

UC Berkeley

UC Berkeley Previously Published Works

Title

Aerobic Biotransformation of Fluorotelomer Thioether Amido Sulfonate (Lodyne) in AFFF-Amended Microcosms

Permalink

<https://escholarship.org/uc/item/0kq802z7>

Journal

Environmental Science and Technology, 49(13)

ISSN

0013-936X

Authors

Harding-Marjanovic, Katie C
Houtz, Erika F
Yi, Shan
[et al.](#)

Publication Date

2015-07-07

DOI

10.1021/acs.est.5b01219

Peer reviewed

Aerobic Biotransformation of Fluorotelomer Thioether Amido Sulfonate (Lodyne) in AFFF-Amended Microcosms

[Katie C. Harding-Marjanovic](#)[†], [Erika F. Houtz](#)[†], [Shan Yi](#)[†], [Jennifer A. Field](#)[‡], [David L. Sedlak](#)[†], and [Lisa Alvarez-Cohen](#)^{†§}

[†] Department of Civil and Environmental Engineering, University of California at Berkeley, Berkeley, California 94720, United States

[‡] Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon 97331, United States

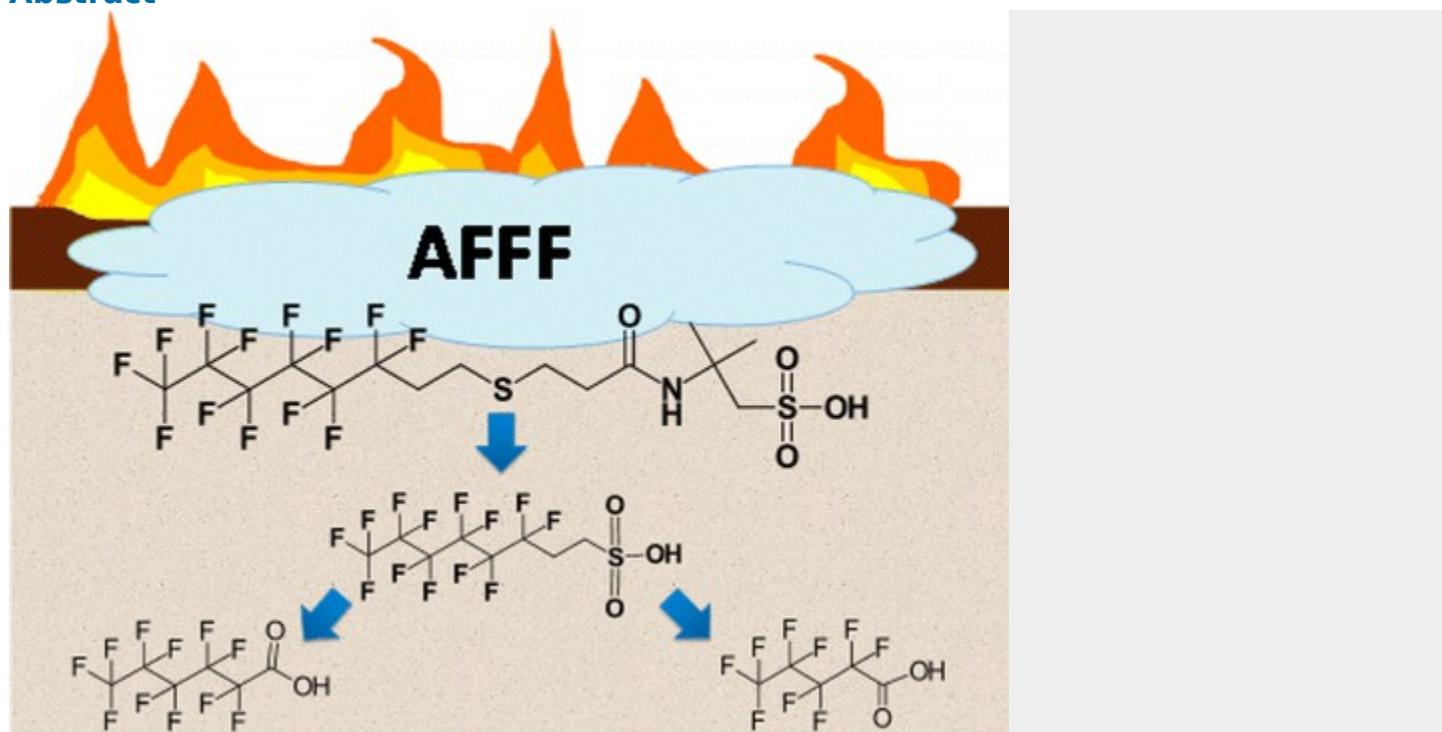
[§] Earth Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, California 94720, United States

*L. Alvarez-Cohen. Phone: 510-643-5969. E-mail: alvarez@ce.berkeley.edu

DOI: 10.1021/acs.est.5b01219

Publication Date (Web): June 4, 2015

Abstract



The aerobic biotransformation pathways of 4:2, 6:2, and 8:2 fluorotelomer thioether amido sulfonate (FtTAoS) were characterized by determining the fate of the compounds in soil and medium microcosms amended with an aqueous film-forming foam (AFFF) solution. The biotransformation of FtTAoS occurred in live microcosms over approximately 40 days and produced 4:2, 6:2, and 8:2 fluorotelomer sulfonate (FtS), 6:2 fluorotelomer unsaturated carboxylic acid (FtUCA), 5:3 fluorotelomer carboxylic acid (FtCA), and C₄ to C₈ perfluorinated carboxylic acids (PFCAs). Two biotransformation products corresponding to singly and doubly oxygenated forms of 6:2 FtTAoS were also identified through high resolution mass spectrometry (MS) analysis and liquid chromatography tandem-MS. An oxidative assay was used to indirectly quantify the total concentration of polyfluorinated compounds and check the mass

balance. The assay produced near complete mass recovery of FtTAoS after biotransformation, with 10% (mol/mol) of the amended FtTAoS accounted for in FtS, FtCA, and PFCA products. The transformation rates of identified products appear to be slow relative to FtTAoS, indicating that some intermediates may persist in the environment. This study confirms some of the sources of FtS and PFCAs in groundwater and soil at AFFF-impacted sites and suggests that fluorinated intermediates that are not routinely measured during the biotransformation of PFASs may accumulate.

