Aerobic Soil Biodegradation of 8:2 Fluorotelomer Stearate Monoester

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Supporting Information

ABSTRACT: A laboratory investigation on the biotransformation of 8:2 fluorotelomer stearate monoester (8:2 FTS) in aerobic soils was conducted by monitoring the loss of 8:2 FTS, production of 8:2 fluorotelomer alcohol (8:2 FTOH) and stearic acid, which would be released by cleavage of the ester linkage, and subsequent degradation products from FTOH for 80 d. Soil microcosms were extracted with ethyl acetate followed by two heated 90/10 v/v acetonitrile/200 mM NaOH extractions. 8:2 FTS was degraded with an observed half-life (t_{1/2}) of 10.3 d. The rate of 8:2 FTS biotransformation substantially decreased after 20 d with 22% of 8:2 FTS still remaining on day 80. No biotransformation of 8:2 FTS occurred in autoclaved soil controls, which remained sterile with 102 ± 6% recovery, through day 20. 8:2 FTOH was generated with cleavage of the ester linkage of 8:2 FTS followed by a rapid decline (t_{1/2} ∼ 2 d) due to subsequent biodegradation. All the expected 8:2 FTOH degradation products were detected including 8:2 fluorotelomer unsaturated and saturated carboxylic acids, 7:2s FTOH, 7:3 acid, and three perfluoroalkyl carboxylic acids with the most prominent being perfluorooctanoic acid (PFOA). PFOA consistently increased over time reaching 1.7 ± 0.07 mol % by day 80. Although cleavage of the ester linkage was evidenced by 8:2 FTOH production, an associated trend in stearic acid concentrations was not clear because of complex fatty acid metabolism dynamics in soil. Further analysis of mass spectrometry fragmentation patterns and chromatography supported the conclusion that hydrolysis of the ester linkage is predominantly the first step in the degradation of 8:2 FTS with the ultimate formation of terminal products such as PFOA.

INTRODUCTION

The widespread detection of anthropogenic polyfluoroalkyl and perfluoroalkyl substances (PFASs), especially perfluorokane sulfonic acids [PFSAs, F(CF_{2})_{n}SO_{3}H] and perfluorooalkyl carboxylic acids [PFCAs, F(CF_{2})_{m}CO_{2}H] in several environmental compartments including biota, soil, and water1−6 and in municipal effluent, sludge, and land-applied biosolids6−11 has led to investigations about their sources12, environmental fate and transport,13,14 and routes of human exposure.15−17 Historically, the primary discharge of PFSAs and PFCAs to the environment is through direct emissions from the related industries with secondary exposures from the use and disposal of consumer products containing PFSAs or PFCAs.18 With recent dramatic deductions in direct emissions,18 understanding the significance of indirect sources of PFCAs or PFSAs, such as fluorotelomer substances used in the manufacturing process and present in final commercial products, is becoming increasingly important.

Fluorotelomer alcohols [FTOHs, F(CF_{2})_{n}CH_{2}CH_{2}OH, n = 2−8]19 are a recognized indirect source of PFCAs and are the key building blocks used to produce fluorotelomer-based polymers and surfactants offering stain repellent properties.20 Studies have particularly elucidated 8:2 FTOH degradation pathways (see Figure SI-1 in the Supporting Information) in aerobic soils, mixed bacterial culture, and pure bacterial cultures.21−24 Perfluorooctanoic acid [PFOA, F(CF_{2})_{7}CO_{2}H] has been validated to be a major terminal degradation product in all studies with a maximum yield of ∼40%, which was achieved within the first 2 weeks of a 7-month aerobic soil study.22 Fluorotelomer polymers as well as many surfactants are made by linking FTOHs to hydrocarbon backbones with ester, ether, or urethane linkages, which if hydrolyzed, release FTOHs that eventually degrade to PFOA or other PFCAs. Quantitative assessments are sparse on the fate of most fluorotelomer products, particularly fluorotelomer surfactants and intermediate materials with relatively low molecular weight. Studies on polyfluorooalkyl phosphates, the surfactants used in paper coatings, have confirmed the susceptibility of the phosphate ester linkage to hydrolysis in both a simulated wastewater treatment process25 and a rat oral feeding test.26 A better understanding of the rate and extent of fluorotelomer degradation is needed, especially in landfills and soils where most of these chemicals will eventually be deposited. The present work focuses on quantifying the stability and biotransformation of 8:2 fluorotelomer stearate monoester
(8.2 FTS) (Figure 1) in a loam soil by measuring the temporal concentrations of parent compound, degradation intermediates, and terminal products. Fluorotelomer stearate esters may be used in water and oil-repellent textiles as indicated by numerous patents.27−29 Great care was taken in using appropriate methodologies to effectively extract FTOHs and subsequent metabolites without artifacts such as solvent-enhanced ester hydrolysis of the test compound as shown in our previous study.30 Recommended names and acronyms for PFASs follow the recommendations provided in a recent review targeted to provide consistent terminology and classification of perfluoroalkyl and polyfluoroalkyl substances.31

