Characterization of the Fate and Biotransformation of Fluorochemicals in AFFF-Contaminated Groundwater at Fire/Crash Testing Military Sites

SERDP Project ER-2128

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ABBREVIATIONS AND ACRONYMS

AEC  Anion exchange capacity
AFB  Air Force Base
AFFF  Aqueous film forming foam
CEC  Cation exchange capacity
CO₂  carbon dioxide
DGBE  Diethylene glycol butyl ether
ECF  Electrofluorination
FAB-MS  Fast atom bombardment mass spectrometry
FOSA  Perfluorooctane sulfonamide
FtB  Fluorotelomer betaines
FTCA  Fluorotelomer carboxylates
FT-ICR  Fourier transform ion cyclotron resonance
FTSaB  Fluorotelomer sulfonamido betaine
FTAoS  Fluorotelomer thioamido sulfonate
FTSAO₂AoS  Fluorotelomer sulfoxide amido sulfonate
FTSAO₃AoS  Fluorotelomer sulfone amido sulfonate
FTTHN  Fluorotelomer thio hydroxy ammonium
FTTPA  Fluorotelomer thioether propanyl alaninate
FTSaAm  Fluorotelomer sulfonamide amine
FTSA  Fluorotelomer sulfonate
FTUCA  Fluorotelomer unsaturated carboxylate
HCl  Hydrochloric acid
HPLC  High performance liquid chromatography
Kd  Soil:water partition coefficient
Kf  Freundlich sorption coefficient
Koc  Organic carbon:water partition coefficient
LC-MS/MS  Liquid chromatography tandem mass spectrometry
MilSpec  Military specification
N₂  Nitrogen
NaOH  sodium hydroxide
NH₄OH  Ammonium hydroxide
pKa  Acid dissociation constant
PFASs  Perfluorooalkyl substances
PFBA  Perfluorobutanoate
PFBS  Perfluorobutane sulfonate
PFCA  Perfluorinated carboxylate
PFDA  Perfluorodecanoate
PFDoA  Perfluorododecanoate
PFDS  Perfluorodecane sulfonate
PFetS  Perfluoroethane sulfonate
PFHpA  Perfluoroheptanoate
PFHpS  Perfluoroheptane sulfonate
PFHxA  Perfluorohexanoate
PFHxS  Perfluorohexane sulfonate
PFHxSA  Perfluorohexane sulfonamide
PFNA  Perfluorononanoate
PFNS  Perfluorononane sulfonate
PFOA  Perfluorooctanoate
PFOS  Perfluorooctane sulfonate
PFPeA Perfluoropentanoate
PFPeS Perfluoropentane sulfonate
PFPrS Perfluoropropane sulfonate
PFSA  Perfluoroalkyl sulfonate
PFSaAm Perfluoroalkyl sulfonamido amine
PFSaAmA Perfluoroalkyl sulfonamide amino carboxylate
PFTrA Perfluorotridecanoate
PFTeA Perfluorotetradecanoate
PFUnA Perfluoroundecanoate
QPL   Qualified products list
QTOF  Quadrupole time of flight
rDNA  Ribosomal deoxyribonucleic acid
TCE   Trichloroethene
TOP   Total oxidizable precursor

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EXECUTIVE SUMMARY

Objectives: The overall objective of this study is to fully delineate the per- and polyfluoroalkyl substances (PFASs) that persist in aqueous film-forming foam (AFFF)-contaminated groundwater, sediment, and soil and evaluate their impact on priority pollutant biotransformation. We intend to characterize the composition of individual PFASs and their precursors in AFFF formulations and delineate the total organic fluorine concentrations of AFFF-contaminated groundwater, sediment, and soils. To achieve this overall goal, we had the following technical objectives (Tasks):

1. Characterize the concentration/composition of individual PFASs and their precursors in AFFF formulations.
2. Characterize the individual PFASs and total organic fluorine composition of AFFF-contaminated groundwater, sediment, and soil at military fire-training sites; determine field-based estimates of PFAS transport; and evaluate the spatial relations with priority pollutant distributions in groundwater at military fire-training sites.
3. Determine the potential for the biotransformation of partially-fluorinated substances and AFFF formulations and the impact of AFFF and its components on TCE transformation under redox conditions that are representative of groundwater at fire-training sites.
4. Characterize the sorption of cationic and zwitterionic PFASs to soils and sediments.

Technical Approach. For Task 1, the PFAS composition of 3M and fluorotelomer-based AFFFs was determined using a number of mass spectrometric approaches. The total oxidizable precursor assay was adapted for use with AFFF-contaminated groundwater, soil, and sediment. For Task 2, the developed analytical tools were applied to environmental samples from US military bases. Microcosm experiments were performed for Task 3 to determine the biotransformation pathways of polyfluoroalkyl substances in fluorotelomer-based Ansul AFFF under anaerobic and aerobic conditions. The impact on TCE reductive dechlorination was also evaluated in laboratory batch microcosm experiments. For Task 4, a series of laboratory batch sorption experiments were conducted using a fluorotelomer-based AFFF from National Foam, which contained anionic, zwitterionic, and cationic PFASs, on six soils that varied in physical properties.

Results. Over 50 classes of PFASs, comprised of several individual homologs, were identified in AFFF formulations and groundwater over the course of this project. The composition of AFFFs was defined as either 3M or fluorotelomer-based AFFFs (Ansul, Chemguard, National Foam, Buckeye Fire Equipment, and Angus) with dates of manufacture dating back to 1989. Many of the newly-identified classes are 3M-derived and are cationic or zwitterionic. The TOP assay was modified for use with AFFF-contaminated media and precursors make up a significant fraction of total PFASs in groundwater, soil, and sediment. However, perfluoroalkyl carboxylates (PFCAs) (e.g., perfluorooctanoate, PFOA), perfluoroalkyl sulfonates (PFSAs) (e.g., perfluorooctane sulfonate, PFOS), and fluorotelomer sulfonates (FTSAs) remain the most abundant individual PFASs in AFFF-contaminated groundwater. The biotransformation of fluorotelomer thioamido sulfonates (FtTAoS) occurs under anaerobic and aerobic conditions; however, the two conditions produce different transformation products. Aerobic conditions produced FTSAs; however, they were further transformed to PFCAs. Some AFFFs and the solvent present in AFFF, diethyl glycol butyl ether (DGBE) promoted reductive dechlorination of trichloroethene when provided as the sole carbon and energy source. Low $K_d$ values for the anionic 6:2 FTSA in sorption experiments are consistent with the detection and mobility of 6:2
SA is groundwater. Higher $K_d$ values indicate that the anionic 8:2 FTSA, zwitterionic FTSaBs, and cationic 6:2 fluorotelomer sulphonamido amine (FTSaAm) are more likely to be associated with soil and sediment of source zones. Complete removal of the cationic FTSaAm indicates potential for strong sorption to source zone soils and sediments at some sites. The lack of correlations between the sorption of anionic FTSAs, zwitterionic FTSaBs, and cationic 6:2 FTSaAm and parameters including organic carbon content, CEC, and AEC, indicates that the bulk parameters do not adequately predict sorption. More information is needed on the conditions (e.g. pH and ionic strength) that promote desorption of zwitterionic and cationic PFASs in order to determine the potential for source zone soils and sediments to act as long-term PFAS sources.

**Benefits:** The analytical tools developed for this project, including methods for quantifying individual PFASs as well as precursors by the total oxidizable precursor (TOP) assay, we have provided analytical advances for more complete characterization of AFFF-contaminated media. Using these tools, we have generated information that has significantly improved our understanding of the PFASs present in groundwater, sediment, and soil at AFFF-contaminated sites. Having identified precursors at AFFF-contaminated sites, efforts now focus on understanding the process that retain PFASs in source zones and the conditions that may mobilize them. Identifying precursors will lead to a better of understanding of the effectiveness of treatment technologies, such as the use of granulated activated carbon and other sorbents for their removal. The biotransformation pathway of the polyfluorooalkyl substances in Ansul AFFF provides a framework for understanding the fate of the precursor and insight into the conditions (anaerobic) that lead to high concentrations of persistent FTSAs and the potential for intermediates to be ultimately transformed to persistent PFCAs.

**Transition Plan:** We worked closely with Wellington Laboratories to identify and name new PFASs and to advocate for the synthesis of high quality authentic standards for perfluoroethane sulfonate (PFEtS). We participated in a number of webinars with DoD participants. Information gained from this project was used to inform current SERDP and ESTCP projects including SERD ER- ER-2720 and ESTCP 201633.
OBJECTIVES and BACKGROUND

PROJECT OBJECTIVE AND TASKS

The overall goal of this project is to better understand the occurrence, behavior, and transport of per- and polyfluoroalkyl substances (PFASs) that are associated with aqueous film forming foam (AFFF) and AFFF-contaminated groundwater, sediment, and soil at military sites where fire-training activities or crashes have occurred and to evaluate their impact on trichloroethene (TCE) biotransformation.

To achieve this overall goal, the four specific tasks of this SERDP project are to:

1. characterize the concentration/composition of individual PFASs and their precursors in AFFF formulations,
2. characterize the individual PFASs and total organic fluorine composition of AFFF-contaminated groundwater, sediment, and soil at military fire-training sites and to evaluate the spatial relations of PFASs with priority pollutants at military fire-training sites, and
3. determine the biotransformation of partially-fluorinated substances and other AFFF formulation components and the relation with TCE transformation under redox conditions relevant to fire-training/crash sites.
4. characterize the sorption of cationic and zwitterionic PFASs

This final report for SERDP ER-2128 describes the activities conducted to complete **Task 1** (characterization of AFFF), **Task 2** (groundwater, sediment/soil characterization and spatial relations of PFASs and priority pollutants at a field site), **Task 3** (biodegradation of AFFF components in relation to TCE biodegradation), and **Task 4** (sorption of cationic and zwitterionic PFASs). Completion of these tasks addresses the Statement of Need (ERSON—11-02) because the information provided will improve our fundamental understanding of the identity, fate, and transport of PFASs at AFFF-contaminated at military sites and their impact on the biotransformation of the chlorinated solvent, TCE.

BACKGROUND

**Task 1.** From the early 1960s until present, Military Specification (MilSpec) requirements and AFFF formulations sold on MilSpec have changed. The original MilSpec specified only performance parameters (e.g., surface tension) but ‘environmental limits’ were added in 1977 with upper limits set < 500,000 mg/L for biological and chemical oxygen demand. By 1981, toxicity limits were in place. Two main types of AFFFs (6% and 3%) are qualified under US MilSpec. The composition of the 3 and 6% types are similar for a given manufacturer, with the difference being the degree of dilution required to achieve the performance speculations. For example, 6% AFFF product must be diluted to 6% with water and 3% AFFF formulations must be diluted to 3% with water. The U.S. Navy developed and uses the equipment necessary to deliver 6% AFFF while the US Air Force primarily uses equipment aimed at delivering 3% AFFF. MilSpec history indicates that AFFFs were sold by 3M from the mid 1960 to the early 1970s and were the sole source of AFFF to the US military. In 1973, National Foam introduced a MilSpec AFFF and in 1976 Ansul qualified an AFFF for MilSpec while it wasn’t until 1994 that other fluorotelomer-based AFFFs were introduced by Angus, Chemguard, Buckeye, and Fire Service Plus. In 2015 and 2016, AFFFs were placed on the QPL by ICL, Tyco/Ansul,
OBJECTIVES and BACKGROUND

Amerex/Solberg.\textsuperscript{8,9}

Once water is added, the 3 and 6\% AFFF formulations for a given manufacturer give similar PFAS concentrations in the final diluted product. From 1970 to present, multiple manufacturers released multiple AFFFs that met MilSpec and that were placed on the qualified products list (QPL).\textsuperscript{8,9} At the onset of this SERDP project it was not known if field sites would be contaminated by a wide array of PFASs or only a few. Therefore, Task 1 was designed to reverse engineer the proprietary PFAS composition of AFFF formulations stockpiled and actually used at field sites using multiple complementary analytical approaches.

Task 2. After use in firefighting and in training exercises, AFFF waste frequently was discharged to surface-holding ponds or groundwater along with residual solvents that were not consumed during combustion. On the basis of a limited amount of monitoring data, it appears that perfluorooctanoate (PFOA), perfluorooctane sulfonates (PFOS), and Fluorotelomer sulfonates (FTSAs) are transported in groundwater from the initial locations of contamination. For example, our previous monitoring efforts\textsuperscript{10} at Tyndall AFB, Wurtsmith AFB, and NAS Fallon indicate very high concentrations (e.g., 14.6 mg/L) within 50-120 m of fire-training pads, with transport up to 540 m with concentrations of 4-9 \(\mu\)g/L that remain above health advisory levels of 1 \(\mu\)g/L.\textsuperscript{11} These areas also may serve as reservoirs for fluorinated compounds because partially-fluorinated precursors of the more persistent forms have the potential to adsorb onto soils and aquifer materials. For example, amphoteric surfactants have cationic functionalities that may undergo cation exchange reactions onto sediments and soils. Furthermore, the high biological oxygen demand loads entering the subsurface are likely to have resulted in strongly reducing conditions in the areas of highest contamination. Under such conditions it is likely that many of the biological processes that could result in release of the persistent perfluoroalkyl carboxylates (PFCAs) (e.g., PFOA), perfluoroalkyl sulfonates (PFSAs) (e.g., PFOS), and FTSA forms will slow down. Thus, any effort to remediate AFFF-contaminated groundwater would need to address contaminated source zones even if they do not contain elevated concentrations of PFOA, PFOS, and FTSA. To assess the potential for source zones to serve as reservoirs of precursors, Task 2 was designed to measure concentrations of PFASs and the precursors in groundwater and soils and sediments collected from areas immediately adjacent to locations where runoff from fire-fighting activities was discharged to the subsurface.