Introduction

Aqueous film-forming foams (AFFFs) are water-based chemical mixtures applied to liquid-fuel fires, such as ignited petroleum hydrocarbons or chlorinated solvents, to extinguish flames and prevent fuel reignition.[\(1, 2\)](#) Although AFFF is typically a proprietary mixture that varies according to manufacturer and year of production, most formulations consist of a glycol ether-based solvent, hydrocarbon surfactants, and various poly- and perfluoroalkyl substances (PFASs).[\(1\)](#) AFFF use at military, industrial, and municipal sites has led to widespread groundwater contamination with PFASs, as well as their occurrence in soil, surface water, and aquatic organisms.[\(3-9\)](#) Repeated AFFF application at training facilities where fire-fighting exercises were conducted in unlined pits has led to particularly high environmental contamination with PFASs.[\(6, 7, 9-11\)](#)

Perfluorocarboxylates (PFCAs) and perfluorosulfonates (PFSAs) are persistent,[\(5, 7, 10\)](#) resistant to biodegradation,[\(13\)](#) and cause adverse human health effects.[\(12\)](#) The 3M company manufactured AFFF with eight-carbon (C_8) perfluorosulfonamides, perfluorosulfonic acid (PFOS), and perfluorooctanoic acid (PFOA) until 2001 when rising concerns over their health and environmental effects led them to be phased out of production.[\(14, 15\)](#) The PFASs in formulations manufactured by other companies, such as Ansul, Chemguard, National Foam, Angus, and Buckeye, consisted of fluorotelomer-based compounds containing C_4 to C_{10} perfluorinated chains linked to an ionic alkyl group by two to three nonfluorinated carbons.[\(18\)](#) The nonfluorinated alkyl groups may have carboxylic acid, tertiary amine, dimethyl quarternary amine, and/or sulfide moieties.[\(18, 19\)](#) Despite growing knowledge of the composition of AFFF, little is known about the environmental fate of these PFASs, including their potential to biotransform under conditions representative of the top-soil, surface water, and aquifer systems in which they are found.

Fluorotelomer thioether amido sulfonate (FtTAoS, also sold under the trade name Lodyne, with a structure shown in Figure [4](#)) is a PFAS present in several widely used AFFF formulations made by at least three manufacturers (i.e., Ansul, Chemguard, and Angus) and was used as early as 1984.[\(18\)](#) Although the 6:2 fluorotelomer appears to be the most abundant FtTAoS homologue in many formulations, 4:2, 8:2, 10:2, 12:2, and 14:2 FtTAoS have also been detected in some AFFFs.[\(18, 19\)](#) FtTAoS has also been measured in groundwater and soil collected from firefighter-training sites.[\(10, 11\)](#) At one U.S. military base, FtTAoS was not detected in soil, despite extensive use of AFFF and detection of other PFASs.[\(10\)](#) At two different sites where the application of FtTAoS-containing AFFF was known to

occur, concentrations of 4:2 and 6:2 FtTAoS in groundwater were low relative to the measured concentrations of fluorotelomer sulfonates (FtSs) and PFCAs. Together, the FtS/FtTAoS and PFCA/FtTAoS ratios were much greater than the ratios measured in the AFFF formulations applied at the site, suggesting that FtTAoS may have been transformed to FtS and PFCAs after its release.[\(11\)](#)

Although PFCAs and PFASs have been shown to be completely resistant to microbial biotransformation under a variety of growth conditions,[\(13\)](#) the larger nonfluorinated alkyl groups of the AFFF-derived PFASs, including FtTAoS, are likely amenable to biotransformation, especially by the diverse metabolic capacity of a soil microbial community. Some fluorotelomer compounds with more simple nonfluorinated functional groups, such as 4:2, 6:2, and 8:2 fluorotelomer alcohol (FtOH), 6:2 FtS, and 6:2 polyfluoroalkyl phosphate esters (mono- and di-PAP) undergo biotransformation to terminal C₄–C₉ PFCAs as well as a number of other products.[\(20-26\)](#) However, slow rates of transformation and challenges in recovering transformation products have made it difficult to differentiate PFAS loss due to physical mechanisms (i.e., sorption) from loss due to biotransformation. Thus, given that the potential conversion of AFFF-derived PFASs like FtTAoS may constitute a significant future source of PFCAs in the environment and that PFCAs have varying toxicities and mobility, understanding the biotransformation potential of PFASs and their expected product formation is a topic of high importance.

Here the aerobic biotransformation of 4:2, 6:2, and 8:2 FtTAoS was investigated in soil slurries constructed with AFFF-impacted topsoil from a U.S. military base and enriched with an FtTAoS-containing Ansul AFFF formulation. Although 6:2 FtTAoS removal was previously observed in activated sludge,[\(32\)](#) it is unknown if other FtTAoS congeners are capable of transforming and whether their presence in an AFFF solution affects their transformation. Additionally, in the previously published study, the disappearance of 6:2 FtTAoS was observed in autoclaved controls, indicating that sorption or abiotic transformation may have constituted a significant portion of the observed FtTAoS mass loss.[\(32\)](#) In this study, the transformation and sorption of three FtTAoS congeners were examined and differentiated with live, sterile, and soil-free microcosms and quantitative mass balances of amended FtTAoS recovery were enabled using a high pH, heat-activated persulfate oxidation assay.[\(33\)](#) High resolution mass spectrometry was also used to identify two previously hypothesized biotransformation intermediates. By amending a native soil microbial community with a historically used AFFF formulation, FtTAoS transformation reactions can be described under conditions that more closely represent contaminated soil and groundwater, and the fate of other constituents of AFFF can be assessed.

Materials and Methods

Chemicals and Standards

AFFF manufactured by Ansul with an estimated 2008 manufacture date was obtained from a U.S. military base as previously described (Place et al. 2012).[\(18\)](#) It was stored in the laboratory in a sealed polyethylene tube at room temperature in the dark. The measured chemical composition of the formulation used in this study is provided in

the [Supporting Information](#), Table S2. The fluorinated surfactant constituents of the AFFF sample consisted of 4:2, 6:2, and 8:2 FtTAoS, as well as molecular ion (m/z) 602. No other fluorinated surfactants were detected in the formulation, including 6:2 fluorotelomer thiohydroxy ammonium (6:2 FtTHN⁺), which was described as a trace constituent in some Ansul AFFF formulations in Houtz et al. 2013, but was not detected in the stock solution used in this study. The concentration of diethylene glycol butyl ether (DGBE) in the sample was 220 g/L, which constituted over 80% of the total organic carbon. A commercial source material containing 6:2 FtTAoS was donated to Oregon State University from the Fire Fighting Foam Coalition and used as a standard reference material for quantitative analysis. The concentration of the analyte was determined in the material using patent data and manufacturer MSDS information, as described previously. [\(11, 40\)](#) C₄–C₁₀ perfluorocarboxylates, 4:2 FtS, 6:2 FtS, 8:2 FtS, 5:3 FtCA, 7:3 FtCA, 6:2 FtCA, 8:2 FtCA, 6:2 FtUCA, and 8:2 FtUCA for use as liquid chromatography-tandem mass spectrometry (LC-MS/MS) analytical standards were purchased from Wellington Laboratories (Guelph, Ontario, CA). All isotopically labeled internal standards used for LC-MS/MS analysis were also purchased from Wellington as previously described. [\(10, 33\)](#) Oxygen (>99.5%) and DGBE were obtained from Sigma-Aldrich (St. Louis, MO), whereas all other chemicals were purchased from either Fisher Scientific (Waltham, MA) or Sigma-Aldrich and of the highest purity possible.

