, the concentrations of sulfinates are so low that even with complete transformation to PFOS, the change in PFOS concentrations is not likely to be statistically significant. The data in Woudneh et al. (2019), coupled with our own data, indicate that there is no difference in bottle material type, especially when whole bottle analyses are conducted, which is now required by the Department of Defense (DoD) quality systems manual (QSM) (DoD, 2019).

In our original proposal we discussed the need to determine the impacts of particulate matter and the potential for iron oxidation during sample storage to impact PFAS analytical results for water samples. In the context of routine analysis of water samples for the DoD, the QSM stipulates that whole bottle analysis must be performed. If > 1% particulate matter is present, centrifugation is allowed. On a practical scale, the centrifuged solids are co-eluted from particulate matter along with the aqueous fraction of the sample right before elution of the solid phase extraction cartridge (U.S. EPA, 2019).

Based on the current literature, and current practices among contract laboratories supporting the DoD, additional experiments to further evaluate bottle type, particulate matter, and are no longer needed. For this reason, no additional activities were completed under this project task.

4.3. Summary

The following summarizes key findings from a study conducted by Woudneh et al. (2019) following submission of the original proposal.

- 29 PFAS from six classes were stable over a period of 180 days at -20°C regardless of the sample matrix.
- Current hold times and storage practices were found to be unlikely to bias analytical results, except for sulfonamido ethanols and 8:2 FTS were the only PFAS that decreased significantly when refrigerated over the 14-day hold time.
- Given current protocols prescribed by the QSM that mandate whole bottle analyses, particulate matter, such as iron precipitates that may form during storage, are already included. Contract laboratory methods capture particulate matter as part of the solid phase extraction methodology and co-elute the PFAS associated with captured particulate matter.

- Current sample hold times for PFAS samples are unlikely to introduce bias, and could be extended without introducing bias, provided that samples are frozen upon receipt at the laboratory.

These findings are reflected in U.S. EPA method 1633, in which longer hold times are specified and hold times vary depending on whether samples are kept refrigerated or frozen. The extended hold times provide site investigators and researchers with more flexibility and provide options to reduce analytical costs (U.S. EPA, 2004).

5. TRANSLATE RESEARCH FINDINGS AND INFORM SAMPLING AND ANALYTICAL PRACTICES

5.1. Background

As described in Section 4, a variety of agencies and organizations have published guidance documents on PFAS sampling, including the DoD, U.S. EPA, state regulatory agencies, commercial laboratories, and consulting firms. Each of the guidance documents promote awareness of the potential presence of PFAS in a variety of commonly used materials, sampling equipment, personal care products, and consumer products that are used during field sampling events. However, based on our review of peer-reviewed literature, equipment blank datasets, and laboratory results from testing additional field equipment, materials, and products (Section 4), there are opportunities to improve existing guidance documents.

As Task 4 of the project, results from the project team's review of scientific literature and laboratory experiments were summarized, broadly shared with a variety of stakeholders, and used to develop recommendations for improving existing PFAS sampling guidance. Task 4 activities served to coordinate and integrate the activities and results from Tasks 1 through 3 and summarize key messages to communicate to stakeholders.

5.2. Technical Approach and Results

5.2.1. Gather knowledge of current practices (Task 4.1)

A list of existing PFAS sampling guidance documents was compiled and reviewed to understand which recommendations were common and which were presented in a limited number of sources, reflecting a lack of consensus within the industry:

- Published papers were reviewed that provided scientific support or refutation of PFAS sampling restrictions and recommendations.
- Unpublished data sets were reviewed that included analytical results from field equipment blank samples.
- Industry practitioners and regulators were surveyed through the Interstate Technology and Regulatory Council (ITRC) PFAS team to solicit input on each step of the process and request other relevant resources.