Task 3. Previous research has established the fact that PFCAs and PFSAs are stable with respect to biotransformation. Available data also suggest that PFSAs do not undergo biotransformation at appreciable rates, but the number of prior studies that investigated this issue is limited, and no prior studies address the anaerobic conditions typically encountered in AFFF-contaminated groundwater. FTSAs and related compounds (e.g., fluorotelomer thioamidosulfonates or FTTAoS) contain functional groups that are amenable to biotransformation. The FTSAs undergo aerobic biotransformation to form PFCAS.\textsuperscript{12} Only recently were FTSAs to be persistent under anaerobic conditions.\textsuperscript{13} Prior to the onset of this project, no information was available on the biotransformation of FTTAoS but formation of FTSAs from FTTAoS has been proposed.\textsuperscript{10} Therefore, Ansul AFFFs that contained FTTAoS and 3M AFFF were identified for study in this project.

The rates of transformation and the identity of the microbial communities responsible for these reactions and product yields have never been investigated. To date, a few studies report the

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biologically-mediated production of PFOS from partially-fluorinated sulfonamide-based substances. However, potential abiotic processes were not investigated and the associated microbial community was not characterized. Therefore, because it is critical to understand the mechanisms of AFFF transformation in the subsurface under a variety of relevant environmental conditions and because it may be advantageous to stimulate key reactions prior to application of other remedial approaches (e.g., activated carbon), we will evaluate the biodegradability of PFASs within AFFF and associated solvents under redox conditions representative of groundwater at fire/crash training sites. Task 3 was to determine the biodegradability of polyfluoroalkyl substances in the AFFF formulation produced by Ansul under anaerobic and aerobic conditions and in the presence of TCE.

The application of AFFFs to extinguish chlorinated solvent-based fires resulted in co-contamination of PFASs and chlorinated solvents, such as TCE, in groundwater and soil. Although reductive dechlorination of TCE by Dehalococcoides mccartyi is a frequently-used remediation strategy, the effects of AFFF and PFASs on TCE dechlorination are not well understood. AFFFs contain PFASs and non-fluorinated hydrocarbon surfactants and one or more glycol ether-based solvents (8-20%) that were used to quickly verify that the target mixing ratios were achieved prior to the application of AFFF to fires. Historically, the most commonly used solvent was diethylene glycol butyl ether (DGBE). Because chlorinated solvents were frequently used to create fires for fire-fighter training exercises, AFFF waste water must have been comprised of PFASs and unburned solvents, such as TCE. Thus, many fire-fighter training areas are likely co-contaminated by TCE and PFASs.

In situ remediation strategies for TCE-contaminated groundwater is conducted by promoting the reductive dechlorination of TCE by adding substrates that ferment to generate hydrogen and acetate. Repeated AFFF applications at training sites resulted in the repeat addition of AFFF containing fermentable substrates, such as the DGBE and, in doing so, created reducing conditions in subsurface environments that support TCE reductive dechlorination by D. mccartyi. Prior to the onset of this project, the impacts of AFFF on dechlorination by D. mccartyi had not been described. To address this data gap in Task 3, we investigated the impacts of three AFFF formulations and of DGBE by itself on the reductive dechlorination of TCE.

Task 4. The sorption of PFCAs and PFSAs increases with the organic carbon content in soils and sediments, as measured by solid-water partition coefficients ($K_d$) and organic carbon normalized solid-water partition coefficients ($K_{oc}$). When organic carbon is low (0-0.78%), PFSA and PFCA sorption is due to electrostatic interactions with mineral phases. Within the PFCA or PFSA homologous series, longer-chained homologs have higher partition coefficients than short-chained homologs.

In contrast, the anionic FTSAs and zwitterionic and cationic PFASs present in groundwater, soil, and sediments have received little attention. Two studies report field-derived $K_d$ values for the 6:2 FTSA, while only one study reports field-derived $K_d$ values for the zwitterionic 6:2 fluorotelomer sulfonamido betaine (FTSaB) and the cationic 6:2 fluorotelomer sulfonamido amine (FTSaAm).

In laboratory batch sorption experiments with PFCAs and PFSAs, multi-compound studies are executed under controlled conditions. However, a similar experimental set-up for sorption...
OBJECTIVES and BACKGROUND

of zwitterionic and cationic PFASs is challenging due to the lack of authentic standards and mass-labeled internal standards for the zwitterionic and cationic PFASs, and few reference materials are available. Furthermore, mass balance of the PFCAs and PFSAs is typically achieved by extracting the soil or sediment. To date, all studies examining concentrations of zwitterionic and cationic PFASs on soil and sediment have relied on a similar extraction method, which has only been validated with methanolic standards added to dry soil. However, previous studies of organic cations in soil and sediment have suggested that stronger extraction conditions are typically necessary, particularly if the cations have been allowed to equilibrate with the soil and/or sediment. If a stronger soil extraction holds true for zwitterionic and cationic PFASs, then both concentrations and field-derived $K_d$ estimates of zwitterionic and cationic PFASs may be significantly underestimated due to incomplete extraction of the (presumably) equilibrated soil and sediment.

Due to the complexity of AFFFs and AFFF-impacted field sites, one study attempted to simulate the conditions of an AFFF-impacted fire training area by conducting sorption experiments with a mixture of PFCAs and PFSAs with and without a non-aqueous phase liquid. Several studies have examined the impact of PFCA and PFSA sorption on the presence of ionized hydrocarbon surfactants, which only partially represents an AFFF discharge event. One study found an increase in the sorption of a single compound, PFOS, in the presence of a single cationic surfactant, while a single anionic surfactant decreased sorption of PFOS (single compound). However, a single sorbate was probed by a single surfactant, which may have a greater sorption impact than the presence of many sorbates (i.e. an AFFF). By contrast, a different anionic surfactant caused no change or a slight decrease in sorption for long-chained (n ≥ 6) perfluoroalkyl acids when present in a mixture of PFASs. However, the scenario doesn’t fully capture the complexity of AFFFs, which contain various solvents and a suite of hydrocarbon surfactants in addition to a mixture of PFASs.

To best replicate the first application of AFFF to pristine soils at a field site, National Foam AFFF, which contains anionic FTSAs, zwitterionic FTSaBs, and cationic FTSaAms (Fig. 2), was selected for use in batch sorption experiments. National Foam AFFF was chosen since it was approved for use by the U.S. military since 1976 and contains a diverse array of anionic, zwitterionic, and cationic PFASs. To determine the soil properties that drive sorption of the anionic FTSAs, the zwitterionic FTSaBs, and the cationic 6:2 FTSaAm, blank soils were selected to encompass a range of organic carbon, cation exchange capacity (CEC), and anion exchange capacity while constraining soil pH to ~ pH 5. Since the FTSaBs and the 6:2 FTSaAm have ionizable functional groups that change speciation with pH (Fig. 2), ion exchange may drive sorption in a manner analogous to that of zwitterionic pharmaceuticals given these data gaps, Task 4 was to characterize the sorption behavior of cationic and zwitterionic on soils/sediments and Task 5 was to model the sorption characterized in Task 4.
METHODS and MATERIALS

MATERIALS AND METHODS

Task 1: Characterize AFFF Formulations

Donald Warner, the Fire Chief of the Air Force, assisted our SERDP project by helping to organize the collection of AFFF samples from Air Force bases around the United States. With his assistance, we received shipment of 65 individual samples of AFFF that are currently stored at Air Force and Naval Bases in the United States. We now have a complete archive of AFFF formulations that are listed on the QPL that meet current Mil Spec. There are six manufacturers of AFFF that have products on the QPL including 3M (although their AFFF formulations are no longer manufactured), Angus, Ansul, Buckeye, Chemguard, and Kidde Fire Fighting. In addition, we have learned that several bases received AFFF formulations that were not listed on the Mil Spec, including First Strike and FireAid.

The strategy for Task 1.1 was first to define the composition of AFFF formulations by fast atom bombardment mass spectrometry (FAB/MS) and LC with high mass accuracy quadrupole time of flight (QTOF) MS (Fig 1). FAB/MS was used to characterize the major PFASs and their respective fluorinated chain-length distributions in AFFF formulations. FAB/MS is rapid, inexpensive, and ideally suited for the detection of surfactant classes. LC-QTOF analyses were performed to determine the identity of unknown classes of PFASs in AFFF formulations. The identification of the newly-identified PFASs was then checked against patent information on AFFFs. LC-MS/MS, the most commonly applied analytical technique for the determination of PFASs, was used to identify the minor classes of PFASs in the AFFF formulations.

After the 2010 publication by Place et al., additional research was undertaken to identify unknown PFASs in AFFFs and groundwater. High-mass accuracy QTOF mass spectrometry was applied to samples of AFFF and groundwater. An ABS Science Triple TOF 5600 instrument, fitted with electrospray ionization was operated in negative ion mode, was used for measurements was located at the Colorado School of Mines, under the direction of Dr. Christopher Higgins (PI, SERDP ER-2126).

For Task 1.1, an extraction method was developed for the analysis of groundwater for the array of newly-identified PFASs. Preliminary work indicated that large volume, direct injection does not provide benefits for PFAS analysis over solid phase extraction. However, we found that direct aqueous injection was not appropriate for water samples containing C8 and longer-chained...
PFASs because the longer-chained forms partition to the air-water interface or to autosampler vials while samples are waiting for analysis. Because PFASs identified in Task 1.1 ranged in chain length from two carbons up to 14 carbons, new extraction and separation approaches were needed, since hydrophobic interactions with C-18-based solid phase extraction media and analytical columns is not sufficient to isolate and separate short-chained PFASs. We found that by adding salt and acidifying a small (3 mL) volume of groundwater, a small volume of organic solvent (ethyl acetate and trifluoroethanol) efficiently extracted anionic, zwitterionic, and cationic PFASs. In addition, an ion-exchange separation system was developed to concentrate short-chained PFASs with various charged (anionic, zwitterionic, and cationic) head groups. An orthogonal chromatographic system was developed for the separation and quantification of 49 individual PFASs found in AFFFs. The extraction and chromatographic methods where optimized, validated, and applied for analysis of groundwater samples for task 2.1.

For Task 1.2, the total oxidizable precursor (TOP) assay was developed and then applied to characterize the production of dead-end products of the partially-fluorinated precursors present in the AFFF formulations. The US military AFFF formulations were analyzed by the TOP assay to determine precursor concentrations by first diluting the AFFF formulations 10,000 fold and then oxidizing them with potassium persulfate under highly basic conditions in the presence of heat. The hydroxyl radicals produced during the oxidation step are unselective oxidants that are capable of oxidizing almost every type of organic compound. PFCAs and PFSAs are among the limited number of compounds that are essentially unreactive with hydroxyl radical. Thus, measurements of the increase in concentrations of PFOS, PFOA, and related compounds after the oxidation step should provide a measure of the concentrations of precursors.

Because the zwitterionic and cationic PFASs were discovered during this project, extraction methods for sediment/soil needed to be developed for these PFASs. Existing methods for soils and sediments were adapted to include the newly-discovered PFASs. A number of individual standards were added to analytical methods and the conversion of individual standards to PFCAs under TOP assay conditions was assessed.

Task 2: Characterize Groundwater, Soil, and Sediment at AFFF-Contaminated Sites

For Task 2.1, a limited number of groundwater samples collected from Ellsworth and Randolph Air Force Bases (AFB) were analyzed by the quantitative analytical method developed for Task 1.1.

For Task 2.2, Groundwater (n=26) was collected in 2011 from a 1200 m by 600 m area encompassing the burn pit. The groundwater depth was 2 to 8 m below ground surface. Groundwater was stored at 4°C prior to analysis. Twenty-two groundwater samples were analyzed. A 500-µL aliquot of groundwater collected from 5-cm below the liquid surface was added to 500 µL of methanol in a 2-mL microcentrifuge tube. Samples were centrifuged at 15,000 rpm for 5 min. Stable-isotope labeled internal standards were added to groundwater after the final dilution.

Soil (n=16) samples were collected 0.6 m below ground surface and sediments (n=10) were collected approximately 5 to 6 m bgs. The soil extraction method was similar to the approach used by Higgins et al. Briefly, a 500 mg soil sample was combined with 2.5 mL of 0.1%
ammonium hydroxide (NH₄OH) in methanol and mixed. The mixture was centrifuged and the supernatant transferred. The extraction was repeated two more times. The combined extract was evaporated to dryness and then reconstituted in 1.5 mL of 0.1% acetic acid in methanol. Cleanup was achieved by passing the extract through ENV1-CARB. For Task 2.2, soil and sediment extracts and TOP assay samples were analyzed by LC-MS/MS as described in Houtz et al.¹

For the TOP assay, groundwater was combined with 60 mM potassium persulfate in 0.125 M NaOH followed by heating for 6 h at 85°C. Soil and sediment extracts were evaporated to dryness with N₂ before adding 6 mL of 60 mM persulfate and 0.125 M NaOH before heating for 6 h at 85°C. After reaction, all samples were neutralized with concentrated HCl and amended with methanol and internal standards prior to analysis by LC-MS/MS.¹ for treatment of the TOP assay day, the total molar concentration of PFCAs produced by oxidation was reported as total PFAS concentrations.

For Task 2.3, our team members participated in a field study at Ellsworth AFB in collaboration with Chris Higgins, the PI on SERDP project ER-2126, in which the PFAS occurrence and distribution was described. Individual PFASs and the TOP assay precursors were mapped.¹⁶

**Task 3: Biotransformation of AFFF PFASs and Impact on TCE Biotransformation**

**Task 3.1 and 3.2 Anaerobic Microcosms with Ansul AFFF**

Anaerobic microcosms were constructed to test for potential AFFF biotransformation under different electron acceptor regimes that are comparable to those that occur in deep groundwater or sediments.

**Chemicals and Standards.** The Ansul AFFF used in this study contained 4:2, 6:2, and 8:2 FTTOaoS as the primary PFASs and was obtained from the US Air Force.⁶ Unlabeled and stable-isotope standards for PFCAs, PFSAs, and 6:2 FTSA were purchased from Wellington Laboratories (Guelph, Ontario, CA), and Zonyl FSA was purchased from Sigma-Aldrich. Zonyl FSA is a proprietary mixture containing n:2 (n = 6, 8, 10) fluorotelomer thioether propionate (FTTPA). High performance liquid chromatography (HPLC)-grade water and methanol were purchased from Fisher Scientific. All other chemicals and solvents were purchased from either Fisher Scientific or Sigma-Aldrich at the highest possible purity.