Microcosm Set-up and Growth Conditions

The microcosms consisted of 250 mL glass bottles containing 60 mL of a 30 mM bicarbonate-buffered mineral medium, [\(34\)](#) 60 µL of neat Ansul AFFF, and 5 g of soil collected from a firefighter training area at the Ellsworth Air Force Base, South Dakota, USA. The 60 µL AFFF dose yielded initial amended concentrations of approximately 26 µM 6:2 FtTAoS, 0.06 µM 8:2 FtTAoS, and 0.005 µM 4:2 FtTAoS. This soil-to-medium ratio in the microcosms created slurries that allowed for complete mixing of the contents on a shaker table, which facilitated the maintenance of oxic conditions while still representing potential conditions that may be encountered in the environment (e.g., top-soil impacted with diluted AFFF following firefighting activities). The medium was modified from that described previously [\(34\)](#) by preparing aerobically and omitting cysteine sulfide, resazurin, and TES buffer, which is a potential microbial carbon source under oxic conditions. A screw-top push-button valve previously washed in methanol was used to ensure gastight bottle closure (Vici mininert valve, F/24 mm, Fisher Scientific). A set of sterile controls was prepared by first subjecting the soil to three autoclave cycles followed by overnight freezing at –20 °C after each, and then amending the medium with 0.5 g/L sodium azide and 60 µL of AFFF. Additionally, soil-free medium controls containing only sterile medium, sodium azide, and AFFF were also prepared to assess potential impacts of the medium on the fluorinated surfactants and to determine losses of the compounds due to the experiment container. All experiments were run in triplicate in separate microcosms and shaken at 100 rpm in a 30 °C incubator for 60 days with samples periodically collected from the headspace or liquid slurry with syringes. Oxygen was measured by injecting 250 µL of microcosm headspace into a Hewlett-Packard 5890 GC-TCD equipped with an Alltech HAYESEP Q column (80/100 mesh, 0.85" ID × 6') operated isothermally at 30 °C. Headspace oxygen was kept between 15 and 25% (v/v) in live microcosms by periodically amending the bottles with pure oxygen using a 0.2 µm-filtered gastight syringe and then depressurizing the bottles for several seconds with a

sterile needle inserted into the cap valve. The oxygen concentrations in all microcosms are provided in Figure S2 of the [Supporting Information](#). Two, 60 μ L aliquots of AFFF were added to the live microcosms over the course of the experiment: on day 0 and again on day 18 after the 6:2 FtTAoS had been depleted, whereas only one aliquot of AFFF was added to autoclaved and medium controls (day 0). On day 40, approximately 300 mg/L DGBE was added to live microcosms to provide 175 mg/L of additional organic carbon. Dissolved organic carbon was measured in all bottles by diluting 200 μ L of microcosm supernatant (slurry previously centrifuged at 15000g for 10 min) in deionized water and analyzing on a Shimadzu TOC-V analyzer. A lack of microbial activity in the autoclaved and medium controls was confirmed when no significant organic carbon or oxygen consumption was observed after 60 days (Figures S1 and S2 of the [Supporting Information](#)).

All microcosms were equilibrated with AFFF for 24 h prior to collection of the first sample and prior to sample collection following subsequent additions of AFFF. At each time point, between 0.5 and 1.5 mL of a well-mixed soil and media slurry was removed with a 3 mL sterile syringe (BD Luer-Lok disposable syringe) and wide gauge needle to minimize selection of small soil particles (BD 18G \times 1" needle). The slurry was then stored in a 2 mL polypropylene centrifuge tube. Approximately 200 μ L of the slurry was immediately diluted in an equal volume of methanol and reserved for LC-MS/MS analysis, whereas 100 μ L was aliquoted into a 7 mL HDPE vial for the total oxidizable precursor assay. The remaining slurry was centrifuged at 15000g for 10 min, aliquoted for dissolved organic carbon analysis, and subsequently stored at -20 °C.

LC-MS/MS and HRMS Analyses

The 50:50 methanol:aqueous samples were vortexed for 30 min at room temperature using a Vortex-Genie 2 affixed with a MO BIO 24-tube adapter, and then centrifuged at 15000g for 10 min. The supernatant was amended with 50 μ L of an internal standard stock containing 20 to 40 μ g/L of mass-labeled internal standards([10, 33](#)) and diluted with HPLC-grade methanol and water to produce 500 μ L of a 50:50 methanol:aqueous mix containing a target analyte concentration in the range of the established calibration curves. FtTAoS, FtS, and PFCA compounds were quantified on an Agilent 6410 LC-MS/MS operating in negative electrospray ionization mode as described previously,[\(10, 33\)](#) except for the addition of 4:2 FtS. The MS parameters and monitored ion transitions for 4:2, 6:2, and 8:2 FtS are provided in Table S2 of the [Supporting Information](#). As a quantitative standard was not available for 4:2 and 8:2 FtTAoS, the 6:2 FtTAoS calibration curve was used to estimate their concentrations after normalization to the mass-labeled 6:2 FtS internal standard (described in greater detail in the [Supporting Information](#) and Table S5).

All fluorotelomer carboxylic acid (FtCA) compounds were measured on a Shimadzu Nexera X2 UHPLC/ABSciEX 5500 Triple Quad MS system operating in negative ionization mode. The same 50:50 methanol:sample mix was first spiked with 2 μ g/L of the internal standard stock and diluted with methanol and water to yield target analyte concentrations in the range of calibration curves. A gradient solvent program was operated with 2 mM ammonium acetate in water and 2 mM ammonium acetate in methanol and a flow rate of 0.5 mL/min (Table S3 of the [Supporting Information](#)). Chromatographic separation was achieved on a Waters ACQUITY UPLC BEH C18

column, 130 Å, 1.7 µm, 2.1 mm × 50 mm following a sample injection of 10 µL. One or two MRM transitions were measured per FtCA compound and were quantified with isotope dilution (Table S4 of the [Supporting Information](#)).

To identify biotransformation products of the polyfluorinated compounds in AFFF, the methanol:sample mix containing internal standard stock was analyzed on the Agilent 6410 LC-MS/MS operating in a molecular ion scanning mode (m/z 50 to 1000) in both positive and negative electrospray ionization modes. Prominent molecular ions were identified and their instrumental responses were normalized to the mass-labeled 6:2 FtS internal standard response to evaluate their relative production and consumption in the microcosms. The LC-MS/MS product ion mass spectra of two of the prominent apparent biotransformation products were acquired to help elucidate their molecular structures. The compounds were also analyzed by high resolution mass spectrometry (HRMS) to confirm their molecular compositions. HRMS analysis was conducted by QB3 Mass Spectrometry Facility at UC Berkeley. Samples were purified with solid phase extraction (SPE) using Oasis WAX SPE cartridges (6 cm³, 150 mg, 30 µM; Waters, Milford, MA) and the conditioning and elution methods previously described.[\(10\)](#) 50 µL of the extracted sample was directly injected onto a Thermo Scientific Finnigan LTQ FT HRMS operating in negative electrospray ionization over a scan range of 150 to 850, and the exact masses of the ions were confirmed within 5 ppm accuracy.