To gain an understanding of PFAS sampling guidance being followed by field teams, the team compared PFAS recommendations listed in readily available guidance documents and highlighted areas of commonality and areas where there was a broader range of recommendations. The PFAS guidance documents that were reviewed are summarized in **Table 18**. Each of the guidance documents promote awareness of the potential presence of PFAS in a variety of commonly used materials, sampling equipment, personal care products, and consumer products that are used during field sampling events. Some of the guidance documents also raise awareness of potential field sources of low bias in PFAS results, including field filtration and sorption to low density polyethylene tubing or to the sides of containers that are used for sample collection (e.g., field

composites). The guidance documents provide differing recommendations on how to best reduce sample bias, typically by categorizing materials and equipment into allowable and prohibited items (i.e., do's and don'ts) or specifying whether they can be used in direct contact with a sample, adjacent to the sample, or in the staging area only. Guidance documents also provide brand-specific information on products that have been tested at least once in the past and found to be PFAS-free. Examples of recommendations in current PFAS sampling guidance documents are provided in **Table 19**.

In general, earlier sampling guidance (produced prior to 2018) was more precautionary and restrictive of materials that could be used during PFAS field sampling. This approach can be beneficial because it bolsters confidence in sampling results, reduces the need for discussions regarding the acceptability of data for decision-making, and avoids the potential need to re-sample or to collect additional samples. However, some of the earlier and more restrictive precautions still remain in use. Overly precautious guidance can increase the cost and duration of field sampling events and increase the amount of waste generated. Many state guidance documents were updated in 2019 or 2020, building on significant engagement of state regulators in the development of ITRC team's technical and regulatory guidance for PFAS (ITRC, 2020). Inter-agency initiatives such as ITRC and cross-DoD working groups appear to be effective in reaching consensus on PFAS sampling best practices and developing and communicating industry best practices. Despite this convergence, questions still remain regarding the scientific basis for PFAS sampling precautions.

The project team prepared and reviewed a spreadsheet matrix that summarized information gathered from a variety of state and Federal guidance documents. The spreadsheet summarized current PFAS sampling and analytical precautions that are required or recommended. In addition, the spreadsheet summarized data supporting these precautions that had been published in peer-reviewed publications. The project team reviewed and supplemented this information with other unpublished data to assess the scientific basis, or lack thereof, for precautionary guidelines. For example, project team experience and database mining was used to identify equipment associated with positive PFAS detections in equipment blanks. Geosyntec completed a literature review to identify other key publications that may provide a scientific basis for assessing PFAS sampling practices.

Following internal project team review, the team conducted external outreach to improve the assessment of PFAS guidance. The project team contacted drilling contractors and equipment manufacturers to obtain information about products used in the field and any intentional changes in material components to remove Teflon™ components or other fluorotelomers. The project team also designed a survey and surveyed members of the ITRC PFAS team to solicit their input. Outreach to the ITRC PFAS team members summarized task activities, guidance documents and datasets reviewed, peer-reviewed publications identified, and asked for input on ongoing or planned updates to guidance documents, scientific publications, or unpublished relevant data. Input was also requested on potential technology transfer and outreach venues and activities. ITRC PFAS team survey responses were summarized upon receipt and used to inform additional activities under Task 4.

Table 18. Guidance documents on PFAS sampling materials, equipment, and procedures