**Microcosm Setup.** To understand the biotransformation of PFASs in AFFF under anaerobic conditions, microcosms were constructed with pristine or AFFF contaminated solids under sulfate-reducing conditions. Pristine solids were collected from the sediment of a creek on UC Berkeley campus and AFFF contaminated solids were from Ellsworth Air Force Base (South Dakota). The initial 6:2 FTTOaoS concentration in the microcosms was approximately 20 µM. Ten g of pristine solids and five g of AFFF-contaminated solids were used as microbial inocula.

Triplicate live and autoclaved microcosms were prepared in 160 mL glass serum bottles with a N₂/CO₂ headspace (80/20 (v/v) and 50 mL basal medium containing cysteine sulfide as reducing reagent and resazurin as redox indicator.⁴ Also, 50 mM of sodium sulfate was amended as electron accepter along with 50 µL neat AFFF and 1.5 mM DGBE, the primary organic solvent in the AFFF formulation) as electron donor and carbon source for the microcosms at the
beginning of incubations. Autoclaved controls were constructed using autoclaved soils and media-only controls consisted of media, but no solids. All microcosms were mixed by gentle swirling to avoid the formation of foams prior to incubation at 30 °C in the dark without shaking. Periodically, 50 mM sodium sulfate and 1.5 or 3 mM DGBE were amended to ensure that sufficient carbon source, electron donor and acceptor were present in the microcosms during the incubation period. Slurry samples were analyzed for transformation products by LC-MS/MS.

**Analysis.** Analytes including 6:2 FTTAoS, 6:2 FTTPA, 6:2 FTSA, PFCAs, and PFSAs were analyzed by LC-MS/MS. Concentrations of FTTPAs in Zonyl FSA were estimated assuming an equimolar response to that of the corresponding FTSA. The TOP assay was used to quantify the total amount of precursors in the microcosms. Samples were dried and then reconstituted in a solution containing 116 mM sodium hydroxide and 51 mM potassium persulfate and incubated for 12 h at 85 °C (water bath). The reacted solutions were then diluted, vortexed, and analyzed for total PFCA concentrations using LC-MS/MS.

**Identifying transformation products.** Samples were analyzed with both high-resolution Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry. FT-ICR analysis was performed with a Thermo Finnigan LTQ FT Ultra High Performance Mass Spectrometer (Thermo Finnigan, San Jose, CA) in the UC Berkeley QB3/Chemistry mass spectrometry facility. Full-scan MS data were acquired in the negative electrospray ionization mode over the m/z range of 100 - 1000 with a resolution setting of 100,000 (at m/z = 400). Xcalibur™ software (version 2.0.7 SP1, Thermo Scientific, Waltham, MA) was used to extract full scan mass spectra from FT-ICR.

**Task 3.1 and 3.2 Aerobic Microcosms with Ansul AFFF**

**Chemicals and Standards.** Ansul AFFF was obtained from the Air Force and contained 4:2, 6:2, and 8:2 FTTAoS, as well as the sulfone form of 6:2 FTTAoS along with 220 g/L DGBE, which corresponded to 80% of the total organic carbon in the AFFF. A commercial source of 6:2 FTTAoS was used as a standard reference material for quantitative analysis.

**Microcosm Setup.** Microcosms were constructed using 250 mL glass bottles, 60 mL of a 30 mM bicarbonate-buffered mineral medium, 60 µL of Ansul AFFF, and 5 g of soil from Ellsworth Air Force Base. Sterile controls consisted of autoclaved soil amended with medium along with 0.5 g/L sodium azide and 60 µL AFFF. Soil-free media controls contained only sterile medium, sodium azide, and Ansul AFFF. All microcosms were run in triplicate while shaking at 100 rpm at 30° C for 60 d. Headspace and slurry samples were taken with syringes. Headspace oxygen was maintained at 15 and 25% (v/v) by adding pure oxygen periodically. Live microcosms received two additions (60 µL) of Ansul AFFF on days 0 and 18. Autoclaved and media-only controls received only 1 Ansul AFFF addition. On day 40, additional carbon in the form of DGBE (300 mg/L) was amended to the live microcosms. No microbial activity was observed in the autoclaved and media-only controls, as determined by the absence of organic carbon and oxygen consumption.

**Analysis.** The concentrations of PFCAs; 4:2, 6:2, and 8:2 FTSA; saturated fluorotelomer carboxylates (FTCA), and unsaturated fluorotelomer carboxylates (FTUC) acids were determined by LC-MS/MS. Slurry samples also were analyzed by the TOP assay. Briefly, 100 µL of slurry was combined with 3 mL HPLC-grade water, and 3 mL of 120 mM potassium...
persulfate in 0.25 M NaOH. The reaction was then carried out at 85°C for 12 h. Prior to LC-MS/MS analysis, the reaction media was neutralized and then combined with 1 mL methanol. Headspace oxygen was measured by gas chromatography flame ionization detection. Dissolved organic carbon was measured using a Shimadzu TOC-V analyzer.

**Task 3.3: Impact of AFFF on TCE Biotransformation**

**AFFF, DGBE, and TCE Microcosms.** Various AFFF formulations, PFASs, and ethylene glycol were amended to the growth medium of a D. mccartyi-containing enrichment culture to determine the impact on dechlorination, fermentation, and methanogenesis as described in Harding Marjanovic et al.³ Three characterized AFFFs (3M, National Foam, and Ansul) were used for the microcosm experiments. Commercial source materials containing FTTAoS, FTSaBs, FTSaAm, perfluorooalkyl sulfonamide amines (PFSaAm), and perfluorooalkyl sulfonamide amino carboxylates (PFSaAmA) were obtained from the Fire Fighting Coalition,⁶ and the concentrations of 6:2 FTTAoS, 6:2 FTSaB, and 6:2 FTSaAm in various stock solutions were previously determined by Backe et al.² All other standards were purchased from Wellington Laboratories (Guelph, Ontario, CA), while TCE and DGBE were purchased from Sigma Aldrich (St. Louis, MO).

All microcosm experiments were conducted in 160 mL glass serum bottles with 100 mL of a reduced basal medium with a vitamin solution containing 100 µg/L vitamin B₁₂ and a N₂/CO₂ (90:10) headspace. Serum bottles received 20 to 30 μmoles of neat TCE equilibrated 24 h prior to adding 5% (vol/vol) of an active D. mccartyi-containing enrichment culture.²⁰

**AFFF Amendment.** For experiments with AFFF and TCE, the experiments were conducted in triplicate with 300 µL of either Ansul, 3M, or National Foam AFFF added to the growth medium and equilibrated for 24 h before inoculating with 5 mL of D. mccartyi. Sterile controls consisted of adding a previously-autoclaved culture to bottles containing AFFF and TCE and a second set that contained only inoculum-free medium and no microbial culture. Live controls consisted of growth medium, 2 mmole lactate, 25-30 µmoles TCE, but no AFFF. At each sampling point, 1-1.5 mL of culture was removed and of that volume, 200 µL was diluted with 200 µL methanol for LC-MS/MS analysis.

**DGBE Amendment.** Triplicate 160 mL bottles contained 100 mL growth medium, 25 µmoles TCE, and either 1) 250 µmoles DGBE, 2) 250 µmoles sterile DGBE stock solution and 2 mmole lactate, or 3) 2 mmole lactate. The DGBE concentration was selected to model the concentration associated with the 300 µL volume of AFFF as described above. Bottles initially amended with DGBE received additional DGBE amendments on days 28, 36, 92, and 118. Bottles with lactate + DGBE received one additional DGBE on day 92. All bottles received additional TCE (25 µmoles) on day 92. All DGBE amendments were periodically sampled by removing 1-1.5 mL of culture.

**PFAS and AFFF Amendments.** Triplicate 60 mL bottles were prepared with growth medium (50 mL) with 3% (vol/vol) of the D. mccartyi culture in a N₂/CO₂ (90:10) headspace along with 2 mmole lactate, 25 µmoles TCE, and either 18 mg/L ethylene glycol, 12 mg/L 1-propanol, 45 mg/L FTTAoS, 16 mg/L 6:2 FTSaB, or 32 mg/L 6:2 FTSaAm. Controls consisted of lactate-only bottles. Concentrations of the PFASs were selected to mirror those in the AFFF amendments described above. All bottles were incubated in the dark for five days at 34 °C.

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To further assess the impact of PFSAs on TCE dechlorination, three different PFSA amendments were tested with enrichment cultures fed with 2 mmoles lactate and 22 μmoles TCE. These amendments contained 7.3, 22, and 36.7 mg/L of PFBS, PFHxS, and PFOS each, yielding three tested conditions with total PFSA concentrations of 22, 66, and 110 mg/L, respectively. In addition, bottles were constructed with the addition of a suite of PFCAs and PFSAs at concentrations of 2, 6, and 10 mg/L of each PFAS, yielding total PFAS concentrations of 22, 66, and 110 mg/L. The methanol in the stock solutions was removed by evaporation prior to adding anaerobic growth medium, 1 mmole lactate, and 22 μmoles TCE. The mixture was inoculated with 1.5 mL D. mccartyi enrichment culture and incubating for seven days at 34 ºC. Controls consisted of bottles that received all materials except the mixture of perfluorinated acids.

Analytical Methods. Chloroethene, methane, and hydrogen were quantified by gas chromatography flame ionization detection; PFASs were quantified by LC-MS/MS; and organic acids (i.e., lactate, acetate, butyrate, and propionate) were quantified by HPLC. The 16S ribosomal deoxyribonucleic acid (rDNA) genes of D. mccartyi genes were quantified by polymerase chain reaction.

**Task 4 Sorption of Cationic and Zwitterionic PFASs to Soils and Sediments**

Soil Sample Collection and Characterization. Six blank soils (Soils 1-6) were selected from an archived soil collection at Oregon State University that had pH values of 5.0-5.5, but covered a range of soil characteristics, including organic carbon, CEC, and anion exchange capacity (AEC) (Table 1). Soils were sieved to < 2 mm and air dried prior to homogenization with a mortar and pestle before use in isotherm experiments.

<table>
<thead>
<tr>
<th>Soil #</th>
<th>pH</th>
<th>% OC</th>
<th>% N</th>
<th>C/N Ratio</th>
<th>CECb (meq/100g)</th>
<th>AECd (meq/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.1</td>
<td>1.5</td>
<td>0.13</td>
<td>13</td>
<td>8.7</td>
<td>0.20</td>
</tr>
<tr>
<td>2</td>
<td>5.5</td>
<td>0.12</td>
<td>0.02</td>
<td>6.8</td>
<td>19</td>
<td>0.58</td>
</tr>
<tr>
<td>3</td>
<td>5.1</td>
<td>2.3</td>
<td>0.20</td>
<td>13</td>
<td>6.8</td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>5.0</td>
<td>7.7</td>
<td>0.43</td>
<td>21</td>
<td>7.1</td>
<td>2.2</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>1.0 ± 0.19f</td>
<td>0.084 ± 0.0087</td>
<td>12 ± 1.1</td>
<td>9.5 ± 1.9</td>
<td>0.28g</td>
</tr>
<tr>
<td>6</td>
<td>5.2</td>
<td>0.098</td>
<td>0.004</td>
<td>30</td>
<td>35</td>
<td>0.33</td>
</tr>
</tbody>
</table>

- Percent organic carbon (% OC) and nitrogen (N) as determined by the method of Goni et al. Cation exchange capacity measured by summation method, pH 7. Anion exchange capacity at native soil pH (using water).

Batch Sorption Experiments. All isotherms, with the exception of the preliminary experiment involving a commercial product, used National Foam AFFF that contained anionic, zwitterionic, and cationic PFASs. The pKas for each chemical form (Fig. 2) were derived by comparing the molecules to model compounds in the literature.
A 21-point isotherm was constructed using Soil 1, with initial aqueous phase concentrations \( C_w \) of 1,000 – 138,000,000 ng/L for the 6:2 FTSaB. Eleven-point isotherms were constructed using Soils 2-6 and two additional isotherms were constructed with Soil 1 that was titrated to pH 4 and 7. For the 11-point isotherms, \( C_w \) ranged from 1,000 to 250,000 ng/L for the 6:2 FTSaB. The upper-end \( C_w \) for the 21-point and the 11-point isotherms were selected based on the 3% AFF in water used in firefighting and the mid-range concentration was selected to represent the 6:2 FTSA concentration in AFF-impacted groundwater (8,900 – 220,000 ng/L), respectively. Replicates, which consisted of homogenized soil spiked with the same volume of National Foam AFF stock solution, were created for 6:2 FTSaB \( C_w \) of 5,000 ng/L (n=4), 60,000 ng/L (n=3), and 138,000,000 ng/L (n=3). All other data are derived from individual reactors.

Homogenized soil was weighed \((1.00 \pm 0.02 \, \text{g})\) into each 50 mL polypropylene centrifuge tube and autoclaved for 40 min at 121 °C with a 20-min drying time (Consolidated Sterilizer Systems Model SSR-2A-ADVVPB, Allston, MA). Vials contained the autoclaved soil and 10 mL 0.5 mM calcium chloride, to which the diluted National Foam AFF was spiked. Positive controls consisted of National Foam AFF prepared at the concentration of the replicates but no soil. One negative control, consisting of the autoclaved soil and 0.5 mM calcium chloride was prepared with each isotherm. Vials were allowed to equilibrate for 24 h on a wrist action shaker at 10° rotation (Burrell Corporation, Model 75, Pittsburg, PA).

Following equilibration, batch reactors were centrifuged at 2808 G for 20 min (Eppendorf, Model 5810R, Hauppauge, NY), and the supernatant was decanted into a separate 15 mL centrifuge tube. The soil was transferred into a separate 50 mL centrifuge tube using deionized water. The equilibrated vial phase (hereafter referred to as vial phase) and the aqueous phase were stored at -20 °C. The soil was freeze dried for 48 h and subsequently stored in the dark at 22 °C until extraction.

Aqueous Sample Preparation and Analysis. After equilibration, the aqueous phase was diluted 1-5,520-fold in groundwater surrogate for the Soil 1 native pH isotherm and 1-10-fold for the remaining isotherms (Soils 2-6, pH 4, pH 7). The diluted aqueous phase was extracted using a micro liquid-liquid extraction as described elsewhere with slight modifications.