Figure 1. Chemical structure of 8:2 fluorotelomer stearate monoester (8:2 FTS). The double slash indicates the site of ester hydrolysis, which would release 8:2 FTOH and a stearic acid.

#### MATERIALS AND METHODS

**Chemicals.** Purified 8:2 FTS (99.8%), 8:2 fluorotelomer alcohol (8:2 FTOH, 99%), 8:2 fluorotelomer carboxylic acid (8:2 FTCA, 97%), 8:2 fluorotelomer α,β-unsaturated carboxylic acid (8:2 FTUCA, 98%), 2H,2H,3H,3H-pentadecafluorodecanoic acid (7:3 Acid, 98%), 2H,2H,3H,3H-pentadecafluorodecenoc acid (7:3 UAcid, 98.4%), 2H-pentadecafluoro-2-nonanol (7:2 sFTNOH, 80%), and the internal standards [1,1,2,2-D₄-3,3,3-C] 8:2 FTOH (96%) and [1,2,2,2-D₄] PFOA (96.4%) were obtained from DuPont (Wilmington, DE). Perfluorohexanoic acid (PFHxA, 97%) and PFOA (97%) were purchased from Fisher Scientific (Pittsburgh, PA). Additional details on other materials are provided in the Supporting Information.

**Soil.** Biotransformation studies were conducted with a loam soil (Raub-42P) collected immediately below the vegetated zone of a turf grass area in March (2010) from the Purdue Agronomy Farm (West Lafayette, IN). Raub-42P consisted of 35% sand, 44% silt, and 21% clay with an organic matter content of 2.7% and a soil pH (soil/water ratio of 1:2) of 6.5. The gravimetric moisture content for this soil at field capacity (corresponds to a −0.03 MPa matrix potential) was estimated to be 25.6%, which was used as the initial soil moisture level in all experiments. The soil mass throughout the text refers to the oven-dry mass at 105 °C.

**Soil Microcosm Setup.** Microcosms consisted of 125 mL crimped amber bottles with ~10 g of soil in each vessel with airtight closures (aluminum faced silicone septa or rubber stoppers) to allow for monitoring analytes both in the soil microcosm and the headspace. 8:2 FTS was amended to live soil and autoclaved soil as abiotic control, which was prepared by autoclaving moist soil three times at 0.1 MPa and 121 °C for 1 h with 1−3 d moist soil incubation (at 22 °C ± 2) before each autoclaving. Blank controls contained only live soil without 8:2 FTS.