Total Oxidizable Precursor Assay

To assess the PFAS mass balance in the microcosms before and after biotransformation and to ensure that observed FtTAoS disappearance was not due to physical losses (e.g., volatilization), a previously developed oxidative precursor assay was applied to microcosm samples.[\(33\)](#) Briefly, the oxidative precursor assay employs the reaction of polyfluorinated compounds (termed perfluoroalkyl acid precursors, or PFAA precursors) with hydroxyl radical to generate corresponding PFCAs of related perfluorinated chain length through the chemical oxidation of its nonfluorinated functional group.[\(33\)](#) The samples containing PFAA precursors were exposed to an excess of hydroxyl radicals produced through persulfate thermolysis at pH >12. PFAA precursors were converted to a suite of PFCAs, and the molar quantity of the precursors was estimated by quantifying the molar quantity of PFCAs produced. For C₆ fluorotelomer compounds, approximately 75% (mol/mol) of the compound was recovered as PFCAs, indicating that some of the C₆ telomer compounds were oxidized to C₂ and C₃ PFCA products that were not measured by the LC-MS/MS method used in this study. For C₈ fluorotelomers, greater than 90% of the initial mass was recoverable as PFCAs after oxidation.[\(33\)](#) For the microcosm samples in this study, the reaction was conducted in 7 mL HDPE vials containing 100 µL of a soil-media slurry, 3 mL of HPLC-grade water, and 3 mL of 120 mM potassium persulfate in 0.25 M NaOH. The vials were submerged in an 85 °C water bath for approximately 12 h. After cooling, the base was neutralized with HCl and amended with 1 mL of methanol before LC-MS/MS analysis.

Results and Discussion

Dissolved Organic Carbon Disappearance and FtTAoS Biotransformation

The amended AFFF and DGBE constituted the majority of the dissolved organic carbon present in live microcosms (Figure S1 of the [Supporting Information](#)). The dissolved organic carbon disappeared within 3 to 5 days in live bottles, whereas no change in concentrations were observed in autoclaved or medium controls (Figure S1 of the [Supporting Information](#)). Correspondingly, the headspace oxygen concentrations decreased in live microcosms (Figure S2 of the [Supporting Information](#)), suggesting that the degradation of the AFFF organic solvents, including DGBE, occurred rapidly by the soil microbial community.

Complete disappearance and biotransformation of 4:2, 6:2, and 8:2 FtTAoS occurred in live microcosms when two aliquots of Ansul AFFF were added to the bottles (Figure 1). Approximately 50 μM 6:2 FtTAoS, the most abundant FtTAoS congener, was biotransformed within 45 days (Figure 1A) in live microcosms, whereas concentrations in autoclaved and medium controls remained nearly constant over the incubation period. The lower quantified concentrations of 6:2 FtTAoS in live and autoclaved microcosms than in medium controls (Figure 1A) is likely due to incomplete extraction of the compound off of soil particles during the methanol dilution and vortexing steps conducted prior to LC-MS/MS quantification. Because autoclaving produced additional dissolved organic carbon in the slurry phase (Figure S1 of the [Supporting Information](#)), greater sorption and lower extracted concentrations of 6:2 FtTAoS were observed in the autoclaved microcosms than in live bottles. The lower total amended concentrations of 4:2 and 8:2 FtTAoS may have produced higher overall extraction efficiencies, yielding similar FtTAoS concentrations among live, autoclaved, and medium control experiments (Figure 1B,C). Because analytical standards were not available for 4:2 and 8:2 FtTAoS, their concentrations were determined semiquantitatively by calibrating their internal standard-normalized LC-MS/MS responses to the 6:2 FtTAoS calibration curve. Complete disappearance of these compounds was observed in live microcosms, whereas concentrations in control experiments remained nearly constant (Figure 1B,C). The apparent lack of an observable increase in 4:2 FtTAoS concentrations following the second AFFF amendment (Figure 1C) could be attributed to difficulties in quantifying very low concentrations of the compound. The compound's instrumental response approached fluorotelomer detection limits in this study. The rapid biotransformation of the compound in the time period immediately following the second AFFF amendment and before the microcosm slurry was sampled for LC-MS/MS analysis may have contributed to the compound's low concentration.

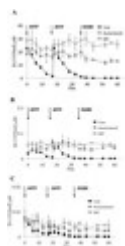


Figure 1. Biotransformation of 6:2 FtTAoS (A), 8:2 FtTAoS (B), and 4:2 FtTAoS (C) in live microcosms. Note the y-axis scales in the B and C plots are less than the scale in the A plot. The second AFFF dose and DGBE amendment were made to live microcosms only and not autoclaved or medium controls (MC). Error bars represent the standard deviation of triplicate experimental bottles.

6:2 FtS was the most abundant biotransformation product detected in live microcosms (Figure 2A), accounting for 8% of the total mass of FtTAoS biotransformed on day 60. 4:2 and 8:2 FtS were also detected in live microcosms; however, together they accounted for less than 1% of total FtTAoS biotransformed (Figure S3 of the [Supporting Information](#)). The production of 5:3 FtCA and 6:2 fluorotelomer unsaturated carboxylic acid (6:2 FtUCA) was also observed in live microcosms, accounting for approximately 0.5% and 0.18% of total FtTAoS biotransformed, respectively (Figure 2B). Some production of 8:2 FtUCA appeared to have occurred in live microcosms relative to autoclaved controls (Figure S4 of the [Supporting Information](#)). No discernible trend was observed for 7:3 FtCA or 6:2 FtCA concentrations in live microcosms compared to autoclaved controls (Figures S4 and S5 of the [Supporting Information](#)), whereas no 8:2 FtCA was detected in live or autoclaved microcosms (Figure S5 of the [Supporting Information](#)). Production of several PFCAs was also observed in live microcosms. PFHxA, PFPeA, and PFBA (Figure 2C) accounted for the majority of PFCA products detected (48%, 40%, and 10% of total PFCAs quantified on day 60, respectively), whereas lesser amounts of PFHpA and PFOA were detected (Figure S6 of the [Supporting Information](#)). PFCA products accounted for approximately 1.5% of the total FtTAoS biotransformed by day 60, whereas all produced FtS, FtCA, and PFCA compounds together accounted for just over 10% of total FtTAoS transformed. No FtS, FtCA, FtUCA, or PFCA biotransformation products were detected in medium controls.



Figure 2. Production of major transformation intermediates identified by direct LC-MS/MS measurement: 6:2 FtS (A), 6:2 FtUCA and 5:3 FtCA (B), and PFBA, PFPeA, PFHxA (C). Note the y-axis scales in the B and C plots are one-tenth of the scale in the A plot.