**Mass Balance.** Mass balance experiments consisted of the 60,000 ng/L 6:2 FTSaB \( C_w \) (n = 4) conditions with Soil 1 that were set up and treated in the same manner as the isotherms. Mass balance experiments were conducted using the aqueous phase extraction and a soil extraction
METHODS and MATERIALS

typically used for cationic and zwitterionic PFASs.\textsuperscript{1,29,30} The vial and soil phases were extracted using 1.5 mM NH\textsubscript{4}OH in methanol as described elsewhere.\textsuperscript{1} Preliminary experiments indicated poor recovery of the zwitterionic 6:2 FTSaB and the cationic 6:2 FTSaAm (data not shown) when soil and analytes were equilibrated in an aqueous phase (and not methanolic spikes) and extracted using relatively mild conditions (1.5 mM NH\textsubscript{4}OH in methanol,\textsuperscript{1} 20 mM sodium hydroxide (NaOH) in methanol,\textsuperscript{30} and 1\% acetic acid in methanol.\textsuperscript{29} Soil extraction methods for cationic, non-fluorinated surfactants typically use either 0.5 M\textsuperscript{50} or 1 M HCl\textsuperscript{34,51,52} in methanol. The 1 M HCl in methanol extraction conditions proved to be problematic (unpublished data). Therefore, 0.5 M HCl in methanol was used in all subsequent soil extractions.

Mass balance experiments were repeated using the acidic methanol extraction for the vial and soil phases. Recoveries improved significantly for the 6:2 FTSaB and the 6:2 FTSaAm (95-105\% recovery). Mass balance experiments also indicated that sorption to the vial walls was minimal for all analytes (unpublished data). Therefore, only the aqueous and soil phases were extracted for each vial (not the vial itself). Due to the high concentration of the stock solution and subsequent variability of the mass of analyte added to each vial, mass balance on each vial was not feasible. Since separate mass balance studies with a known mass of analyte added achieved mass balance, mass balance was assumed in all vials. The analytes were quantified by LC-MS/MS.\textsuperscript{2}

Data Treatment. The fraction of pore water following equilibration was determined so soil phase concentrations for each analyte could be adjusted to account for the mass of each analyte associated with remaining pore water. Sorption isotherms were fitted using the following Freundlich isotherm model equations:

\[
C_s = K_f C_w^n \quad (\text{Eqn 1})
\]

\[
log C_s = n log C_w + log K_f \quad (\text{Eqn 2})
\]

where $K_f$ is the Freundlich sorption coefficient and $n$ is a measure of nonlinearity and represents the free energy associated with adding more sorbate to the sorbent.\textsuperscript{53} $K_f$ values were converted to the concentration-specific $K_d$ values to enable comparisons between isotherms and across other sorption studies for other PFASs. To incorporate the greatest amount of uncertainty, the $K_d$ values from the 60,000 ng/L $C_w$ are used in the discussion below.
RESULTS AND DISCUSSION

Task 1: Characterize AFFF formulations

Information, including figures and tables, contained in this section is documented in the following published papers:


Task 1.1 Define PFAS Composition of AFFFs

For Task 1.1, a total of 11 classes of PFASs were initially identified in AFFF formulations stockpiled at US military sites (Fig 3 and 4).

Fig 3. Major and trace level classes of PFASs identified in ECF-based AFFFs.

\begin{align*}
\text{i' Perfluoroalkylsulfonates} & \quad \text{ii' PrSA\textsuperscript{m}A} \\
\text{iii' Trace} & \quad \text{iv' PrSA\textsuperscript{m}}
\end{align*}
RESULTS and DISCUSSION

All the PFASs shown in Fig. 3 are derived from electrofluorination (ECF) chemistry by 3M. In addition to PFSAs, which were expected, three additional zwitterionic and cationic classes were identified (Fig 3). Examination of the new structures and their fluorinated chain lengths reveal their potential to form PFSAs. The PFASs in 3M AFFF all had perfluorinated chains directly bonded to a sulfur atom, which indicate the potential to degrade to PFASs. Six major classes and one minor class were identified in the fluorotelomer-based AFFFs (Fig 4). Only the FTTAoS (Fig 5iii)\textsuperscript{10,54} and (FTSaB (Fig 5i)\textsuperscript{55} have since been identified by others in AFFFs.\textsuperscript{55,56} The polyfluorinated forms contained the characteristic fluorotelomer-based chain with an even number of fluorinated carbons bonded to two carbons bearing hydrogens. In terms of nomenclature for Fluorotelomer-based substances, ‘6:2’ refers to six perfluorinated carbons and two methylene carbons. For example, 6:2 FTSA represents $\mathrm{F}_3\mathrm{C}(\mathrm{CF}_2)_5\mathrm{CH}_2\mathrm{CH}_2\mathrm{SO}_3\textsuperscript{-}$.

Quantitative analytical method for groundwater. Once the PFASs were identified in the 3M and fluorotelomer-based AFFFs, the next step was to quantify the individual PFASs in the AFFF formulations. However, at the time these PFASs were identified, analytical methodology for these chemicals had not been developed. It is for this reason that a new analytical method was developed as part of Task 1 to quantify these newly-identified PFASs.

The final method briefly consists of adding salt and then acidifying a small (3 mL) volume of groundwater that is then extracted with ethyl acetate. This approach was selected over more conventional water sample extractions for PFASs, such as EPA method 537, because it avoids the use of solid phase extraction, thus minimizing the potential for sample contamination and analyte loss and the generation of solid and liquid waste. The method is efficient and cost effective because it involves minimal handling. For example, once the ethyl acetate extract is generated, 0.9 mL or 60% of the 1 mL extract is injected directly into the LC-MS/MS system.
RESULTS and DISCUSSION

Application of Analytical Method to PFASs in AFFF. AFFF formulations were analyzed by LC-MS/MS in order to determine the proportions of the chemical classes for each AFFF. The 3M formulations contained only ECF-based PFASs (Fig 3) and the mixtures were predominantly C4-C10 PFSAs with lesser levels of the C4-C8 PFCAs (Table 2). The ratio of PFSAs to PFCAs in the 3M formulations was ~ 20:1 (Table 2), with PFOS as the primary component. The composition of 3M AFFF formulations changed around 1993 to contain greater proportions of PFSaAm and PFSaAmA classes that are primarily C6 in chain length (Table 2). However, it is important to note that we only obtained AFFF formulations dating back to 1989. Discussions with the Fire Fighting Foam Coalition, who provided a letter of support for this SERDP project, indicate that 3M revealed in meetings that potentially higher levels of PFCAs were used in their older AFFF formulations. Efforts to obtain older AFFF formulations were unsuccessful.

Only polyfluoroalkyl substances were quantified in fluorotelomer-based AFFFs varies by manufacturer (Table 3). PFCAs were not detected in the telomer-based AFFF formulations (Table 3) at the dilution levels employed (1:100,000 – 1:1,000,000). The FTSAs were only quantified at relatively low levels in telomer-based AFFFs, which indicates that FTSAs in groundwater are likely due to the biotransformation of higher molecular weight precursors, including FTTAoS, FTSaB, and FTSaAms (Fig. 2). Within the FTSaB class, the polyfluorinated chain lengths ranged from 6:2 to 12:2. The longer chained 8:2, 10:2, and 12:2 FTSaBs are potentially of interest since the structures indicate the potential to form long chain (≥C8) PFCAs.
Table 2. Concentrations in mg/L of newly-identified and legacy PFASs in 3M AFFF formulation by year of manufacturing. PFCAs above C8 (PFOA) were not detected.²

<table>
<thead>
<tr>
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<LOD = less than the limit of detection
Table 3. Concentrations in mg/L of polyfluorinated chemicals in fluorotelomer-based AFFFs by manufacturer.2

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<th>Buckeye Fire Equipment</th>
<th>Angus Fire Equipment</th>
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<td>56</td>
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Additional PFASs Discovered in AFFF and Groundwater. Two separate additional studies were conducted after the first round of PFAS discovery in 2010.6 Using high mass accuracy QTOF-MS, perfluoroethane sulfonate (PFETs) and perfluoropropane (PFPrS) sulfonate were identified in AFFF. The analytical method developed for the PFASs found in AFFF and groundwater in 2010 was then adapted to quantify PFETs and PFPrS in AFFF and groundwater.40 Concentrations of PFETs and PFPrS ranged from 7-13 mg/L and 120-270 mg/L, respectively, in 3M AFFFs. Eleven groundwaters collected from AFFF-contaminated sites gave PFETs at concentrations of 11-7, 500 ng/L and PFPrS at concentrations from 19-63,000 ng/L. These ultra-short chained forms are characterized by high aqueous solubility and mobility in the subsurface, which will likely make them difficult to remove by conventional activated carbon treatment.

The final phase of discovery, was more thorough in the number and type of PFASs identified.41 The result is that 40 classes of novel anionic, zwitterionic, and cationic PFASs were discovered. In addition, 17 previously-reported classes were observed for the first time in AFFF and AFFF-impacted groundwater. With the assistance of Wellington laboratories, all 57 classes were
assigned an acronym. Thirty four of the 40 newly-identified PFAS classes derive from the 3M ECF-based process. Of the 13 classes identified only in AFFF impacted groundwater, 11 were ECF-derived, while the two remaining classes are associated with fluorotelomer-based chemistry. The frequency of occurrence and concentrations are not determined for this study and will be part of future work conducted under ESTCP ER-201633 and SERDP ER-2720. With the identification of these additional classes, it may now be possible to close the mass balance on highly-fluorinated substances in US military groundwater. Additional work is needed to compare the estimated concentrations of the PFASs to other indicators, such as total fluorine by PIGE and the TOP assay. Given the structures, most are water-soluble, mobile, and contribute to the total mass of highly-fluorinated substances to be removed from potential drinking water sources. Work is underway with AECOM under the AFCEC Contract FA8903-12-C0005 to determine the removal of the newly-identified PFASs from Wurtsmith groundwater by granulated activated carbon.

Task 1.2 Total Oxidizable Precursor Assay

For Task 1.2, the precursors present in AFFF formulations were found to convert only to perfluorinated carboxylates. Analysis before and after persulfate treatment gave significant increases in the concentrations of the PFCAs in all AFFF formulations (Fig. 6). Concentrations of PFSAs were unchanged by oxidation (compare Fig 6a and 6b). In the 3M formulations, PFHxA was the main PFCA produced during oxidation, which indicates a predominance of C6 chain-length precursors in 3M formulations. Chemguard, Ansol, National Foam, and Buckeye AFFF formulations also produced relatively high concentrations of PFHxA, as well as other short-chain PFCAs upon oxidation. Ansol formulations from the mid-1980s and National Foam...
and Buckeye formulations produced PFOA upon oxidation. Neither the 3M formulation nor the AFFFs from Ansul and Chemguard consistently generated significant amounts of PFOA upon oxidation, which suggests that these formulations contain precursors with less than eight perfluorinated carbons.

In addition to the AFFF formulations, reference materials including Forafac 1157 and FTTAoS, which are found in the AFFFs sold by National Foam and Ansul, respectively, were analyzed by the TOP assay. A mixed suite of PFCAs evolved with complete disappearance of the monitored parent precursor compounds. The molar profiles of the reference materials were consistent with the PFCA product profile of corresponding AFFF formulations upon oxidation. The concentrations of PFCAs evolved in an AFFF formulation after oxidation is used as a conservative quantitative estimate for the concentration of precursor compounds initially present in the AFFF formulations because recovery of standards as PFCAs was variable and significantly less than 100%.

**Task 2: Characterize Groundwater, Sediment and Soil at AFFF-Contaminated Sites**

The information, including figures and tables, contained in this section is documented in the following published papers:


**Task 2.1 Analysis of Groundwater from Field Sites**

Under Task 2.1, the new analytical method developed in Task 1.1 was applied to field site groundwater from Ellsworth and Randolph AFBs. The method detection limits ranged from 0.71 ng/L to 67 ng/L and whole-method accuracy ranged from 96±8.4% to 106±9.1% for analytes with authentic analytical standards. For analytes without authentic analytical standards, whole-method accuracy ranged from 78% to 144%. Method precision for all analytes was less than 15%. For some of the newly-identified analytes, the AFFF formulation represents the only source of the newly-identified PFASs. This means that the resulting data for the newly-identified PFASs will be semi-quantitative until high quality standards become available.

*OSU ER-2128 Final Report*
commercially.

Application of the developed methodology to groundwater collected from Ellsworth AFB (Table 4) and Randolph AFB (Table 5) gave quantifiable concentrations of legacy (PFCAs, PFSAs, FTSAs). Groundwater from Ellsworth AFB (Table 4), Randolph AFB (Table 5), and sites previously sampled including Wurtsmith AFB, Naval Air Station Fallon, and Tyndall AFB, gave quantifiable levels of 3M precursors (PFSaAm and PFSaAmA) as well as the FTTAoS (Appendix Table A7). However, at both the Ellsworth and Randolph sites, the concentrations of these precursors were significantly lower than those of PFSAs and PFCAs, which ranged in concentration up to a maximum of 360,000 ng/L (Table 4) at Ellsworth AFB and up to hundred and 170,000 ng/L at Randolph AFB (Table 5). These two data sets provide further evidence that the 6:2 FTSA occurs at concentrations similar to that of PFOA and PFOS. At Tyndall AFB, NAS Fallon, and Wurtsmith, the newly-identified chemicals (Appendix Table A7) were significantly lower than that of PFCAs, PFSAs, and FTSA, which ranged into the mg/L (10^6 ng/L) range.