Live soils were preincubated for 6 d at field capacity prior to 8:2 FTS addition. 8:2 FTS was added to each soil microcosm through a talc carrier. FTS-coated talc (~ 50 μg FTS mg⁻¹ talc) was prepared by mixing talc powder with an acetone solution containing 8:2 FTS followed by acetone evaporation. FTS-coated talc powder (~50 mg) was mixed manually using sterile spatulas resulting in a soil concentration of ~250 μg FTS g⁻¹ soil. All incubations were conducted under static conditions in the dark at 22 °C ± 2. For compounds with low aqueous solubility, talc has been successfully used as the carrier in several other biodegradation studies to allow for even chemical distribution throughout the soil by visual inspection.21,32 This replaces the addition of solvent that could serve as a carbon source or inhibitor for microbial communities.21 The small amount of talc added (0.5% of the soil weight) was assumed to exert no influence on soil properties. Residual 8:2 FTOH present in the 8:2 FTS-coated talc prior to application to the soils was 0.035 ± 0.003 wt % (0.05 ± 0.004 mol %) of the total 8:2 FTS.

**Sampling and Sample Treatment.** Triplicate soil microcosms were destructively sampled at selected times. Prior to soil extraction, headspace gas (10 mL) was drawn with a gastight syringe through two 600 mg C₁₈ dry solid phase extraction (SPE) cartridges in serial followed by elution of the cartridges with 5 mL of acetonitrile (ACN). The measured capture efficiency of 8:2 FTOH in headspace using C₁₈ SPE cartridges is close to 100% at a loading rate of 10 μg of 8:2 FTOH per 600 mg C₁₈ sorbent similar to what has been observed for 6:2 FTOH.33 7:2 sFTOH was assumed to be efficiently captured by the SPE cartridges because of its structural similarity (and chemical properties) to 6:2 and 8:2 FTOHs. CO₂ and O₂ levels in the headspace of the live and autoclaved soils were monitored in a separate set of microcosms identical to those used for chemical analysis as detailed above.

Soils were extracted with ethyl acetate (EA) followed by two sequential extractions with 20 mL of 90/10 v/v ACN/200 mmol NaOH on a shaker at 45 °C for ~18 h. The initial extraction with EA targeted efficient recovery of 8:2 FTS and 8:2 FTOH. In our previous work, we observed solvent-enhanced ester hydrolysis of the FTS ester linkage with several other solvents of high dielectric constants.30 Such artifacts were validated minimal with EA to make it the best solvent for assessing 8:2 FTS and 8:2 FTOH levels in the soil microcosms. EA (20 mL) was added directly to the soil microcosms, which were then capped with new septa and extracted on a rotary shaker for ~18 h. Following solid−liquid separation via centrifugation (700g for 30 min), the EA layer was saved for analysis of 8:2 FTS and expected degradation products, as well as for other potential unknown products. The basic ACN extractions targeted recovery of any remaining acid degradation products. Each extraction was followed by neutralization with equal moles of HCl after solid−liquid separation via centrifugation (700g for 30 min). The original septa used during the incubations were extracted for 8:2 FTOH and 7:2 sFTOH using ACN as described previously by Liu et al.21 Additional solvent−FTS controls containing only FTS-coated talc in 20 mL of EA were sampled at 0, 20, and 80 days to monitor stability of 8:2 FTS in the solvent in the absence of soil. All soil extracts were cleaned with graphitized carbon
adsorbent (ENVI-Carb) prior to analysis to minimize matrix effects23,34 and stored at ≤−10 °C in the dark until analysis.

**Chemical Analysis.** EA extracts were analyzed for 8:2 FTS using a Shimadzu gas chromatographic system with a quadrupole mass spectrometer (GC/MS-QP 2010) as detailed in the Supporting Information. EA extracts diluted 1:9 with ACN and all ACN extracts were analyzed for 8:2 FTOH, 7:2s FTOH, and all acid degradation products by high performance liquid chromatography tandem electrospray ionization mass spectrometry (HPLC/ESI-MS/MS) using a Shimadzu HPLC system (HTA autosampler with binary SCL-10ADvp pumps) coupled to a API3000 mass spectrometer operated in multiple reaction monitoring mode (details in the Supporting Information). An internal standard mixed solution (50 μL) of [1,1,2,2-D₄; 3-¹³C] 8:2 FTOH (for the FTOHs) and [1,2-¹³C₂] PFOA (for the acid degradation products) was added to sample extracts immediately before analysis. Standard curves corrected for recovery of 8:2 FTOH using EA from similar soils were not corrected for recovery.