Intermediate Product Identification

LC-MS/MS molecular ion scans indicated the presence of four polyfluorinated compounds in the microcosms for which no analytical standards were available: molecular ions 602, 618, 702, and 718. The proposed structures for 602 and 618 possess one or two oxygens (respectively) on the thioether functional group of 6:2 FtTAoS. These compounds are designated 6:2 fluorotelomer sulfoxide amido sulfonate (6:2 FtSOAoS) and 6:2 fluorotelomer sulfone amido sulfonate (6:2 FtSO₂AoS), and their structures are shown in Figure 4. Similarly, molecular ions 702 and 718 are designated 8:2 FtSOAoS and 8:2 FtSO₂AoS, respectively (Figures S9 and S10 of the [Supporting Information](#)). LC-MS/MS retention times and chromatograms are shown in Table S6 and Figures S7 and S8 of the [Supporting Information](#), respectively. Molecular ion 602 was observed in live, autoclaved, and medium-control microcosms (Figure 3A), whereas molecular ions 618, 702, and 718 were observed only in live microcosms (Figures 3B, S9, and S10 of the [Supporting Information](#), respectively). Molecular ion 602 was also present in the AFFF formulation. The MS response of the ion on day 0 in the microcosms was equivalent to the medium control and approximately doubled in the live microcosms after a second aliquot of AFFF was added (Figure 3A). The

analyte response of 602 appeared to increase slightly in live microcosms over time and then decrease, which is consistent with a compound that is both produced and consumed. The response of the ion also increased slightly in autoclaved microcosms but not medium controls, suggesting that its production could have occurred through an abiotic reaction involving the solid phase. The abiotic production of ion 602 is further supported by the slight loss of 6:2 FtTAoS in autoclaved controls that was observed over the incubation period. The analyte response of m/z 618 increased linearly in live microcosms over the incubation period, but always remained less than that of 602 (Figure 3B). The responses of ions 702 and 718 increased over the course of the incubation (Figures S9 and S10 of the [Supporting Information](#)), consistent with a biological production of these species. The instrumental analyte response of m/z 586 (6:2 FtTAoS) and m/z 686 (8:2 FtTAoS) obtained during the same MS analyses are plotted for comparison in Figures S11 and S12 of the [Supporting Information](#), respectively.

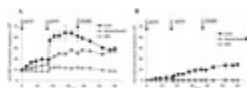


Figure 3. Average LC-MS/MS analyte responses (peak area) of molecular ions 602 (A) and 618 (B) normalized to the mass labeled-6:2 FtS internal standard response (peak area) in live, autoclaved, and medium-control (MC) microcosms. The second AFFF and DGBE amendments were made to the live microcosms only. Error bars represent the standard deviation of triplicate microcosms. The exact mass measurements and proposed structures of the compounds represented by each ion are shown.

The exact mass measurements of 602 and 618 were obtained through high-resolution mass spectrometry of live-microcosm sample extracts after 38 days. The molecules had the same atomic composition as 6:2 FtTAoS with one (602) or two (618) additional oxygen atoms (Table S5 of the [Supporting Information](#)). Once the atomic compositions of the molecular ions were known, sample extracts were analyzed on the LC-MS/MS operating in the selected ion mode. The mass spectrum for each of the two molecular ions is included in Figure S13 of the [Supporting Information](#). Key structural features were proposed based on the mass spectrum observed for each molecular ion (Figure S13 of the [Supporting Information](#)). The product ion scans of both 602 and 618 showed fragments corresponding to the entire nonfluorinated part of 6:2 FtTAoS adjacent to the thioether moiety (m/z 206) as well as the terminal sulfonate moiety (m/z 135) contained within m/z 206 (Figure S13 of the [Supporting Information](#)). Because these two fragments were also observed in the mass spectrum of 6:2 FtTAoS, it is likely that the oxygen additions occurred on the part of the molecule containing the fluorotelomer thioether group. Although exact mass measurements for molecular ions 702 and 718 were not obtained due to their low relative abundance in live microcosm samples, the mass spectra generated from MS product ion scans on 686, 702, and 718 produced mass fragments m/z 206 and 135, suggesting oxygen addition on the 8:2 fluorotelomer thioether moiety. Molecular ions 502 and 518, which would correspond to the singly- and doubly oxygenated species of 4:2 FtTAoS (4:2 FtSOAoS and 4:2 FtSO₂AoS, respectively), were not detected in these microcosms with either LC-MS/MS molecular ion scans or HRMS. This is likely due to the low initial abundance of 4:2 FtTAoS in the AFFF.

Although Weiner et al. (2013) proposed the occurrence of a sulfoxide and sulfone species as potential biotransformation products of 6:2 FtTAoS, it was not determined whether 6:2 FtSOAoS was a biotransformation product as no production of the compound was observed in live microcosms.⁽³²⁾ 6:2 FtSO₂AoS was also not

detected in their study. Here, the exact molecular masses of the biotransformation products were determined, evidence supporting their proposed molecular structures was obtained, and time course trends confirming their role as FtTAoS biotransformation products were constructed. The identification of these species describes the mechanism through which multiple congeners of FtTAoS may aerobically biotransform to FtS compounds.

FtTAoS Biotransformation Pathways

The proposed biotransformation pathways for 4:2, 6:2, and 8:2 FtTAoS (Figure 4) were based on quantification of 4:2, 6:2, and 8:2 FtS, 5:3 FtCA, 6:2 and 8:2 FtUCA, and C₄–C₈ PFCAs with certified analytical standards, as well as the detection of intermediate compounds identified by HRMS and molecular fragmentation patterns. Because more 6:2 FtSOAoS was produced in live microcosms than in autoclaved controls in the 20 days following the first AFFF amendment, it is likely that this reaction was attributable to both microbial and abiotic activity. Thus, the first two steps in the biotransformation reactions of each FtTAoS compound appear to involve sequential oxygen additions on the thioether group to form FtSOAoS and FtSO₂AoS, which are then followed by the formation of FtS through a third oxygen addition and cleavage of a carbon–carbon bond, resulting in a fluorotelomer sulfonate and an alkyl amidosulfonate group. The aerobic biological oxidation of a thioether group to its corresponding sulfoxide and sulfone has been previously reported with a variety of sulfide-containing compounds, including dimethyl sulfide, dibenzothiophene, and bis-(3-pentafluorophenylpropyl)sulfide (PFPS), and across diverse groups of microorganisms, such as *Nitrosomonas*, *Rhodococcus*, and *Gordonia* spp. (36-38) The abiotic oxidation of thioethers through this mechanism has also been reported, such as the environmental photooxidation of dimethyl sulfide. (39) The oxidation of 6:2 FtS to 6:2 FtUCA, 5:3 FtCA, PFHxA and PFPeA in aerobic microcosms inoculated with activated sludge has been reported previously. (24) In this study, the production of PFHpA, PFOA, and 8:2 FtUCA (Figures S6 and S4 of the Supporting Information, respectively), which were the likely products of 8:2 FtS biotransformation (Figure S3 of the Supporting Information), were consistent with those observations and formed an analogous oxidation pathway for 8:2 FtTAoS (Figure 4). Production of PFBA and some 4:2 FtS was also observed in live microcosms (Figures 2C and S3 of the Supporting Information, respectively). Although PFBA is expected to be a terminal oxidation product of 4:2 FtS, its concentration in live microcosms at the end of the 60 day incubation period (0.5 μM) was much greater than the total estimated 4:2 FtTAoS biotransformed (<0.01 μM), suggesting that it was also a biotransformation product of 6:2 FtS produced from 6:2 FtTAoS.