Type	Organization	Year	Title
Industry	ITRC	2020	PFAS technical and regulatory guidance document and fact sheets, PFAS-1. Washington, D.C. https://pfas-1.itrcweb.org/
	National Groundwater Association (NWGA)	2018	Groundwater and PFAS: State of knowledge and practice
Federal	U.S. EPA Region 4	2015	Field equipment cleaning and decontamination at the FEC, SESDPROC- 206-R3, Science and Ecosystems Support Division, Athens, Georgia
	U.S. EPA	2020	PFAS technical brief
	U.S. DoD Environmental Data Quality Workgroup (EDQW)	2017	Bottle selection and other sampling considerations when sampling for PFAS
	Naval Facilities Engineering Command (NAVFAC)	2017	Interim PFAS site guidance for NAVFAC remedial program managers
States	California State Water Resources Control Board (CA SWRCB)	2020	PFAS sampling guidelines for non-drinking water
		2019	Drinking water sample collection for PFAS sampling guidance
	Florida Department of Environmental Protection (FDEP)	2019	Draft SOP—PFAS sampling
	Maine Department of Environmental Protection (Maine DEP)	2019	Addendum A—Development of a sampling and analysis plan, additional requirements for the sampling of PFAS, and Attachment A - PFOA and PFOS sampling and analysis plan form template, SOP No. RWM-DR-014-ADDENDUM
	Massachusetts Department of Environmental Protection (Maine DEP)	2020	Fact sheet. Interim guidance on sampling and analysis for PFAS at disposal sites regulated under the Massachusetts Contingency Plan. October 21
	Michigan Department of Environment, Great Lakes, and Energy (EGLE)	2018	Groundwater PFAS sampling
		2018	General PFAS sampling guidance
		2018, 2019	Guidance specific to PFAS sampling of residential wells, groundwater, surface water, surface water foam, wastewater, and more
	Minnesota Pollution Control Agency (MPCA)	2018	Currently using Michigan's 2018 guidance and may develop guidance for sampling foam on surface waters

Type	Organization	Year	Title
	New Hampshire Department of Environmental Services (NHDES)	2019	Laboratory testing guidelines for PFAS at waste sites
		2018	Master quality assurance project plan of the Hazardous Waste Remediation Bureau Waste Management Division
		2017	Frequently asked questions (FAQs) for sampling and analysis of PFAS at waste management and disposal sites
	New York State Department of Environmental Conservation (NYSDEC)	2020	Sampling, analysis, and assessment of PFAS under NYSDECs Part 375 Remedial Programs. October
	Ohio EPA	2020	DDAGW SOP for PFAS sampling at public water systems, Ohio EPA LOE contractors, Revision 1.1, Final. March
	Utah Department of Environmental Quality	2020	Sampling and analysis plan, Statewide PFAS monitoring Phase I: Drinking water systems. October.
	Washington Department of Ecology (Ecology)	2016	Quality assurance project plan, Statewide survey of PFAS in Washington state rivers and lakes
		2020	PFAS draft chemical action plan. Publication 20-04-035. October

Table 19. Examples of differing recommendations for PFAS sampling materials in various guidance documents

Example Topic	Guidance from CA RWQCB (2020)	Guidance from EGLE (2018)	Guidance from NAVFAC (2017) and EDQW (2017)
Markers for labeling and field notes	Acceptable to use ballpoint pens or pre-printed labels from the laboratory. Avoid regular or thick-size markers (Sharpie® or otherwise as they may contain PFAS). Acceptable to use Fine or Ultra-Fine Sharpie® markers to label empty sample bottles in the staging area provided that the lid is on the sample bottle and that gloves are changed following sample bottle labeling.	Acceptable to use ballpoint pens, pencils, and Fine or Ultra-Fine Sharpie® markers. Other markers need screening, i.e., equipment blank samples should be taken to verify that the product is PFAS-free prior to use during sampling.	Waterproof pens may contain PFAS. EDQW guidance is referenced (EDQW, 2017). Markers are prohibited; pens are recommended (EGLE, 2018).
Plastic bags	LDPE should not be used for any items that will come into direct contact with sample media (e.g., plastic bags, tubing, containers and bottles). Samples and ice should be double-bagged using LDPE bags (e.g., Ziploc®) (CA SWRCB, 2019). *Note that this 2019 guidance was replaced in 2020 with the following:* Sampling equipment that have parts made of LDPE should be avoided if the part comes in direct contact with the sample. However, if it is absolutely necessary, equipment that have parts made of LDPE may be used if an equipment blank has confirmed it to be PFAS-free. LDPE bags should be kept separate from other sampling supplies in the staging area and should not come into direct contact with the sample media. Gloves are changed after handling LDPE bags (CA SWRCB, 2020).	LDPE should not be used for any items that will come into direct contact with sample media (e.g., plastic bags, tubing, containers and bottles). However, LDPE may be used if an equipment blank has confirmed it to be PFAS-free. LDPE does not contain PFAS in the raw material but may contain PFAS cross-contamination from the manufacturing process. LDPE bags (e.g., Ziploc®) that do not come into direct contact with the sample media and do not introduce cross-contamination with samples may be used (EGLE, 2018). Surface water foam has been successfully sampled using various high density polyethylene (HDPE) bottles and polyethylene plastic bags (e.g., Ziploc®). Polyethylene plastic bags are preferred for sample collection because the wide openings facilitate the placement of surface water foam (EGLE, 2019).	Plastic bags may contain PFAS. EDQW guidance is referenced. LDPE or polypropylene containing materials (e.g., bags or containers used to transport samples) are prohibited. HDPE and silicon materials are recommended. Acetate liners are recommended for direct push technologies. Bags of ice are recommended (EDQW, 2017).