The groundwater data from Ellsworth and Randolph AFBs indicate a predominance of the C6 over that of the C8 chain lengths. The PFSAs can only originate or derive from 3M AFFF. However, AFFF formulation data indicates that PFHxS is lower in abundance than PFOS. Therefore, the predominance of PFHxS at field sites could be due to either biodegradation of C6-based precursors (PFSaAmA and PFSaAm), AFFF formulations enriched in PFHxS (prior to 1989), or differences in solubility and/or transport between the two homologs. This question was further examined as part of Task 2.3 in an effort led by Chris Higgins, the PI of the SERDP project ER-2126. In contrast, PFCAs are not present in ECF-based AFFFs at levels comparable to PFSAs nor are they in telomer-based AFFF formulations at the dilutions used for the measurements. As such, PFCAs may arise from the biodegradation of fluorotelomer-based precursors or 3M AFFF formulations older than 1989 that are relatively enriched in PFCAs. Given the listing of Ansul AFFF on the QPL and the contracts held by Ansul in the 1980s, it is likely that the 6:2 FTTAoS may have biodegraded to form PFCAs. In Ansul AFFF formulations, the 6:2 FTTAoS is greater in abundance than the 8:2 form, so more C6 products would be expected and they would be expected to be only linear and not branched.
Table 4. Concentrations of PFASs quantified in groundwater from Ellsworth AFB.\(^2\)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>ng/L</td>
<td>ng/L</td>
<td>ng/L</td>
<td>ng/L</td>
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<td>490</td>
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</table>

< LOD = less than the limit of detection; \(^a\) No 8:2 FTTAoS, 6:2 FTTHN\(^c\), fluorotelomer sulfonamido betaines, fluorotelomer sulfonamido amines, nor fluorotelomer betaines were detected; \(^b\) calculated assuming equal molar response to 6:2 FTTAoS (see main text); \(^c\) calculated assuming equal molar response to PFOS; \(^d\) concentration above limit of quantification but below the lowest calibration standard.
Table 5. Concentrations of PFASs quantified in groundwater from Randolph AFB.²

<table>
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<tr>
<th>Analyte</th>
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<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
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< LOD = less than the limit of detection; a no 8:2 FTTAoS, 6:2 FTHN+, fluorotelomer sulfonamido betaines, fluorotelomer sulfonamido amines, nor fluorotelomer betaines were detected; ‡ calculated assuming equal molar response to 6:2 FTTAoS; ‡ calculated assuming equal molar response to PFOS; d concentration above LOQ but below the lowest calibration standard.
Task 2.2 Analysis of Sediment/Soil from Field Sites

For Task 2.2, a total of 26 groundwaters, 16 soils, and 10 sediment samples from Ellsworth AFB were analyzed by the TOP assay for Task 2.2. After oxidation, the groundwaters all gave increased PFCA concentrations, indicating the presence of precursors. The C6 carboxylate increased to the greatest extent, indicating specifically the presence of C6 precursors in groundwater. A maximum of 50% of the total precursor concentrations in groundwater could be accounted for by individual, measured PFASs. This finding indicates that there are additional PFAS species present in groundwater that remained to be identified.

The PFASs that can be measured as individual components by LC-MS/MS account for ~ 50% of the precursors at this site (Fig. 7). Additional analytical work to identify precursors in soil and sediment is needed and will be part of SERDP ER-2720. As expected, oxidation of sediment/soil resulted in the production of only PFCAS and the formation of primarily the PFHxA indicated that precursors in soil/sediment are predominantly C6 compounds. Nearly all of the PFOA production was accounted for by the two C8 precursors measured, 8:2 FTSA and perfluorooctane sulfonamide (FOSA). FOSA is a known intermediate in the degradation of sulfonamides and is present on soil and sediments at the site but not in groundwater.

When the concentrations of individual PFASs and total oxidizable precursors for groundwater and sediment/soil are examined together, several conclusions can be drawn. First, the newly-identified PFASs in AFFF formulations can be found in groundwater and soil/sediment at the Ellsworth AFB site but only at trace levels. For this reason, it would appear that the precursor components of AFFF have undergone some level of biodegradation at this site or are sorbed to soil/sediments. Second, the predominance of C6 forms (e.g., PFHxA and PFHxS) at Ellsworth and Randolph AFBs (Table 4 and 5) and predominance of C6 precursors, as determined by the TOP assay, in groundwater, sediment, and soil are viewed as consistent with biodegradation of the C6 PFASs that are present in MilSpec AFFF.
formulations or, alternatively, originate from AFFF formulations that differ in composition from those tested for this study.

As part of Task 2.3 (analysis of spatial distribution and co-contamination with priority pollutants) this project collaborated with SERDP project number ER-2126, led by Dr. Christopher Higgins of the Colorado School of Mines.

Task 3: Biotransformation of AFFF PFASs and Impact on TCE Biotransformation

The information, including figures and tables, appear in the following publications:


Task 3.1 and 3.2 Anaerobic microcosms with Ansul AFFF

Preliminary results of a 35 d anaerobic incubation with the Ansul AFFF indicate that the soil microbial community can actively use the organic carbon in AFFF under all electron acceptor conditions (data not shown). Visible color changes resulting from the utilization of electron acceptors were apparent in bottles containing live cultures compared to autoclaved controls. Analysis of PFAS concentrations in active and control bottles on days 0, 15 and 35 indicated no significant production of FTSAs or PFCAs under any electron acceptor condition.

Anaerobic biotransformation of the Ansul AFFF formulation was analyzed in a 317-day incubation. PFAS analyses of the microcosms indicated that the 6:2 FTTAoS, the principal fluorotelomer precursor in Ansul AFFF formulation, was gradually transformed under every tested redox condition, compared to the respective autoclaved control microcosms (Fig. 8).

The most active transformation was observed under nitrate-reducing conditions, where the live cultures successfully transformed two consecutive doses of 6:2 FTTAoS in the Ansul AFFF during the incubation period. Compared to the 159 day incubation for the first dose of Ansul AFFF, it took only 61 days for live cultures to transform a similar amount of 6:2 FTTAoS in a second dose of Ansul AFFF, indicating increased microbial transformation of FTTAoS. In contrast, a much slower biotransformation occurred under the other three redox conditions with only about 45-70% removal of the 6:2 FTTAoS in the first dose of Ansul AFFF.
RESULTS and DISCUSSION

Fig 8. Anaerobic biotransformation of 6:2 FT-TaO in the Ansul AFFF. Note ZFAS is an early acronym for FTTPA.

The TOP assay was performed on all control and live microcosm slurries in order to quantify PFAS mass balances. Compared to the respective autoclaved controls, 121±15%, 115±7% and 96±8% of PFASs were recovered on days 0, 194, and 276 from the live microcosms inoculated with pristine solids, and 114±10%, 98±13% and 67±6% of PFAS mass was removed on days 0, 135, and 282 with contaminated solids (data not shown). These results suggest that while mass balances were achieved in the pristine solid microcosms, biotransformation in the contaminated microcosms eventually led to non-detectable products.

The identity of 6:2 FTTPA was confirmed by the authentic standard Zonyl FSA. The formation of this intermediate was not observed in the medium or autoclaved controls, but accumulated in the live

Fig 9. Production of 6:2 FTTPA (m/z 451) during 6:2 FtTaO biotransformation. Error bars represent the standard deviation of averages from triplicate microcosm.
culture (Fig. 9). At the end of the incubation, insignificant difference in the final 6:2 FTTPA concentrations was observed between pristine and contaminated microcosms. The 6:2 FTTaoS was predominantly transformed to 6:2 FTTPA, likely via a hydrolysis reaction that is catalyzed by a microbial amidase. Two additional identified products, fluorotelomer thioether propanoyl alaninate (FTTPIA) and 6:2 fluorotelomer thioether propanoylalanylalaninate (FTTPIAA) were identified (Fig. 10).

**Aerobic microcosms with 3M AFFF**

Although the experimental procedures were not described in earlier sections, preliminary results of a 20 d aerobic incubation with the 3M AFFF formulation indicated that the soil microbial community can actively use the organic carbon in AFFF to support their growth. Cell counts in the microcosms revealed that initial bacterial numbers in the medium containing only AFFF accounted for 34% of total cells, but this percentage increased to almost 100% in subsequent days, indicating that the organic carbon in AFFF can effectively serve as an energy and carbon source for the microcosm organisms. Analysis of PFAS concentrations in active and control samples on day 0 and day 20 demonstrated no significant increase in PFCAs and PFSAs, even though 3M AFFF is known to contain C4-C8 precursors.\(^2,^6\) Significant decreases in PFOS were observed both in active and autoclaved soils, suggesting that the PFOS loss is due to abiotic processes, such as sorption to soils, rather than biotransformation. No further experiments were conducted with 3M AFFF for this SERDP project. However, the biotransformation of 3M AFFF will be investigated under SERDP ER-2720, “Key Fate and Transport Processes Impacting the Mass Discharge, Attenuation, and Treatment of Poly- and Perfluoroalkyl Substances and Comingled Chlorinated Solvents or Aromatic Hydrocarbons,” which is being led by Dr. C. Higgins of the Colorado School of Mines.

**Aerobic microcosms with Ansul AFFF**

Live microcosms amended with Ansul AFFF containing DGBE gave organic carbon concentrations that disappeared within 3 to 5 d, while no similar change was observed for autoclaved/medium controls (Fig. 11). Similar trends in FTTaoS disappearance were observed for 4:2 and 8:2 FTTaoS; however, these forms occurred at lower concentrations in AFFF relative to 6:2 FTTaoS (data not shown).\(^4\) In live microcosms, oxygen concentrations declined, which indicates that microbial community was active. In contrast,
RESULTS and DISCUSSION

concentrations of FTTAoS remain nearly constant in controls (Fig. 11).

The most abundant transformation product formed was 6:2 FTSA (Fig. 12a), presumably because the 6:2 FTTAoS was the most abundant precursors in the National Foam AFFF, but only accounted for 8% of the initial mass of 6:2 FTTAoS added. The 4:2 and 8:2 homologs also were detected in the microcosm experiments, but accounted for less than 1% of the corresponding parent FTTAoS (data not shown). In addition, the 5:3 FTCA and 6:2 FTUCA were formed but only accounted for ~ 0.5% and 0.18% of FTTAoS precursors (Fig 11b). Formation of 8:2 FTUCA was observed but at low concentrations and there were no trends observed for 7:3 FTCA or 6:2 FTCA, relative to autoclaved controls (data not indicated)

Several persistent PFCAs were observed including PFHxA, PFPeA, and PFBA (Fig. 12C) that were 48%, 40%, and 10% of total PFCAs produced, which was ~ 1.5% of the total FTTAoS transformed.

It is interesting to note that PFBA is expected to be a product of 4:2 FTTAoS and, by analogy, 4:2 FTSA. However, the concentration of PFBA at the end of the experiments (0.5 µM) was greater than the amount of 4:2 FTTAoS biotransformed (<0.01 µM). This finding indicates that PFBA may be a biotransformation product of other chain lengths (e.g., 6:2 and 8:2). However, specific mechanism is not identified. In addition, PFHpA and PFOA were detected. PFCAs were not detected in controls.

An attempt was made to identify additional information products in the live microcosms. Four polyfluorinated QTOF-MS as the sulfone (-SO-) sulfoxide (-SO2-) and intermediates of the 6:2 and 8:2 FTTAoS. The structures of 6:2 fluorotelomer sulfoxide amido sulfonate (6:2 FTSAOAoS) and 6:2 fluorotelomer sulfone amido sulfonate (6:2 FTSAO2AoS) are indicated in the overall pathway (Fig 12). Interestingly, the 6:2 sulfone (6:2 FTSAOAoS) was also observed in the original National Foam AFFF as well as in autoclaved
controls but not in medium controls, which suggests an abiotic pathway for the production and loss of the 6:2 sulfone (6:2 FTSAO₂AoS).⁴

Others report the biological oxidation of thioether-containing compounds to sulfones and sulfoxides⁵⁷-⁵⁹ and abiotic oxidation the thioether, dimethyl sulfide.⁶⁰ The formation of analogous 4:2 FTSAO₂AoS and 4:2 FTSAO₂AoS were not detected in any microcosms, presumably due to the low concentrations of the 4:2 FTTAoS precursor in National Foam AFFF. This study extends the findings of Weiner et al.,⁵⁴ who proposed the sulfone and sulfoxide products. However, they were unable to ascertain whether the sulfone was a product of biotransformation because it was observed in both live and control microcosms and no 6:2 FTSAO₂AoS (sulfone) was detected in their study.

**Mass Balance.** All the transformation products observed accounted for an estimated 10% of the transformed FTTAoS. Please note that accurate measurements of the sulfone and sulfoxide intermediates are confounded by the lack of standards. Mass balance calculations are also challenging because only an authentic reference material was available for this 6:2 FTTAoS precursor, and not for the 4:2 and 8:2 analogs. Further, stable-isotope labeled standards were not available for any of the FTTAoS precursors. Because mass balance was not obtained, the TOP essay was applied to microcosm samples. The TOP assay recovered 75-85% of the added FTTAoS in the form of PFCAs and up to 80-100% during the second AFFF additions.⁴ Additional research would be required to identify the remaining transformation products that are detected by the TOP assay. Application of the TOP assay to the microcosm study, demonstrates its potential utility for closing the mass balance, although it cannot be used to identify additional transformation products.

**Environmental Implications.** The initial biotransformation of FTTAoS occurs in a matter of weeks under aerobic conditions, but subsequent conversion to PFCAs is slow, even under aerobic conditions. The identification of sulfones, sulfoxides, and FTSAOs in microcosms is important, because it rationalizes their occurrence in anoxic AFFF-contaminated groundwater.⁴¹ Although FTSAOs undergo anaerobic transformation to FTCAs and PFCAs,¹² they do not biotransform under anaerobic conditions.¹³ The lack of anaerobic FTSA biotransformation is consistent with the high (ug/L to mg/L) levels of FTSAOs found in anoxic AFFF-contaminated groundwater.²,¹⁰ Based on the proposed pathway (Fig 13), FTSAOs in an anoxic groundwater that reach aerobic receiving waters, will potentially undergo biotransformation to dead-end PFCAs, thus acting as a long-term source of PFCAs to surface waters.
RESULTS and DISCUSSION

Task 3.3 Impact of AFFF on TCE biotransformation

AFFF Amendment Experiments. Reductive dechlorination was observed in microcosms containing 3M AFFF, but not in those amended with either Ansul or National Foam AFFF (Fig 14 A-C). Subsequent amendments of DGBE and 3M AFFF (Fig 14A) indicated that hydrogen limitation after DGBE amendment was responsible for the slow dechlorination rate and lack of methanogenic activity (Fig 14D). Low concentrations of hydrogen and slow rates of dechlorination after DGBE amendment compared to that after addition of 3M AFFF indicates that other fermentable substances are present in 3M AFFF, which form acetate and hydrogen. However, the addition of 3M AFFF to the systems containing National Foam and Ansul did not result in increased rates of TCE dechlorination. Autoclaved controls and growth medium only controls did not produce significant amounts of methane and TCE dechlorination did not occur.
RESULTS and DISCUSSION

Although acetate was produced by cultures receiving National Foam AFFF, it did not result in TCE reductive dechlorination. Increased acetate production in the National Foam amended systems is attributed to ethylene glycol, which is transformed to acetate under anaerobic conditions.\(^{61,62}\) No significant growth of D. mccartyi as detected, as measured by 16S rRNA gene copy numbers. None of the PFAS concentrations declined significantly for any of the test systems as well as the controls.