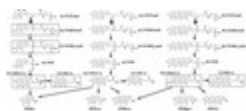


Figure 4. Proposed biotransformation pathways of 4:2, 6:2, and 8:2 FtTAoS by aerobic soil microcosms. Compounds in dashed boxes are proposed biotransformation intermediates and were not directly detected in microcosms. The double arrow indicates that the reaction occurs both biologically and abiotically.

The cleavage of the carbon–carbon bond during the oxidation of fluorotelomer sulfonates and alcohols has been observed in previous studies. For example, Wang et al. (2011) detected PFBA formation (accounting for approximately 0.14% mol/mol) when 6:2 FtS was aerobically oxidized, (24) whereas production of PFBA was also observed when 6:2 FtOH was biotransformed under aerobic conditions. (23, 25, 26) In this study, PFBA accounted

for approximately 0.1% (mol/mol) of the total amended 6:2 FtTAoS at the end of the incubation. It is possible that 8:2 FtS biotransformation also produced the C_{n-2} PFCA product, PFHxA, as aerobic 8:2 FtOH biotransformation to PFHxA has been previously observed.[\(25\)](#) Although approximately 0.3 μ M PFHxA was formed, it is likely that the majority of it was produced from 6:2 FtS oxidation, as the concentration of 8:2 FtTAoS in the AFFF in this study was much lower than that of 6:2 FtTAoS. For both 6:2 and 8:2 FtTAoS biotransformation in these microcosms, the terminal C_n and C_{n-1} PFCA products (PFHxA/PFPeA and PFOA/PFHpA, respectively) were produced in approximately equimolar quantities. Similar findings were reported for the biotransformation of 6:2 FtS and 6:2 FtOH.[\(23, 24\)](#)

Generally, the rate of FtTAoS disappearance observed in this study was faster than the rate of transformation of intermediate compounds, indicating that the rate-limiting step in PFCA production likely occurs after FtSOAoS production. It is unknown whether the observed FtTAoS biotransformation occurs metabolically or cometabolically by the soil microbial community in these microcosms. Biotransformation of FtTAoS and production of FtS and PFCA products continued to occur after the labile organic carbon compounds disappeared. Although dissolved organic carbon may only represent a certain fraction of the total bioavailable organic carbon in the system, high concentrations of amended labile carbon did not appear to have a significant impact on PFCA production, suggesting some factor other than organic carbon may control the rate of FtTAoS biotransformation.

Precursor Oxidation Assay and Mass Balance

The FtS and PFCA biotransformation products that could be quantified directly by LC-MS/MS (4:2, 6:2, 8:2 FtS, 6:2 FtUCA, 5:3 FtCA, and C_4 – C_8 PFCAs) accounted for approximately 10% of the total FtTAoS transformed. The PFCA products alone accounted for approximately 1.5% of the FtTAoS transformed, a recovery within the range of those observed in other aerobic FtS and FtOH biotransformation studies (0.3–10% mol/mol).[\(20-26\)](#) It is unclear whether the new intermediate products described constitute the remainder of the FtTAoS mass balance.

Because a complete mass recovery of 6:2 FtTAoS was not obtained, the total oxidizable precursor assay was employed on microcosm samples to check the material balance. Seven samples extracted from each live, autoclaved, and medium control bottle over the 60 day incubation period were subjected to the assay. In live microcosms, these samples represented time points before, during, and after the two aliquots of FtTAoS were biotransformed. Of the approximately 26 μ M 6:2 FtTAoS amended to all experimental conditions, the precursor assay recovered 75–85% of the compound as C_4 – C_8 PFCA products (Figures [5](#), S14, and S15 of the [Supporting Information](#)). After the second AFFF addition in live microcosms, the recovery ranged from 80 to 100%. As described by the oxidation model presented in Houtz et al.,[\(33\)](#) these results confirm that approximately 75% of a fluorotelomer PFAA precursor is recoverable in PFCA products using this assay. The incomplete recovery of the precursor in some samples may be due to the production of PFCAs with fewer than four carbons that are not quantified by this LC-MS/MS method.[\(10, 33\)](#) The concentration distribution of PFCA congeners (C_4 : C_5 : C_6) produced from the precursor assay is also consistent with products of C_6 fluorotelomer precursor oxidation, confirming that 6:2 FtTAoS and its related C_6 transformation products were the primary oxidizable PFASs in these microcosms.

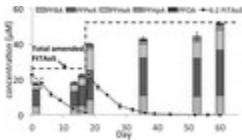


Figure 5. Concentration of 6:2 FtTAoS and PFCAs generated in live microcosm samples after oxidation in the PFAA precursor assay. The dotted line represents the total amended FtTAoS and was obtained by summing the 4:2, 6:2, and 8:2 average FtTAoS concentrations measured in the medium controls. PFNA was not detected.

Although the majority of the total amended FtTAoS was not accounted for as intermediates and terminal products, the precursor assay provided insight into the PFAS mass balance. The nearly complete recovery of amended FtTAoS as oxidized PFAA precursors before, during, and after biotransformation confirmed that all significant quantities of unidentified transformation compounds remained in the soil-media slurry and that substantial quantities of transformation intermediates were not lost through volatilization into the headspace or sorption to bottle walls and cap closures. Although small quantities of volatile intermediate compounds may have been produced, such as the 5:2 ketone or 5:2 sFtOH compounds that were previously reported in 6:2 FtS biotransformation,⁽²⁴⁾ the generation of these compounds likely did not represent a significant sink for the 6:2 FtTAoS disappearance in this study. Additionally, if cleavage of the fluoroalkyl tail to less than a C₄ perfluorinated chain or complete defluorination had occurred, the assay would have likely yielded lower recoveries in PFCAs oxidation products.

Environmental Implications

The results of this study may help to explain the occurrence of FtS and PFCAs compounds in groundwater and soil at sites where AFFF was released.⁽³⁵⁾ As FtTAoS is present in AFFF from several manufacturers, its biotransformation to FtS and PFCAs under conditions representative of soil, groundwater, and surface water is important for understanding the present occurrence and future potential PFCAs influx into the environment. Although FtTAoS is biotransformed over a period of weeks, its conversion to PFCAs appears to be slow. Other intermediate biotransformation products (e.g., 6:2 FtS, 6:2 FtUCA) may constitute a significant source of PFASs in FtTAoS-contaminated environments. Furthermore, measuring only PFCAs and FtS in environmental samples may not completely characterize the potential for long-term release of PFCAs. The total oxidizable precursor assay can be used to obtain an estimate of the concentration of intermediate products that are not quantifiable by direct analysis. Two of the identified intermediate compounds, 6:2 FtSOAoS and 6:2 FtSO₂AoS, appear to have slow transformation rates relative to 6:2 FtTAoS, indicating they may occur in subsurface environments.