**Degradation Rate Analysis.** A first order degradation rate for 8:2 FTS (kₐ, d⁻¹) was estimated by fitting a standard first-order exponential decay model for 0−20 days assuming that 8:2 FTOH is the first degradation product in the aerobic soils.21,22 Stearic acid [CH₃(CH₂)₁₆COOH] along with palmitic acid [CH₃(CH₂)₁₅COOH], a primary metabolite from β-oxidation of stearic acid,35 were also analyzed in the EA extracts using both GC/MS and GC/FID (Shimadzu GC-17A) with myristic acid [CH₃(CH₂)₁₄COOH] as an internal standard. Method details are provided in the Supporting Information. EA extracts were also assessed in a full scan mode on the GC/MS to look for potential degradation products corresponding to the shortening of the 8:2 FTS hydrocarbon chain (stearate group) if microbial attack also occurred at the hydrocarbon end of the 8:2 FTS in an alternative pathway to ester hydrolysis.

**Microcosm CO₂ and O₂ Status.** To assess if microcosms remained aerobic and active during the 80 d incubation, headspace O₂ and CO₂ levels were measured on day 0, 17, 37, and 80 in triplicate live microcosms dosed with 8:2 FTS, using a Hewlett-Packard GC/TCD system as detailed in the Supporting Information.

**RESULTS AND DISCUSSION**

**Biotransformation of 8:2 FTS.** The aerobic biodegradation of 8:2 FTS was observed to follow the expected pathway: 8:2 FTS → 8:2 FTOH → transient degradation products → PFCAs (Figures 2 and 3, and Figure SI-1 of the Supporting Information). Biotransformation of 8:2 FTS was assessed directly by loss of the parent compound and indirectly by the production of 8:2 FTOH as the primary degradation product (Figure 2). Recovery of 8:2 FTOH using EA from similar soils was previously determined to be 99.2 ± 1%.30 The immediate extraction recovery of 8:2 FTS from FTS-coated talc powder mixed in soil was 101 ± 8 wt % in the first EA extraction. Experimental assessments of the extraction recovery of 8:2 FTOH and 8:2 FTS are detailed in the Supporting Information. In addition, the average efficiency of EA to extract 8:2 FTS from the autoclaved soil was 100 ± 6% (n = 21) for the first 20 d (Figure 2), indicating consistent recoveries of 8:2 FTS with EA over time.

A first-order kinetic model applied to 8:2 FTS fits reasonably well up to day 20 with a half-life (t₁/₂) of 10.3 d (R² = 0.91, Figure 2). 8:2 FTOH resulting from hydrolysis of the ester bond in FTS reached 8 mol % within the first 12 h and increased to ~11 mol % by day 2 before gradually decreasing as it was converted into secondary degradation products. The half-life of 8:2 FTOH during 80 days was estimated at ~2 d using eq 1 (R² = 0.91, Figure 2), which is consistent with t₁/₂ values of less than 7 d measured for 8:2 FTOH in several different aerobic soils.21,22

By 80 d, 8:2 FTS had been decreased to 22% of its applied amount; however, the rate of 8:2 FTS biotransformation substantially decreased after 20 d with only an additional loss of ~10% between 20 and 80 d. All soil microcosms remained aerobic during the 80 d study (Table SI-3 of the Supporting Information); thus, the lack of O₂ was likely not the reason for rates declining over time. While no loss of 8:2 FTS was observed during the first 20 d in the autoclaved controls, only 85 ± 8% FTS was recovered on the next sampling point (40 d, Figure SI-2 of the Supporting Information) suggesting a resurgence of microbial activity that was further evidenced by increasing CO₂ production (Table SI-3 of the Supporting Information), despite the initial thorough sterilization procedure using repeated autoclaving. The CO₂ evolution remained low in autoclaved soils through day 37 (1.46 μmol CO₂ g⁻¹ soil versus ≥30 μmol CO₂ g⁻¹ soil in live soils), but by day 80, CO₂...
levels in autoclaved controls increased to levels similar to the live soil (Table SI-3 of the Supporting Information). 8:2 FTS recovery remained unchanged but more variable by day 80 (85 ± 18%); however, 8:2 FTOH and its characteristics degradation products were detected by day 80 (Figure SI-2 of the Supporting Information). The lack of any 8:2 FTS transformation in autoclaved controls during the first 20 d suggests that 8:2 FTS transformation in soils is primarily biological. In solvent−FTS controls containing only FTS-coated talc in 20 mL of EA, 97 ± 3% of 8:2 FTS was recovered on day 80 exemplifying its stability in the EA in the absence of soil.