**DGBE Amendment Experiments.** Experiments were performed to determine if DGBE could act as an electron donor upon fermentation to drive TCE reductive dechlorination. Although cultures were provided enough substrate (lactate or DGBE) to have an excess of electron equivalents to dechlorinate TCE, there was not a significant difference between cultures receiving lactate and DGBE from those amended with only lactate (Fig 14). This finding indicates that lactate is more labile for fermentation than DGBE.

**AFFF Component and PFAS Amendment Experiments.** To gain further insight into the inhibitory effect of National Foam and Ansul AFFFs on TCE dechlorination, individual components of the AFFF were added to culture undergoing lactate- enhanced fermentation. Reductive dechlorination of TCE was not inhibited by the addition of 18 mg/L ethylene glycol, 12 mg/L 1-propanol, or 45 mg/L 6:2 FTTAoS, the main active component in Ansul AFFF. In contrast, the addition of 16 mg/L zwitterionic 6:2 FTSaB slowed the rate of reductive and doubling the concentration of 6:2 FTSaB to 32 mg/L inhibited reductive dechlorination.
altogether (data not shown). National Foam AFFF contains zwitterionic betaines and cationic surfactants. Non-fluorinated zwitterionic and cationic surfactants are established anti-microbial agents.63-65

The addition of a mixture of PFSAs and PFCAs added at 22 and 66 mg/L did not significantly impact the rates of TCE dechlorination (Fig. 15). In contrast, a mixture of PFSAs and PFCAs (100 mg/L) significantly reduced the rate of reductive dechlorination (Fig 15). Given this observation, either or the combination of PFCAs and PFSAs or PFCAs alone cause of inhibition of TCE dechlorination. Weathers et al. 2016 found that the abundance of D. mccartyi decreased when exposed to a mixture of PFSAs and PFCAs (110 mg/L), when compared to controls that contained no PFSAs and PFCAs.47

Environmental Implications. Based on the experiments with AFFF and TCE, it appears that the ability to support reductive dechlorination of TCE depends on the composition of the AFFF. Fermentation of the organic solvents, such as DGBE and ethylene glycol, drives the production of hydrogen and acetate, which are necessary to support TCE dechlorination by D. mccartyi. In the case of DGBE, the rates of reductive dechlorination are lower due to the lower concentrations of hydrogen produced when compared to other potential constituents, such as ethylene glycol. PFCAs may impact reductive dechlorination of TCE. Ongoing transformation of precursors, due to biotransformation or oxidation, to persistent PFCAs may potentially impact the activity of microbial communities carrying out reductive dechlorination.16 Because a range of AFFFs was likely used at sites,2,10,16 the actual impact of AFFF addition of an individual site is difficult to predict.

Task 4 Sorption of Cationic and Zwitterionic PFASs to Soils and Sediments

Sorption Isotherms. Plots of the aqueous phase concentration (C_w) against the soil phase concentration (C_s) were nonlinear for most soils and analytes, as indicated by n ≠ 1 in the linearized Freundlich isotherms (Table 6). The values of n typically ranged from 0.8 to 1.2, which is consistent with other batch sorption experiments for PFCAs and PFSAs.22,23,49

Capstone Product vs. AFFF. Preliminary experiments compared the sorption of the 6:2 FTSaB from Capstone, a commercial mixture that contains 6:2 FTSaB, 6:2 FTS, and 6:2 FtSaAm but is not an AFFF, and from the National Foam AFFF demonstrated that the sorption of the 6:2 FTSaB was not significantly different at the 95% confidence level (CI). Therefore, multiple competing sorption processes (e.g. van der Waals, electrostatics) driving the sorption of 6:2 FTSaB in the presence of hydrocarbon surfactants and additional solvents in the AFFF result in no difference in sorption of the 6:2 FTSaB. Additional analyte comparisons between the commercial product and the AFFF could not be made, since the remaining two analytes in the
commercial product were either not removed (6:2 FTSA) or completely removed (6:2 FTSaAm) from the aqueous phase. Similar phenomena were observed with sorption of PFCAs and PFSAs in the presence of an anionic hydrocarbon surfactant.  

Table 6. Freundlich coefficients ($\log K_f$), $n$ values, and correlation coefficients ($R^2$) for the 6:2 FTSA, 8:2 FTSA, 6:2 FTSaB, 8:2 FTSaB, 10:2 FTSaB, and 6:2 FTSaAm Freundlich isotherms for Soils 1-6, Soil 1 pH 4, and Soil 1 pH 7.  

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<td>0.63 ± 0.72</td>
<td>1.7 ± 0.39</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>0.98 ± 0.060</td>
<td>1.2 ± 0.36</td>
<td>1.5 ± 0.45</td>
<td>1.8 ± 1.1</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>5</td>
<td>$\log K_f$</td>
<td>0.83 ± 0.18</td>
<td>1.6 ± 0.15</td>
<td>1.8 ± 0.24</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>0.88 ± 0.13</td>
<td>1.1 ± 0.18</td>
<td>0.90 ± 0.18</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>6</td>
<td>$\log K_f$</td>
<td>0.84 ± 0.070</td>
<td>1.6 ± 0.12</td>
<td>1.9 ± 0.21</td>
<td>2.4 ± 0.13</td>
<td>n/a</td>
<td>2.2 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>0.95 ± 0.058</td>
<td>1.1 ± 0.13</td>
<td>0.91 ± 0.17</td>
<td>0.41 ± 0.34</td>
<td>n/a</td>
<td>0.17 ± 0.23</td>
</tr>
<tr>
<td>1 pH 4</td>
<td>$\log K_f$</td>
<td>0.83 ± 0.15</td>
<td>1.7 ± 0.19</td>
<td>2.0 ± 0.22</td>
<td>2.3 ± 0.12</td>
<td>2.7 ± 0.19</td>
<td>n/a</td>
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<tr>
<td></td>
<td>$n$</td>
<td>0.93 ± 0.12</td>
<td>0.90 ± 0.20</td>
<td>0.80 ± 0.16</td>
<td>0.85 ± 0.21</td>
<td>0.96 ± 0.37</td>
<td>n/a</td>
</tr>
<tr>
<td>1 pH 7</td>
<td>$\log K_f$</td>
<td>0.78 ± 0.17</td>
<td>1.2 ± 0.14</td>
<td>1.4 ± 0.17</td>
<td>2.1 ± 0.14</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>0.85 ± 0.13</td>
<td>0.97 ± 0.19</td>
<td>0.97 ± 0.11</td>
<td>0.83 ± 0.23</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

$^a$The 12:2 FTSaB is not included since all aqueous phase concentrations were below the limit of detection. $^b$Error represents the 95% CI of the y-intercept ($\log K_f$) or slope ($n$). $^c$Not applicable.

Soil 1 Native pH (5.1) Isotherm. Linearized Freundlich isotherms (Eqn 2; Fig. 15) of the anionic 6:2 and 8:2 FTSA and the zwitterionic 6:2, 8:2, and 10:2 FTSaB for Soil 1 at pH 5.1 indicates the relative sorption strength of each sorbate. The values of the y-intercepts (i.e. $\log K_f$) indicate that the strongest sorbed analyte is the 10:2 FTSaB, followed by the 8:2 FTSaB, 8:2 FTSA, 6:2 FTSaB, and 6:2 FTSA, which is the weakest sorbed analyte. Within each class (FTSA and FTSaB), sorption increases as the fluorinated chain length increases. The increase in sorption with fluorinated chain length for PFCAs and PFSAs was observed in previous sorption studies.  

The vertical increase of the last 3 points in the 6:2 FTSA and 6:2 FTSaB isotherms strongly suggests that sorption is no longer following monolayer sorption but becomes multilayer sorption at the highest sorbate concentrations. The cationic 6:2 FTSaAm and the zwitterionic 12:2 FTSaB were not detected (< LOD) in the aqueous phase and were completely sorbed to the soil. For the purposes of brevity, only the isotherms for Soil 1 at the native pH are discussed here; isotherms for all analytes for Soils 2-6, and for Soil 1 pH 4 and pH 7 are not shown.
FTSA, FTSaB, and 6:2 FTSaAm

Sorption. The $\log K_d$ increase per CF$_2$ group between the 6:2 and 8:2 homologs for the FTSAs and FTSaBs was $0.41 \pm 0.059$ and $0.20 \pm 0.088$, respectively. The increase in $\log K_d$ per CF$_2$ group for the anionic FTSAs is consistent with the 0.5-1 $\log K_d$ increases for the anionic PFCAs and PFSAs.\textsuperscript{23,26,67} The $\log K_d$ increase per CF$_2$ group for the zwitterionic FTSaBs is lower than expected $\log K_d$ increase for the anionic FTSAs. However, the analytical variability was greater for the FTSaBs, due to a lack of internal standards, relative to the FTSAs.

For the same fluorinated chain length, the 6:2 FTSaB has a larger $K_d$ than the 6:2 FTSAs for all soils and pH conditions, despite the greater size and molecular weight of the 6:2 FTSaB. Since the 6:2 FTSaB head group has two positive charges and a terminal negative charge at pH 5.1 (Fig. 2), the head group is contributing to increased interactions, presumably due to cation exchange. The 6:2 FTSaAm, which was completely sorbed to all soils except Soil 6, has two positive charges (Fig. 2), one of which is terminal. The 6:2 FTSaAm has the highest observed $K_d$ value (Table A8), so the number of positive charges cannot explain the difference in sorption. Rather, the position of the charges is important; in this case, the terminal positive charge on the 6:2 FTSaAm leads to greater sorption onto soil.

The single-point $K_d$ values for the 6:2 FTSA (3.1-12 L/kg; Table A8) are in good agreement with the field-$K_d$ values reported (5.8 L/kg\textsuperscript{29} and 3.2 L/kg\textsuperscript{32}) for sediment-water systems. The $K_d$ values for the 6:2 FTSaB range from 23-240 L/kg (Table A8), which are significantly greater than the single field-$K_d$ of 4.5 L/kg.\textsuperscript{29} The single $K_d$ value obtained for the cationic 6:2 FTSaAm was 650 L/kg (Table A8), which is also significantly greater than the single reported field-$K_d$ of 34 L/kg.\textsuperscript{32} The lower values reported by Boiteux et al.\textsuperscript{29} are likely due to incomplete extraction of zwitterionic and cationic PFASs from sediment using the milder extraction conditions (1% acetic acid in methanol) compared to the 0.5 M HCl in methanol that was used in the present study.

Correlations. Sorption of anionic FTSAs does not indicate a trend in $K_d$ with organic carbon (Fig. 16). The $K_d$ values for the longer-chained 8:2 FTSA are consistently greater than those of 6:2 FTSA except for Soil 4 ($K_d = 120$ L/kg), in which the 8:2 FTSA $K_d$ is disproportionately high for an unknown reason. At the lowest levels of organic carbon (0.098 – 0.12%), the $K_d$ values are similar in magnitude to the soils with higher organic carbon (1.0 – 2.3%), which suggests that the mineral phase in the low organic carbon soils (Soils 2, 6) influences sorption.\textsuperscript{24,25,32} The nonpolar fluorinated tail of the anionic FTSAs may interact with the hydrophobic (nonpolar) sites between the charged sites on the mineral surface via weak hydrophobic interactions.\textsuperscript{24,69}
The lack of a correlation in FTSA sorption may be due to the extent of organic matter decomposition of the soils tested. If the C/N ratio is accepted as a rough proxy for the extent of organic matter decomposition, with a wide C/N indicating a relative prevalence of aliphatic C and nonpolar functionality, the quality of organic matter can be determined. Soils 1-3 and 5 have either low organic carbon content or a lower C/N ratio (greater number of carboxyl groups; Table 1), indicating a minimal influence of organic carbon on sorption (Soil 2) or less hydrophobic organic matter (Soils 1, 3, and 5). Therefore, the lack of correlation of FTSA sorption with soil organic carbon may be due to low hydrophobicity of the organic matter of the soils tested, with the exception of Soil 4, which is more hydrophobic (higher C/N ratio; Table 1) with fewer carboxyl groups.

To the best of our knowledge, studies examining PFAS sorption do not consider metrics for the extent to which decaying organic matter that has ionizable, oxygen containing functional groups, which needs to be examined further. Many sorption studies of PFCAs and PFSAs observe an increase in $K_d$ with increasing organic carbon, while a single study found little change in $K_d$ with increasing organic carbon. The sorption of zwitterionic 6:2 and 8:2 FTSaBs also do not correlate with organic carbon (Fig. 17), which is consistent with reports that $K_d$ values for zwitterionic pharmaceuticals do not correlate with organic carbon content. The $K_d$ values for the 8:2 FTSaB are consistently and proportionally higher than the 6:2 FTSaB, due to the increased hydrophobicity of the additional two CF$_2$ groups. Due to the proportionate increase in $K_d$ for the FTSaBs with organic carbon, the disproportionate increase in the 8:2 FTSA $K_d$ in Soil 4 (Fig. 17a) may be an outlier. Interestingly, the magnitude of the $K_d$ values for the zwitterionic FTSaBs are higher than the anionic FTSA, which is likely due to the polar head group, as will be discussed below.