Acknowledgment

This study was supported by the Strategic Environmental Research and Development Program (SERDP), grant number ER-2128. The authors thank Professor Chris Higgins, Keenan Christensen, and Jennifer Guelfo at Colorado School of Mines for providing the soil used to conduct these experiments.

References

This article references 40 other publications.

[1.](#)

Moody, C. A.; Field, J. A. Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams *Environ. Sci. Technol.* 2000, 34, 3864– 3870

[\[ACS Full Text\]](#), [\[CAS\]](#)

[2.](#)

Tuve, R. L.; Jablonski, J. E. Method of extinguishing liquid hydrocarbon fires. U.S. Patent 3,258,423, June 28, 1966.

[3.](#)

Moody, C. A.; Martin, J. W.; Kwan, W. C.; Muir, D. C. G.; Mabury, S. C. Monitoring perfluorinated surfactants in biota and surface waters following an accidental release of fire-fighting foam into Etobicoke Creek *Environ. Sci.*

Technol. 2002, 36, 545– 551

[\[ACS Full Text\]](#), [\[CAS\]](#)

[4.](#)

Oakes, K. D.; Benskin, J. P.; Martin, J. W.; Ings, J. S.; Heinrichs, J. Y.; Dixon, D. G.; Servos, M. R. Biomonitoring of perfluorochemicals and toxicity to the downstream fish community of Etobicoke Creek following deployment of aqueous film-forming foam *Aquat. Toxicol.* 2010, 98, 120– 129

[\[Crossref\]](#), [\[PubMed\]](#), [\[CAS\]](#)

[5.](#)

Awad, E.; Zhang, X.; Bhavsar, S. P.; Petro, S.; Crozier, P. W.; Reiner, E. J.; Fletcher, R.; Tittlemier, S. A.; Brawkevelt, E. Long-term environmental fate of perfluorinated compounds after accidental release at Toronto airport *Environ. Sci.*

Technol. 2011, 45, 8081– 8089

[\[ACS Full Text\]](#), [\[CAS\]](#)

[6.](#)

Karrman, A.; Elgh-Dalgren, K.; Lafossas, C.; Moskeland, T. Environmental levels and distribution of structural isomers of perfluoroalkyl acids after aqueous fire-fighting foam (AFFF) contamination *Environ. Chem.* 2011, 8, 372– 380

[\[Crossref\]](#)

[7.](#)

Moody, C. A.; Hebert, G. N.; Strauss, S. H.; Field, J. A. Occurrence and persistence of perfluorooctanesulfonate and other perfluorinated surfactants in groundwater at a fire-training area at Wurtsmith Air Force Base, Michigan, USA *J. Environ.*

Monit. 2003, 5, 341– 345

[\[Crossref\]](#), [\[PubMed\]](#), [\[CAS\]](#)

[8.](#)

Weiss, O.; Wiesmüller, G. A.; Bunte, A.; Göen, T.; Schmidt, C. K.; Wilhelm, M.; Hölzer, J. Perfluorinated compounds in the vicinity of a fire training area – Human biomonitoring among 10 persons drinking water from contaminated private wells in Cologne, Germany *Int. J. Hyg. Environ. Health* 2012, 215, 212–215

[\[Crossref\]](#), [\[PubMed\]](#), [\[CAS\]](#)

[9.](#)

Schultz, M. M.; Barofsky, D. F.; Field, J. A. Quantitative determination of fluorotelomer sulfonates in groundwater by LC-MS/MS *Environ. Sci. Technol.* 2004, 38, 1828– 1835

[\[ACS Full Text\]](#), [\[CAS\]](#)

[10.](#)

Houtz, E. F.; Higgins, C. P.; Field, J. A.; Sedlak, D. L. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil *Environ. Sci. Technol.* 2013, 47, 8187– 8195

[\[ACS Full Text\]](#), [\[CAS\]](#)

[11.](#)

Backe, W. J.; Christensen, K. E.; Field, J. A. Newly-identified cationic, anionic, and zwitterionic fluorinated chemicals in groundwater at U.S. Military bases by non-aqueous large-volume injection HPLC-MS/MSEnviron. Sci. Technol. 2013, 47, 5226– 5234

[[ACS Full Text](#) , [[CAS](#)]

[12.](#)

Nelson, J. W.; Hatch, E. E.; Webster, T. F. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population Environ. Health Perspect. 2009, 118, 197–202

[[Crossref](#)]

[13.](#)

Lui, J.; Avendaño, S. M. Microbial degradation of polyfluoroalkyl chemicals in the environment Environ. Int.2013, 61, 98– 114

[[Crossref](#)], [[PubMed](#)]

[14.](#)

Kissa, E. Fluorinated Surfactants and Repellents, 2nd ed.; Marcel Dekker, Inc.: New York, NY, 2001.

[15.](#)

Chemical & Material Emerging Risk Alert: Aqueous film forming foam (AFFF); Department of Defense, Chemical and Risk Management Directorate: July, 2011.

[16.](#)

U.S. Environmental Protection Agency. 2010/2015PFOA

StewardshipProgram.<http://www.epa.gov/oppt/pfoa/pubs/stewardship/> (accessed April 16, 2014) .

[17.](#)

Environment Canada. Perfluorooctane sulfonate (PFOS), Its salts and its precursors.<https://www.ec.gc.ca/toxiques-toxics/Default.asp?lang=En&n=98E80CC6-1&xml=ECD5A576-CEE5-49C7-B26A-88007131860D> (accessed April 16, 2014) .