Degradation Products from 8:2 FTOH Biotransformation. The subsequent degradation products expected from 8:2 FTOH biotransformation were observed including 8:2 FTCA, 8:2 FTUCA, 7:2s FTOH, PFOA, PFHpA, PFHxA, and the 7:3 Acid (Figure 3) consistent with previous studies.21−24 Though 8:2 FTCA, 8:2 FTUCA, and 7:2 sFTOH are considered as transient products, they were consistently the primary products observed during the 80 day incubation with the exception of 7:2 sFTOH, which decreased after day 20 with only small amounts remaining by day 80. The persistent and likely terminal products are PFOA, PFHpA, PFHxA, and 7:3 Acid. Their concentrations continued to increase throughout the 80 d incubation with PFOA constituting the greatest portion (1.7 mol %) followed by 7:3 Acid (0.45 mol %) > PFHpA (0.38 mol %) > PFHxA (0.16 mol %). PFNA was also observed and increased over time to 0.009 mol % on day 80 (Figure SI-3 of the Supporting Information). This PFNA is suspected to be from low residuals (method detection limit) of 10:2 FTOH in FTS (MDLs for FTOHs are about an order of magnitude greater than for PFCAs). Martin et al.36 observed PFNA resulting from 8:2 FTOH metabolism in rat hepatocytes; thus, the ability of microbes to do this cannot be ruled out; however, note the yield to PFNA in this study and the Martin et al.36 work was very low compared to other terminal products. Similar trends in the major metabolites secondary to 8:2 FTOH (7:2 sFTOH and the acid metabolites) were observed in an earlier study by Liu37 (Figure SI-4 of the Supporting Information), where assessing the biotransformation potential of 8:2 FTS was first attempted with a similar soil type (Raub-33, details provided in the Supporting Information 2.3). In the earlier study, 8:2 FTS was not measured directly and solvent-induced artifacts during soil extraction impacted an accurate assessment of the 8:2 FTOH levels generated from FTS biotransformation.31

7:2 sFTOH, which is a direct precursor of PFOA,22 increased to 7 mol % by day 20 and then decreased in all subsequent times. PFOA increased throughout the study to 1.7 ± 0.05 mol % by day 80. In the previous 8:2 FTOH biodegradation studies by Wang et al.,22 PFOA reached a plateau at different times, with a different maximum mole percent for the different soils, for example, ~12 mol % by day 140 for a Chalmers soil, ~20 mol % by day 56 for a Manning soil, and ~40 mol % by day 15 for a Sassafras soil. Small amounts of the two fluorinated alcohols were detected in the headspace (sum of 8:2 and 7:2 sFTOH ≤ 0.001 mol %) with levels of 7:2s FTOH being consistently higher than 8:2 FTOH, which is likely due to 7:2 sFTOH’s slower degradation to PFOA and/or higher vapor pressure.22 The sum of the two fluorinated alcohols recovered from the headspace and the septa never exceeded 0.03 mol % (Figure SI-5 of the Supporting Information).