Not surprisingly, the sorption of the anionic 6:2 and 8:2 FTSA does not correlate with CEC (Fig. 18a), due to repulsion between negatively-charged organic matter and mineral surfaces and the negatively charged sulfonate group. The high $K_d$ of 120 L/kg for the 8:2 FTSA in Soil 4 with a CEC of 7.1 meq/100 g is attributed to the more hydrophobic nature of the organic matter in this

**Fig. 17.** No correlation between (A) the anionic FTSA and (B) the zwitterionic FTSaB and cationic 6:2 FTSaAm and soil organic carbon.
soil as discussed above rather than cation exchange processes.

Sorption of the zwitterionic 6:2 FTSaB does not correlate with increasing CEC, while the $K_d$ values for the 8:2 FTSaB may increase with increasing CEC (Fig. 18b). The proportionate increase in $K_d$ from the 6:2 FTSaB to the 8:2 FTSaB may arise from the greater affinity of the 8:2 FTSaB to the hydrophobic sites in between the localized negative charges on the smectite surface.68 Interestingly, the cationic 6:2 FTSaAm was completely depleted from the aqueous phase in all but one soil (Soil 6), which has the highest CEC (35 meq/100 g). The terminal positive charge in the cationic 6:2 FTSaAm may interact more readily with the cation exchange sites than the FTSaBs, in which the terminal carboxyl group of the FTSaBs may repel the negatively-charged CEC sites.73 Regardless, sorption studies with zwitterionic and cationic veterinary pharmaceuticals.36,37 and cationic hydrocarbon surfactants74 report that CEC is the major sorption driver.

The negative charge (cation exchange sites) of sorbent surfaces in soils likely arises from mineral as well as organic matter constituents.75 Soil organic matter develops negative charges as the oxidative decomposition process acting on plant litter adds carboxyl groups with pKas in the range of 4-6.76 Phyllosilicates develop permanent negative charge through isomorphic substitutions within Al-octahedra or Si-tetrahedra of the mineral phase.68,77 The extent of negative charge is conveniently expressed by the capacity of the soil matrix to adsorb and exchange cations with the soil solution (CEC). Soil 6 is unique with regards to CEC, since the clay fraction of Soil 6 is dominated by smectite with low (0.098%) organic carbon. Soil 2, which also has low organic carbon and a high CEC (but half that of Soil 6), contains some smectite as well as pedogenic iron oxides and other clay minerals. Although Soils 2 and 6 have similar organic carbon and CEC, the additional minerals and pedogenic iron oxides present in Soil 2 may provide additional mechanisms necessary to sorb the cationic 6:2 FTSaAm, since sorption of some organic cations is influenced by both CEC and organic carbon.75 The greater complexity of the mineral phase in Soils 1-5 indicates the possibility of sorbate-sorbent interactions through a variety of mechanisms not limited solely to cation exchange, although the exact mechanisms need to be determined.
The lack of a correlation between the zwitterionic 6:2 FTSaB and CEC and the single $K_d$ value for the cationic 6:2 FTSaAm for Soil 6 (highest CEC) suggests that the bulk soil parameters do not fully explain the sorption mechanisms of zwitterions and cations. The easily-measured bulk soil parameters indicate the total number of charged sites, rather than the actual accessibility (spatial and steric effects) of zwitterions and cations to the charged sites (i.e. sites for CEC, cation bridging, anion ligand exchange).\textsuperscript{78}

The $K_d$ model proposed by MacKay and Vasudevan\textsuperscript{78} incorporates the accessibility of charged sites:

$$K_d = \frac{C_{s,\text{Type I}} + C_{s,CE} + C_{s,CB+LE}}{C_w} \quad \text{(Eqn 3)}$$

where $C_{s,\text{Type I}}$ is the analyte concentration on the soil due to hydrophobic interactions. The $K_d$ model of MacKay and Vasudevan\textsuperscript{78} indicates that a combination of hydrophobic interactions (i.e. hydrophobic sites or more hydrophobic organic matter) and exchange processes contribute to the overall sorption of zwitterions and cations, which is likely the case with the zwitterionic FTSaBs and the cationic 6:2 FTSaAm.

Surprisingly, sorption of the anionic FTSAs remains unchanged as AEC increases (Fig. 19a), with the exception of the high $K_d$ value of the 8:2 FTSA for Soil 4 (AEC = 2.2 meq/100 g). The sorption of the 8:2 FTSA is proportionally greater than the 6:2 FTSA, except in Soil 4. Since Soil 4 has both the highest organic carbon content and the highest AEC, the exact sorption mechanism is difficult to determine and may be a combination of the two parameters.

Anion exchange sites are fully protonated ($\text{pH} \leq 6$), singly coordinated hydroxyl groups that arise from poorly crystalline forms of iron oxides (ferrihydrite) and aluminosilicates.\textsuperscript{79} Soil 3 is a particularly oxide rich Ultisol, and Soil 4 has a poorly crystalline mineral phase (“andic” soil properties) that contains singly coordinated hydroxyl groups. In the case of Soil 4 with the anionic 8:2 FTSA, inner sphere complex formation via anionic ligand exchange with the singly coordinated hydroxyl groups\textsuperscript{79} may contribute to the observed increase in sorption. However, if

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Fig19}
\caption{Lack of correlations for the (A) anionic FTSAs and (B) zwitterionic FTSaBs and cationic 6:2 FTSaAm as a function of AEC. Error bars indicate the 95\% CI.\textsuperscript{3}}
\end{figure}
inner sphere complex formation was driving sorption in Soil 4, a proportional increase in the sorption of the 6:2 FTSA would be expected. The lack of correlation between FTSA sorption and AEC is in direct contrast to anionic pesticide sorption experiments, which found an increase in $K_d$ with increasing AEC.\textsuperscript{37,79}

The sorption of the zwitterionic FTSAbs does not change with AEC (Fig. 19b). Similar to previous bulk soil parameters, the 8:2 FTSAB is more strongly sorbed than the 6:2 FTSAB. The magnitude of the $K_d$ values of the zwitterionic FTSAbs are greater than the $K_d$ values for the anionic FTSAbs. AEC trends with zwitterion sorption are less frequently studied, but zwitterionic pesticide sorption experiments found either an increase\textsuperscript{80} or no change\textsuperscript{81} in sorption with increasing AEC. The differing conclusions suggest that the location of the charge may influence zwitterion sorption by anion exchange processes. Although the FTSAbs have a terminal carboxyl group, the two positively charged moieties appear to have a larger impact on sorption.

As pH increases from 4 to 7, $K_d$ values remain unchanged for the anionic 6:2 FTSA but decrease for the anionic 8:2 FTSA (Fig. 20). For the zwitterionic FTSAbs, however, sorption remains unchanged as pH increases (Fig. 20).

The decrease in $K_d$ with increasing pH for the anionic 8:2 FTSA is consistent with observed decreases in sorption for organic acids\textsuperscript{82} and other anionic PFASs\textsuperscript{23} with an increase in pH. The decrease is linear, with a decrease of 12 L/kg in the $K_d$ with a unit increase in pH (-0.17 log units per unit increase in pH), which is less steep relative to other anionic PFASs.\textsuperscript{23} Since the 6:2 and 8:2 FTSA remain anions in the pH range studied, the decrease in sorption is likely due to deprotonation of the negatively charged organic matter (pK$_a$ ~ 4.5) and thus anion repulsion. Alternatively, the singly coordinated hydroxyl groups of the iron and aluminum oxides that contribute to AEC may not be fully protonated (pK$_a$ ~ 8.5),\textsuperscript{79} and anionic ligand exchange may not occur as frequently at pH 7, which would also result in the lowest $K_d$.

The lack of correlation of the zwitterionic FTSAbs with increasing pH may be due to the speciation of the FTSAbs. At pH 4, the FTSAbs have 2 positive charges and ~97% deprotonated carboxyl group (negative charge). At pH 7, the FTSAbs have a negative carboxyl group, a positively charged quaternary amine, and ~95% protonated sulfonamide N. Therefore, the speciation of the zwitterionic FTSAbs does not significantly change in the pH range studied. The minimal change in $K_d$ values suggest that the deprotonation of the negatively charged organic matter has little influence on the sorption of the FTSAbs. The finding contrasts sorption studies of veterinary pharmaceuticals that found pH to play a significant role in zwitterion sorption.\textsuperscript{36,37,81,83}
DELIVERABLES

- Improved understanding of the PFAS components of AFFF formulations stockpiled and potentially released to the environment
- New analytical methodology for a comprehensive list of PFASs that occur in groundwater and soil
- New methodology that quantifies groundwater and sediment mixtures’ potential to form dead-end PFASs
- Improved understanding of the scope and scale of military site contamination by AFFF components
- New information on the biotransformation of precursors in Ansul AFFF that biodegrade to FTSAs, FTCAs, and PFCAs
- New information on AFFF influence on transport of priority pollutants and transformation of TCE
- Interactions with Higgins’ project (ER-2126) for additional leverage and understanding of transport processes
- 8 peer-reviewed articles in Environmental Science and Technology and 1 paper in Environmental Science and Technology Letters
- 5 Ph.D. graduate students and 1 undergraduate received their degrees working on this project
Table A7. Concentrations of the PFASs quantified in archived groundwater samples from Wurtsmith AFB (WAFB), Naval Air Station Fallon (NASF), and Tyndall AFB (TAFB). Legacy PFAS concentrations are reported in Schultz et al.\(^{10}\)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>TAFB TY22FtA</th>
<th>TAFB T 11-2</th>
<th>TAFB PW-7</th>
<th>TAFB PW-10</th>
<th>NASF MW 16</th>
<th>NASF MW 51-U</th>
<th>WAFB FT-3</th>
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</thead>
<tbody>
<tr>
<td>4:2 FTTAoS(^b)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>6:2 FTTAoS</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>8.8</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>PFBSaAm(^c)</td>
<td>4.1(^c)</td>
<td>11</td>
<td>&lt;LOD</td>
<td>720</td>
<td>&lt;LOD</td>
<td>550</td>
<td>26</td>
</tr>
<tr>
<td>PFPeSaAm(^c)</td>
<td>2.8(^c)</td>
<td>7.8</td>
<td>5.1</td>
<td>190</td>
<td>&lt;LOD</td>
<td>61</td>
<td>79</td>
</tr>
<tr>
<td>PFHxSaAm(^c)</td>
<td>5.7</td>
<td>8.3</td>
<td>6.3</td>
<td>260</td>
<td>&lt;LOD</td>
<td>260</td>
<td>36</td>
</tr>
<tr>
<td>PFBSAmA(^c)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>62</td>
<td>660</td>
<td>&lt;LOD</td>
<td>9.7</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>PFPeSaAmA(^c)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>7.9</td>
<td>610</td>
<td>&lt;LOD</td>
<td>5.8</td>
<td>&lt;2.7</td>
</tr>
<tr>
<td>PFHxSaAmA(^c)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>10</td>
<td>590</td>
<td>&lt;LOD</td>
<td>38</td>
<td>&lt;2.7</td>
</tr>
</tbody>
</table>

\(^a\)no 8:2 FTTAoS, 6:2 FTTHN\(^+\), fluorotelomer sulfonamido betaines (FTSaB), fluorotelomer sulfonamido amines (FTSaAm);\(^b\)calculated assuming equal molar response to 6:2 FTTAoS;\(^c\) calculated assuming equal molar response to PFOS; <LOQ = below the lowest level of quantification
Table A8. Computed $K_d$ values$^a$ and the corresponding $C_w$ (nmol/L) for the 6:2 FTSA, 8:2 FTSA, 6:2 FTSaB, 8:2 FTSaB, 10:2 FTSaB,$^b$ and 6:2 FTSaAm using the 5,000 ng/L $C_w$ and the 60,000 ng/L $C_w$.\(^c\)

<table>
<thead>
<tr>
<th>Soil #</th>
<th>$C_w$ in 5,000 ng/L $C_w$ R$^c$</th>
<th>6:2 FTSA</th>
<th>8:2 FTSA</th>
<th>6:2 FTSaB</th>
<th>8:2 FTSaB</th>
<th>10:2 FTSaB$^a$</th>
<th>6:2 FTSaAm</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td>2.4</td>
<td>&lt;LOQ</td>
<td>2.1</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td></td>
<td>$C_w$ in 5,000 ng/L $C_w$ R2</td>
<td>2.6</td>
<td>0.022</td>
<td>2.4</td>
<td>0.21</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td></td>
<td>$C_w$ in 5,000 ng/L $C_w$ R3</td>
<td>2.4</td>
<td>0.019</td>
<td>2.5</td>
<td>0.22</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td></td>
<td>$C_w$ in 5,000 ng/L $C_w$ R4</td>
<td>2.4</td>
<td>0.019</td>
<td>2.4</td>
<td>0.21</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td></td>
<td>$C_w$ in 60,000 ng/L $C_w$ R1$^c$</td>
<td>29</td>
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<td>36</td>
<td>1.8</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td></td>
<td>$C_w$ in 60,000 ng/L $C_w$ R2</td>
<td>29</td>
<td>0.28</td>
<td>35</td>
<td>2.7</td>
<td>1.2</td>
<td>&lt;LOD</td>
</tr>
<tr>
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<td>$C_w$ in 60,000 ng/L $C_w$ R3</td>
<td>29</td>
<td>0.27</td>
<td>35</td>
<td>2.1</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td></td>
<td>$K_d$ (5,000 ng/L $C_w$; L/kg)</td>
<td>5.9 ± 2.0</td>
<td>30 ± 4.2</td>
<td>31 ± 10</td>
<td>120 ± 15</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>$K_d$ (60,000 ng/L $C_w$; L/kg)</td>
<td>8.5 ± 4.6</td>
<td>39 ± 2.0</td>
<td>62 ± 10</td>
<td>220 ± 63</td>
<td>240 ± 83</td>
<td>n/a</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3.0</td>
<td>&lt;LOQ</td>
<td>0.71</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOD</td>
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<tr>
<td></td>
<td>$C_w$ in 5,000 ng/L $C_w$ R2</td>
<td>3.1</td>
<td>0.028</td>
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<td>&lt;LOQ</td>
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<tr>
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<td>$C_w$ in 5,000 ng/L $C_w$ R3</td>
<td>3.0</td>
<td>0.021</td>
<td>0.76</td>
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<td>&lt;LOD</td>
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<tr>
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<td>&lt;LOD</td>
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<td>$C_w$ in 60,000 ng/L $C_w$ R1</td>
<td>42</td>
<td>0.11</td>
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<td>150 ± 34</td>
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<td>170 ± 110</td>
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<td>34 ± 16</td>
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OSU ER-2128 Final Report
## APPENDIX