[18.](#)

Place, B. J.; Field, J. A. Identification of novel fluorochemicals in aqueous film-forming foams used by the U.S. military Environ. Sci. Technol. 2012, 46, 7120– 7127

[[ACS Full Text](#) , [[CAS](#)]

[19.](#)

D'Agostino, L. A.; Mabury, S. A. Identification of novel fluorinated surfactants in aqueous film forming foams and commercial surfactant concentrates Environ. Sci. Technol. 2014, 48, 121– 129

[[ACS Full Text](#) , [[CAS](#)]

[20.](#)

Dinglasan, M. J. A.; Ye, Y.; Edwards, E. A.; Mabury, S. A. Fluorotelomer alcohol biodegradation yields poly- and perfluorinated acids Environ. Sci. Technol. 2004, 38, 2857– 2864

[[ACS Full Text](#) , [[CAS](#)]

[21.](#)

Wang, N.; Szostek, B.; Buck, R. C.; Folsom, P. W.; Sulecki, L. M.; Capka, V.; Berti, W. R.; Gannon, J. T. Fluorotelomer alcohol biodegradation – Direct evidence that perfluorinated chains breakdown Environ. Sci. Technol. 2005, 39, 7516– 7528

[[ACS Full Text](#) , [[CAS](#)]

[22.](#)

Lee, H.; D'Eon, J.; Mabury, S. A. Biodegradation of polyfluoroalkyl phosphates as a source of perfluorinated acids to the environment Environ. Sci. Technol. 2010, 47, 3305– 3310

[[ACS Full Text](#) 

[23.](#)

Liu, J.; Wang, N.; Buck, R. C.; Wolstenholme, B. W.; Folsom, P. W.; Sulecki, L. M.; Bellin, C. A. Aerobic biodegradation of [¹⁴C] 6:2 fluorotelomer alcohol in a flow-through soil incubation system Chemosphere2010, 80, 716– 723

[\[Crossref\]](#), [\[PubMed\]](#), [\[CAS\]](#)

[24.](#)

Wang, N.; Liu, J.; Buck, R. C.; Korzeniowski, S. H.; Wolstenholme, B. W.; Folsom, P. W.; Sulecki, L. M. 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants *Chemosphere* 2011, 82, 853– 858

[\[Crossref\]](#), [\[PubMed\]](#), [\[CAS\]](#)

[25.](#)

Kim, M. H.; Wang, N.; McDonald, T.; Chu, K. H. Biodefluorination and biotransformation of fluorotelomer alcohols by two alkane-degrading pseudomonas strains *Biotechnol. Bioeng.* 2012, 109, 12

[26.](#)

Kim, M. H.; Wang, N.; Chu, K. H. 6:2 Fluorotelomer alcohol (6:2 FTOH) biodegradation by multiple microbial species under different physiological conditions *Environ. Biotechnol.* 2014, 98, 1831– 1840

[\[Crossref\]](#), [\[PubMed\]](#)

[27.](#)

Rand, A. A.; Mabury, S. A. Covalent binding of fluorotelomer unsaturated aldehydes (FTUALs) and carboxylic Acids (FTUCAs) to proteins *Environ. Sci. Technol.* 2013, 47, 1655– 1663

[\[ACS Full Text\]](#), [\[CAS\]](#)

[28.](#)

Guelfo, J. L.; Higgins, C. P. Subsurface transport potential of perfluoroalkyl acids at aqueous film-forming foam (AFFF)-impacted sites *Environ. Sci. Technol.* 2013, 47, 4164– 4171

[\[ACS Full Text\]](#), [\[CAS\]](#)

[29.](#)

Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl acids: A review of monitoring and toxicological findings *Toxicol. Sci.* 2007, 99, 366– 394

[\[Crossref\]](#), [\[PubMed\]](#), [\[CAS\]](#)

[30.](#)

Fei, C.; McLaughlin, J. K.; Tarone, R. E.; Olsen, J. Perfluorinated chemicals and fetal growth: A study within the Danish National Birth Cohort *Environ. Health Perspect.* 2007, 115, 1677– 1682

[\[Crossref\]](#), [\[PubMed\]](#), [\[CAS\]](#)

[31.](#)

Sakr, C. J.; Kreckmann, K. H.; Green, J. W.; Gillies, P. J.; Reynolds, J. L.; Leonard, R. C. Cross-sectional study of lipids and liver enzymes related to a serum biomarker of exposure (ammonium perfluorooctanoate or APFO) as part of a general health survey in a cohort of occupationally exposed workers *J. Occup. Environ. Med.* 2007, 49, 1086– 1096

[\[Crossref\]](#), [\[PubMed\]](#), [\[CAS\]](#)

[32.](#)

Weiner, B.; Yeung, L. W. Y.; Marchington, E. B.; D'Agostino, L. A.; Mabury, S. A. Organic fluorine content in aqueous film forming foams (AFFFs) and biodegradation of the foam component 6:2 fluorotelomermercaptoalkylamido sulfonate (6:2 FTSAS) *Environ. Chem.* 2013, 10, 486– 493

[\[Crossref\]](#), [\[CAS\]](#)

[33.](#)

Houtz, E. F.; Sedlak, D. L. Oxidative conversion as a means of detecting precursors to perfluoroalkyl acids in urban runoff *Environ. Sci. Technol.* 2012, 46, 9342– 9349

[\[ACS Full Text\]](#), [\[CAS\]](#)

[34.](#)

Lee, P. K. H.; Johnson, D. R.; Holmes, V. F.; He, J. Z.; Alvarez-Cohen, L. Reductive dehalogenase gene expression as a biomarker for physiological activity of *Dehalococcoides* spp *Appl. Environ. Microb.* 2006, 72, 6161– 6168

[\[Crossref\]](#), [\[PubMed\]](#), [\[CAS\]](#)

[35.](#)

McGuire, M. E.; Schaefer, C.; Richards, T.; Backe, W. J.; Field, J. A.; Houtz, E. F.; Sedlak, D. L.; Guelfo, J. L.; Wunsch, A.; Higgins, C. P. Evidence of remediation-induced alteration of subsurface poly- and perfluoroalkyl substance distribution at a former firefighter training area *Environ. Sci. Technol.* 2014, 48, 6644– 6652

[\[ACS Full Text\]](#) , [\[CAS\]](#)

[36.](#)

Van Hamme, J. D.; Fedorak, P. M.; Foght, J. M.; Gray, M. R.; Dettman, H. D. Use of a novel fluorinated organosulfur compound to isolate bacteria capable of carbon-sulfur bond cleavage *Appl. Environ. Microbiol.* 2004, 70, 1487– 1493

[\[Crossref\]](#), [\[PubMed\]](#), [\[CAS\]](#)

[37.](#)

Juliette, L. Y.; Hyman, M. R.; Arp, D. J. Inhibition of ammonia oxidation in *Nitrosomonas europaea* by sulfur compounds: Thioethers are oxidized to sulfoxides by ammonia monooxygenase *Appl. Environ. Microbiol.* 1993, 59, 3718– 3727

[\[PubMed\]](#), [\[CAS\]](#)

[38.](#)

Rhee, S. K.; Chang, J. H.; Chang, Y. K.; Chang, H. M. Desulfurization of dibenzothiophene and diesel oils by a newly isolated Gordona strain, CYKS1 *Appl. Environ. Microbiol.* 1998, 64, 2327– 2331

[\[PubMed\]](#), [\[CAS\]](#)

[39.](#)

Yin, F.; Grosjean, D.; Seinfeld, J. H. Photooxidation of dimethyl sulfide and dimethyl disulfide. I: Mechanism development *J. Atmos. Chem.* 1990, 11, 309– 364

[\[Crossref\]](#), [\[CAS\]](#)

[40.](#)

Chemguard, Inc. Chemguard S-103A Material Data Safety Sheet, 2011.