Example Topic	Guidance from CA RWQCB (2020)	Guidance from EGLE (2018)	Guidance from NAVFAC (2017) and EDQW (2017)
Glass	PFAS may adsorb to glass containers and therefore should not be used for water, leachate, or other aqueous samples. Glass containers may be used for dry or solid samples, provided that absorbed PFAS can be extracted by laboratory as part of the sample preparation procedure (CA SWRCB, 2020).	Glass bottles or containers may be used if they are known to be PFAS-free. However, PFAS have been found to adsorb to glass, especially when the sample is in contact with the glass for a long period of time (e.g., being stored in a glass container). If the sample comes into direct contact with the glass for a short period of time (e.g., using a glass container to collect the sample, then transferring the sample to a non-glass sample bottle), the adsorption is minimal (EGLE, 2018).	Drinking water samples must be collected in accordance with U.S. EPA Method 537, which requires sample collection in polypropylene bottles with a polypropylene screw cap. All other samples must be collected in an HDPE container with an unlined plastic screw cap (EGLE, 2018).

5.2.2. Synthesize results and distill key messages and guidelines (Task 4.2)

Geosyntec summarized results from the ITRC PFAS team survey and discussed the survey results internally with the project team. Geosyntec briefed the ITRC PFAS team on survey results and provided them with a general project update to keep survey recipients engaged and encourage any final contributions to the survey. Geosyntec then prepared a detailed summary of key messages for assessing bias during PFAS sampling. The summary provided key questions and topic areas that have been addressed by this project and synthesized key findings from the literature review, unpublished data, and original research being conducted by the project team. Following project team review, this document provided a comprehensive outline of topics that would be covered by an interim guidance document and other outreach materials. The project team invited Dr. Janice Willey, a senior chemist at the Naval Sea Systems Command and SERDP PFAS project technical team member, to participate on a project team conference call to share her perspective on effective outreach strategies and priorities. Dr. Janice Willey also reviewed the summary of key messages for assessing and mitigating bias during PFAS sampling and provided comments.

The project team incorporated the latest findings into a technical report (Deeb et al. 2021), including updated guidance documents from New York State Department of Environmental Conservation, Massachusetts Department of Environmental Protection, and Utah Department of Environmental Quality as well as a PFAS draft chemical action plan from the Washington Department of Ecology, each published in October 2020. An October 2020 project publication in ES&T (Schwichtenberg et al., 2020) was also referenced and integrated into the draft report. Geosyntec expanded the outline prepared during third quarter of 2020 to develop a draft written report that provides a comprehensive overview of potential PFAS bias and synthesizes the current scientific state of knowledge and guidance for PFAS sampling and presents the scientific basis for assessing potential bias (Deeb et al., 2021). The draft report was shared internally with the project team and comments were discussed on a separate team call before the report was revised, submitted again for final team comments, and finalized for submittal to SERDP/ESTCP. The draft report was finalized and submitted to SERDP/ESTCP SEMS management system in January 2021 (Deeb et al., 2021).