<table>
<thead>
<tr>
<th>Soil #</th>
<th>6:2 FTSA</th>
<th>8:2 FTSA</th>
<th>6:2 FTSaB</th>
<th>8:2 FTSaB</th>
<th>10:2 FTSaB</th>
<th>6:2 FTSaAm</th>
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<td>&lt;LOQ</td>
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<td>35</td>
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### APPENDIX

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<th>Soil #</th>
<th>6:2 FTSA</th>
<th>8:2 FTSA</th>
<th>6:2 FTSaB</th>
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<td>1.1</td>
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<td>6.6 ± 1.8</td>
<td>66 ± 10</td>
<td>79 ± 32</td>
<td>230 ± 23</td>
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<td>K&lt;sub&gt;d&lt;/sub&gt; (60,000 ng/L C&lt;sub&gt;w&lt;/sub&gt;; L/kg)</td>
<td>6.3 ± 3.0</td>
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<td>64 ± 41</td>
<td>190 ± 62</td>
<td>520 ± 150</td>
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<td>0.039</td>
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<td>0.37</td>
<td>50</td>
<td>2.3</td>
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<td>C&lt;sub&gt;w&lt;/sub&gt; in 60,000 ng/L C&lt;sub&gt;w&lt;/sub&gt; R3</td>
<td>36</td>
<td>0.37</td>
<td>53</td>
<td>2.9</td>
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<td>19 ± 2.7</td>
<td>30 ± 9.7</td>
<td>140 ± 13</td>
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<td>17 ± 1.5</td>
<td>31 ± 16</td>
<td>110 ± 42</td>
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*Error represents the 95% CI of the n = 4 or n = 3 replicates that incorporates the 95% CI of both K<sub>f</sub> and n; The 12:2 FTSaB is not included since all aqueous phase concentrations were below the limit of detection. R1 indicates replicate 1, R2 is replicate 2, etc. LOQ = less than the limit of quantification. <LOD = less than the limit of detection. n/a= Not applicable.*
REPORTING AND DISSEMINATION

Reporting

Task 6 for this project included non-experimental subtasks including filing of an interim report and final report. In addition, monthly financial reports and quarterly progress reports were filed as required by SERDP. In progress review presentations were given as scheduled. In addition the findings of this research were disseminated through journal manuscripts, oral presentations, posters and webinars. Three PhD theses also resulted from this work.

Dissemination

The results of this project resulted in 27 public presentations and five webinars as provided below:

9. J. Field. Fluorinated Chemical Research Roadmap Meeting, Sponsored by SERDP,
March 7, 2013 [Invited]
10. J. Field. SERDP/ESTCP Contaminated Groundwater Workshop, Arlington, VA 
August 13-14, 2013 [Invited]
11. J. Field. SERDP and ESTCP Workshop on Long Term Management of Contaminated 
Groundwater Sites, October 2013 [Invited]
12. J. Field FCASA Management of Perfluoroalkylated Compounds At Federal 
Contaminated Sites, Ottawa (Ontario) Canada, February 19-20, 2014 [Invited Speaker].
13. J. A. Field “Per- and Polyfluoroalkyl Substances (PFASs) at US Military Sites” 6th 
International Workshop Per- and Polyfluoroalkyl Substances – PFASs, Analysis, Fate, 
Human Exposure, Regulation, June 15-18, 2014, Idstein Germany [Invited Speaker]
14. KA Barzen-Hanson and J Field. “Discovery and Implications of C2 and C3 Sulfonates in 
Groundwater and Aqueous Film-Forming Foams. “ Society of Environmental Toxicology 
15. J. A. Field “State of the Art Knowledge on Per- and Polyfluoroalkyl 
Substances (PFASs) DoD Environmental Monitoring and Data Quality Workshop, 2015 Portland, OR 
April 28 [Invited Speaker] 
16. KA Barzen-Hanson, M Kleber, and J Field. “Sorption of Anionic, Zwitterionic, and 
Cationic Per- and Polyfluoroalkyl Substances to Soil and Sediment. “ Fluoros. Golden, 
17. KA Barzen-Hanson and J Field. “Discovery of Novel Per- and Polyfluoroalkyl 
Substances in Aqueous Film-Forming Foams and Groundwater. “ Invited Seminar at 
18. KA Barzen-Hanson, M Kleber, and J Field. “Sorption of Anionic, Zwitterionic, and 
Cationic Polyfluoroalkyl Substances in AFFF to Soil and Sediment. “ International 
Conference on Chemistry and the Environment 2015. Leipzig, Germany. Sept. 20-24, 
19. KA Barzen-Hanson, S Roberts, C Higgins and J Field. “Towards Closing the Mass 
Balance on Per- and Polyfluoroalkyl Substances in Groundwater at Aqueous Film-
Forming Foam Impacted Sites. “ International Conference on Chemistry and the 
20. KA Barzen-Hanson, S Roberts, C Higgins and J Field. “Discovery of Novel Per- and 
Polyfluoroalkyl Substances in Aqueous Film-Forming Foams and Groundwater. “Invited 
21. KA Barzen-Hanson, S Roberts, G Peaslee, C Higgins and J Field. “Closing the Mass 
Balance on Per- and Polyfluoroalkyl Substances in Groundwater at Aqueous Film-
Forming Foam (AFFF) Impacted Sites. “ American Chemical Society 251st National 
22. KA Barzen-Hanson, M Kleber, and J Field. “Sorption of Anionic, Zwitterionic, and 
Cationic Polyfluoroalkyl Substances in AFFF to Soil and Sediment. “ American 
23. J. A. Field “Per- and Polyfluoroalkyl Substances (PFASs): Frequently Asked Questions 
[Invited Keynote Speaker].
(PFASs)” Navy Remediation Innovative Technology Seminar (RITS) Series. Gave
workshops to Navy program managers in Washington DC (Apr 25), Norfolk, VA (Apr 26), San Diego (May 9), Honolulu, HI (May 18), and Jacksonville, FL (June 1) [Invited Speaker]


26. J. A. Field “State of the Art Knowledge on Per- and Polyfluoroalkyl Substances (PFASs) Australia Air Land Groundwater Association (ALGA) Sponsored Travel: Keynote Speaker, Workshop speaker at EcoForum, Perth Oct 25-27; Keynote speaker at ALGA Events in Melbourne (Oct 31), Sydney (Nov. 1), and Brisbane (Nov. 2) [Invited Speaker]


Webinars

1. J.A. Field participated in the Environmental Committee of the Society of American Military Engineers (SAME) Webinar on November 7, 2012. The talk was on the topic of Perfluorinated Compounds and had 58 participants from a number of private companies, the military, and federal agencies.

2. Dr. Field and Cornell Long of the Air Force participated in a webinar on October 3, 2013 titled “Perfluorinated Compounds”, which was hosted by the Groundwater Resources Association of California (GRACast).

3. Dr. Field gave a talk titled “Analytical Issues & Overview of per- and poly-fluoroalkyl substances (PFAS)“ as part of a Society of American Military Engineers (SAME) Webinar titled ‘DOD emerging Contaminant Programs: Prioritization, Investigation, and Remediation’, held on November 19, 2014. The webinar had 90 participants from a number of private companies, military personnel, and federal agencies.

4. J. Field participated in the SERDP/ESTCP Webinar “Emerging Contaminants: DoD Overview and State of Knowledge on Fluorochemicals and 1,4-Dioxane” on December 3, 2015 as part of SERDP’s Tools and Training Webinar Series

5. J. Field participated in the SERDP/ESTCP Webinar “Emerging Contaminants: DoD Overview and State of Knowledge on Per- and Polyfluoroalkyl Substances (PFASs): Analytical and Characterization Frontiers on January 28, 2016 as part of SERDP’s Tools and Training Webinar Series


Peer-reviewed publications (to date)


Backe, W.J. and Field, J.A. 2012 Is SPE necessary for environmental analysis? A quantitative


**Manuscript in progress**

Barzen-Hanson, K.A., Davis, S.E., Kleber, M., Field, J.A. Submitted. Sorption of the Fluorotelomer Sulfonates, Fluorotelomer Sulfonamido Betaines, and Fluorotelomer Sulfonamido Amine in National Foam Aqueous Film-Forming Foam to Soil. Environmental Science and Technology

Awards/Other Impacts

The manuscript by Barzen-Hansen, K. and Field, J.A titled, “Discovery and implications of C2 and C3 perfluoroalkyl sulfonates in aqueous film forming foams (AFFF) and groundwater” that was published in Environmental Science and Technology Letters in 2015 received the Editor’s Choice award and the 2015 Environmental Science and Technology Letters Best Paper Award.

Ms. Barzen-Hanson won a 2015 National Science Foundation Graduate Research Fellowship Program Fellowship for her proposal titled “Polyfluorinated Alkyl Substances at Aqueous Film-Forming Foam (AFFF) Impacted Sites: Identification of Unknowns and Effective Chemical Oxidation Remediation Development".

The C3-perfluoropropane sulfonate reported in the 2015 in Environmental Science and Technology Letters paper was synthesized for commercial sale by Wellington Laboratories in 2016.

Summary and Conclusions

Key points from Task 1:

- Over 50 classes of PFASs, comprised of several individual homologs, were identified in AFFF formulations and groundwater over the course of this project
- Perfluorinated chain lengths ranged from 4 to 12 but the major perfluorinated chain length had six perfluorinated carbons.
- Carboxylates (e.g., PFOA) and FTSA were only minor components of ECF and telomer-based AFFF formulations, respectively.
- Many of the newly-identified classes of PFASs are cationic or zwitterionic and this may impact their association with sediments and soils in the field, as well as their toxicity.
- TOP assay data indicate that AFFF formulations generate only carboxylates upon oxidation.
- Determining the agreement between the TOP assay and the estimated concentration of newly-identified PFASs has yet to be determined (e.g., mass balance on the TOP assay).

Key Points from Task 2:

- After development of analytical methodology for newly-identified PFASs, PFCAs, and PFASs, and FTSA remain the most abundant PFASs in groundwater.
- Up to half of precursors in groundwater, sediment, and soil, as measured by the TOP assay, cannot be accounted for by precursors measured directly by LC-MS/MS.
- Application of the TOP assay to field samples indicates that PFASs in groundwater are likely characterized by chain lengths < C8 while precursors in soil/sediments are characterized by longer chain lengths

Key Points from Task 3

- 6:2 and 8:2 FTSA degradation to PFCAs occurred in aerobic microcosms prepared with pristine soils and National Foam AFFF.
- 6:2 FTTAoS degradation to FTSA and PFCAs occurred in aerobic enrichments derived from
pristine soil previously cultured with National Foam AFFF.

- Rapid degradation of 6:2 FTTAoS to FTSA and PFCAS occurred in aerobic enrichments prepared with contaminated soil from Ellsworth Air Force Base.
- 6:2 FTTAoS degradation was observed in anaerobic microcosms under nitrate-, sulfate-, iron-reducing, and methanogenic conditions with the greatest degradation rates were observed under nitrate-reducing conditions.
- The formation of perfluoroalkyl thiocarboxylate (FTTCA) was identified for the first time as a transformation product of FTTAoS.
- 3M AFFF and DGBE are capable of promoting reductive dechlorination of TCE in enriched anaerobic microbial communities when they are provided as the sole carbon and energy source.
- Significant quantities of hydrogen are produced when National Foam AFFF is amended to the cultures; however, reductive dechlorination does not occur after 2 weeks with this specific AFFF formulation.
- The microbial community of D. mccartyi demonstrated the capacity to ferment the non-fluorinated organic components (e.g., DGBE) in all four AFFF formulations tested to hydrogen and acetate.
- The products formed varied in concentration with the type of AFFF formulation tested. In the presence of 3M AFFF, TCE was dechlorinated but not in the presence of Ansul and National Foam AFFFs.
- This research indicate that the DGBE stimulates TCE dechlorination while PFAS in some AFFF formulations inhibited reductive dechlorination.

Key Points from Task 4

- Low $K_d$ values for the anionic 6:2 FTSA suggest that the 6:2 FTSA is highly mobile in groundwater.
- Higher $K_d$ values indicate that the anionic 8:2 FTSA, zwitterionic FTSaBs, and cationic 6:2 FTSaAm are more likely to be associated with soil and sediment of source zones.
- Complete removal of the cationic FTSaAm indicates potential for strong sorption to source zone soils and sediments at some sites.
- In contrast, measurement of cationic PFASs in groundwater indicates the potential to impact surface water sources and cationic surfactants are more toxic to aquatic species.
- Removal of zwitterionic and cationic PFASs from drinking water sources by conventional technology (GAC) is unknown.
- The lack of correlations between the sorption of anionic FTSAs, zwitterionic FTSaBs, and cationic 6:2 FTSaAm and parameters including organic carbon content, CEC, and AEC, indicates that the bulk parameters do not adequately predict sorption. More research is needed the factors that control their sorption.
- Sorption of additional PFASs in AFFF to a soil already saturated with PFASs may increase the sorption of the added PFASs (e.g., multi-layer sorption).
- Conditions (e.g. pH and ionic strength) that promote desorption of zwitterionic and cationic PFASs need to be determined to determine potential for source zone sediments to act as long-term PFAS sources.
References


(3) Barzen-Hanson, K. A.; Davis, S. E.; Kleber, M.; Field, J. A. Sorption of the fluorotelomer sulfonates, sulfonamido betaines, and sulfonamido amine in National Foam aqueous film-forming foam to soil. Submitted to Environ Sci Technol.


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(72) Pan, G.; You, C. Sediment-water distribution of perfluorooctane sulfonate (PFOS) in