The technical report summarized key findings and presented the following findings and recommendations to improve PFAS sampling guidance documents:

- Based on our review of scientific studies and consideration of potential pathways for sample cross-contamination, many PFAS sampling restrictions in current guidance are based on the precautionary principle rather than on scientific merit. A limited number of restrictions and recommended best practices are substantiated by scientific studies. Some guidance documents unnecessarily restrict the use of materials and equipment in the field that are never in direct contact with water inside sample bottles and have no credible pathway for biasing sample results.
- In general, earlier sampling guidance (produced prior to 2018) was more precautionary and restrictive of materials that could be used during PFAS field sampling. This approach was beneficial because it bolstered confidence in sampling results, reduced the need for discussions regarding the acceptability of data for decision-making, and avoided the potential need to re-sample or to collect additional samples. However, some of the earlier

and more restrictive precautions still remain in use.

- Guidance that is highly specific and restrictive increases the time and effort required for fieldwork planning and implementation, likely resulting in higher cost and more waste generated.
- Sampling guidance can be improved by differentiating between the limited field practices and equipment that are scientifically known to result in PFAS detections in laboratory tests (e.g., PTFE bailers or tubing) from those that do not.
- Current sampling protocols already provide an additional layer of sample protection by specifying glove changes prior to the collection of each sample and the collection of field equipment blanks.

5.2.3. Outreach and translation (Task 4.3)

In addition to publishing the technical report (Deeb et al., 2021), the project team also published several peer-reviewed papers and conducted multiple platform and poster presentations. A summary of outreach activities is provided as **Appendix B**.

6. CONCLUSIONS

This project contributed original research results and summarized results from previously published and unpublished datasets to evaluate several potential sources of bias in PFAS sampling results.

Findings from laboratory and field research into stratification of PFAS in groundwater and surface waters were presented in Section 2. Results indicate that PFAS stratification does occur at the air/water interface and that PFAS preferentially partition into naturally-occurring foam that may form on some surface water bodies due to the presence of organic compounds in surface waters. In groundwater wells, however, stratification was not significant enough to affect sample concentrations, even under conditions when there is no mixing within the water column prior to sampling. PFAS enrichment at the surface microlayer has been demonstrated using sampling techniques such as a glass plate method. The project team hypothesized that PFAS accumulation in the surface microlayer could affect surface water PFAS sampling results, given that some sampling methods may over-represent the surface microlayer. For example, filling a sample bottle by repeatedly skimming the top of a shallow water body to avoid collecting sediments could have an elevated PFAS concentration relative to sampling that used a peristaltic pump and tubing to extract bulk surface water from a depth of a few inches. However, field sampling results demonstrated that there was no statistically significant difference between samples collected using different techniques. That is, results indicate that the variability in sampling results caused by an over-representation of the surface microlayer is within the range of sample variability due to other factors including analytical variability and spatial distribution of PFAS in surface waters (i.e., variability in field duplicate sample results).

As described in Section 3, the project team's review of various PFAS sampling guidance documents, scientific publications, and unpublished datasets of equipment testing and equipment field blank results demonstrated that there are potential sources of bias during PFAS sampling that have already been recognized and addressed in current guidance documents. Examples include the use of pumps with Teflon™ or PTFE components that have the potential to contribute PFAS to the sample. The project team did not identify other potential sources of sample bias that have not been recognized and included in current guidance documents.

Section 4 summarizes information on potential bias to PFAS sampling results that could result from extended hold time or elevated temperatures. With the publication of U.S. EPA Method 1633, hold times are longer than previously specified under most laboratory SOPs for modified method 537 methods. This change has provided practitioners with more flexibility to analyze some samples from a field event first and hold other samples until the initial set of analytical results are available.

Section 5 describes project team outreach of key results and recommendations. After reviewing the scientific basis for guidance document restrictions, the project team concluded that existing sampling guidance can be overly cautious; that is, materials, equipment and products may be restricted in PFAS sampling guidance without regard to whether a pathway has been demonstrated for those materials, equipment, or products to come into contact with the sample. Because the precautions prescribed by guidance documents increase PFAS field sampling time and expense,

and generate additional waste, guidance document updates are recommended to focus on targeted and common-sense precautions to maintain sample quality. Guidance documents should continue to restrict sampling equipment, materials, and products that are in direct contact with a sample and have the potential to bias PFAS concentrations. Guidance documents should describe and rely on industry standard best practices for environmental sampling and sample handling to avoid incidental contamination from other sources (e.g., field sampler clothing). Finally, guidance documents could be improved by providing more information on equipment decontamination procedures to prevent carryover from one sampling location to another.

By adopting this approach, regulatory agencies will encourage consistency throughout the industry and improve the focus on using common-sense best practices and preventing common pathways for potential sample contamination. Additional details on key findings are presented in a SERDP project ER19-1205 technical report (Deeb et al., 2021).

7. LITERATURE CITED

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APPENDIX A. SUPPORTING DATA

Relevant supporting data from project team activities are summarized as supplemental information for published and accepted peer-reviewed publications. Citations for peer-reviewed publications are provided in Appendix B.

APPENDIX B. PROJECT PUBLICATIONS AND PRESENTATIONS

Over the course of the project, the team prepared multiple presentations, publications, and reports submitted to SERDP, as described below.

Peer-Reviewed Publications

Roark, S.A., Fallon, A., Struse, A., Rectenwald, H., Bogdan, D., Heron, C., and Field, J. **Accepted**. Inclusion of the surface microlayer does not bias surface water bulk water PFAS concentrations. *Int. Environ. Assess. Manage.*

Schaefer, C.E.; Lemes, Maria C.S., Schwichtenberg, T. and Field, J.A. Enrichment of poly- and perfluoroalkyl substances (PFAS) in the surface microlayer and foam in synthetic and natural waters. *Journal of Hazardous Materials* **2022**, 440: 129782. (DOI:10.1016/j.jhazmat.2022.129782).

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Technical Reports and Other Publications

Wanzek, T., McIntyre, H., Hawley, E., Deeb, R., Bogdan, D., Schaefer, C., DiGuiseppi, B., Struse, A. and Field, J. **Accepted.** Assessing potential bias in PFAS concentrations in groundwater and surface water samples, *Ground Water Remediation Journal.*

Deeb, R. A., Hawley, E.L., Sayler, C., Bogdan, D., Schaefer, C.E., DiGuiseppi, B., Struse, A., Field, J.A., and Schwichtenberg, T., Assessing the potential for bias in PFAS concentrations during groundwater and surface water sampling. SERDP Technical Report, May **2021**.

Platform and Poster Presentations

Schwichtenberg, T., Field, J.A., DiGuiseppi, B., Deeb, R., Hawley, E., Schaefer, C., Drennan, D., Nguyen, D. and Bogdan, D., "Assessing and mitigating factors that impact measured PFAS levels during ground and surface water sampling (ER19-C2-1205)", poster presentation at the SERDP/ESTCP PFAS Technical Meeting, July **2019**.

DiGuiseppi, B. "Much ado about (almost) nothing: Field sampling materials unlikely source of PFAS contamination", platform presentation at the Emerging Contaminants Summit in Denver, Colorado, March **2020**.

Field, J.A. "Assessing and mitigating bias in PFAS levels during groundwater and surface water sampling (C2-1205)", virtual presentation to Interstate Technology and Research Council (ITRC) PFAS Team, July **2020**.

Field, J.A., "Assessing and mitigating bias in PFAS levels during groundwater and surface water sampling (C2-1205)", presentation to SERDP/ESTCP PFAS Technical Meeting, July 28, **2020**.

Schwichtenberg, T., Field, J.A., DiGuiseppi, B., Deeb, R., Hawley, E., Schaefer, C., Drennan, D., Nguyen, D. and Bogdan, D., "Assessing and mitigating factors that impact measured PFAS levels during ground and surface water sampling (ER19-C2-1205)", virtual poster presentation at the SERDP/ESTCP PFAS Technical Meeting, July **2020**.

Field, J.A. "Characterizing PFAS enrichment in naturally occurring surface water foams", virtual talk at the National Environmental Monitoring Conference (NEMC), August 17, **2020**.

Field, J.A. "PFAS enrichment in foams on a Northern U.S. freshwater lake", virtual poster presented at the Oregon State Clean Water Virtual Conference, September 1, **2020**.

Field, J.A. "PFAS and dissolved organic carbon enrichment in surface water foams on a Northern U.S. freshwater lake", virtual technical presentation to Interstate Technology and Research Council (ITRC), September 22, **2020**.

Schwichtenberg, T. "PFAS and dissolved organic carbon enrichment in surface water foams on a Northern U.S. freshwater lake", virtual poster presented at the University of Rhode Island Sources, Transport, Exposure & Effects of PFAS (STEEP) PFAS in our World Virtual Conference, October 14, **2020**.

Field, J.A. "New pieces of the PFAS puzzle", invited virtual presentation at the Seminar for the University of North Carolina. October 23, **2020**.

Field, J.A., "Assessing and mitigating bias in PFAS levels during groundwater and surface water sampling (ERSON-C2-1205)", platform presentation at the SERDP/ESTCP Virtual Symposium, December 3, **2020**.

Field, J.A., "Assessing and mitigating factors that impact PFAS levels during ground and surface water sampling (ER19-1205)", poster presentation at the SERDP/ESTCP Virtual Symposium, November 30 – December 4, **2020**.

Field, J.A., "Curious incidences of PFAS at environmental interfaces", invited virtual presentation at the Seminar for Stockholm University. January 26, **2021**.

Field, J.A. Keynote, "PFAS Analytical Challenges and Opportunities", The RemTEC Summit, March 9-11, **2021**.

Field, J.A. Assessing and Mitigating PFAS Sampling Bias, SERDP ESTCP Technical Webinar #138, June 27, **2021**.

Hawley, E.L., Deeb, R., Bogdan, D., DiGuiseppi, B., Struse, A., Rectenwald, H., Schaefer, C., Schwichtenberg, T. and Field, J. "Assessing and mitigating bias in PFAS sampling", virtual platform presentation at Emerging Contaminants in the Environment Conference (ECEC), University of Illinois Urbana-Champaign, April 28, **2022**.

Hawley, E.L., Deeb, R., Bogdan, D., DiGuiseppi, B., Struse, A., Rectenwald, H., Schaefer, C., Schwichtenberg, T. and Field, J. "Assessing and mitigating bias in PFAS sampling", platform presentation at GRA Western Groundwater Congress, September 20, **2022**.

Hawley, E.L., Schwichtenberg, T., Struse, A., Deeb, R., Bogdan, D., DiGuiseppi, B., Rectenwald, H., Schaefer, C., and Field, J. "Assessing and mitigating bias in PFAS sampling", workshop at RemTEC & Emerging Contaminants Summit, October 4-6, **2022**.

Field, J. "Assessing and mitigating bias in PFAS sampling results", PFAS sampling and analysis: A closer look at data defensibility, best practices and interpretation of results. Geosyntec Technical Webinar Series, August **2023**.

Other

Invited review of NIOSH PFAS Topic Page (J Field, January 15, **2021**).