Recoveries of Fluorinated Degradation Products. Immediate extractions of soils amended with a chemical represent the maximum recovery that can be achieved as the chemical has almost no interaction time with the soil before solvent addition. The immediate recoveries were 73−100% (standard errors <10%) for the fluorinated acid degradation products (Table SI-1 of the Supporting Information). True recoveries may decrease over time as noted by Russell et al.38 for 8:2 FTCA and 8:2 FTUCA, which declined from 89% to 50% and from 92% to 79%, respectively by day 180. Decreased recovery could be attributed to strong and potentially irreversible sorption.24

To assess if resistance to extraction increased over time, the mole percent recovered for a given extraction relative to the sum of all three extractions was evaluated (Figure SI-6 and Table SI-6 of the Supporting Information). Trends of the relative amounts with each sequential extraction are similar to those observed for the maximum extraction efficiency tests (Table SI-4 of the Supporting Information) with the exception of 7:2 sFTOH. For 7:2 sFTOH, nearly equal amounts were recovered in the first and second extractions on day 40 unlike what was recovered at earlier times. To assess the potential for a higher recovery, day 80 samples were extracted twice with EA followed by two 90/10 v/v ACN/base extractions (Table SI-7 of the Supporting Information). Although only small amounts
of 7:2 sFTOH remained by day 80, less than 5% of 7:2s FTOH was recovered in the two sequential EA extractions, and the majority (84%) was recovered in the first basic ACN extraction.

Assessing Production of Other Degradation Products. Recoveries of freshly amended stearic acid and its first β-oxidation metabolite palmitic acid from soil are 97 ± 6 and 99 ± 4 wt % in the first EA extraction. The stearic acid levels expected based on the stoichiometry of ester hydrolysis, assuming no stearic acid degradation and taking into account background levels, would have reached ~80 μg/g by day 10 and ~100 μg/g after 40 days (Figure SI-7 of the Supporting Information). However, stearic and palmitic acids were observed at consistently low levels in live and autoclaved soils with no substantial differences in their trends (Figure SI-8 of the Supporting Information). The lack of clear trends in stearic acid levels is likely due to natural soil microbial processes.35,39

Stearic acids as well as other fatty acids are naturally produced microbially from plant material and are subsequently transformed intracellularly.39 Lack of such an observation suggests that either microbes actively consumed these fatty acids or there is another significant 8:2 FTS biodegradation pathway. To verify the latter, we also looked for other possible degradation products formed from the breakdown of the hydrocarbon backbone (stearate group) prior to ester cleavage. EA extracts were assessed with a slow GC temperature ramp to look for peaks associated with compounds reflecting a shortened hydrocarbon chain of the 8:2 FTS. Analysis was done using GC/MS in both full scan mode and SIM mode under electron ionization targeting the expected m/z for the shortened hydrocarbon fragments (loss of one or multiple CH2 units), as well as using GC-FID. Such fragmentation patterns were only observed for the 8:2 FTS peak, but not for any other peaks using GC/MS, and at the same time, no other peaks eluted near the retention time of 8:2 FTS in GC/FID were observed. These results support the conclusion that the hydrocarbon chain of the 8:2 FTS was not attacked by the microbes prior to hydrolysis of the ester linkage.

Mass Balance. Total mass balance in live soils, which includes parent compound and all degradation products quantified in the three solvent extractions, headspace, and extraction of the septa, decreased over time to about 38 mol % by 80 d. Decreasing mass balance in live soils over time may be attributed to several factors, including irreversible sorption and decreasing extraction efficiencies of degradation products over time and formation of additional products that were not quantified or identified.22 Using 14C-labeled 8:2 FTOH, Wang et al.22 recovered 72% on average with nearly half of this quantified or identified.22 Using 14C-labeled 8:2 FTOH, Wang et al.22 showed that the extraction efficiencies of degradation products over time decreased over time to about 38 mol % of the terminal product PFOA (4.8 ± 0.2 μmol PFOA kg−1 soil) was produced. Although PFOA levels did not appear to have reached a plateau, the rate of PFOA production appeared to decrease after 20 d. In Wang et al.,22 PFOA production ranged as high as ~40 mol % by day 15 in one soil type and plateaued as low as ~10 mol % after 140 d in another soil type. The time frames and subsequent degradation rates of FTOH in the current study are similar to those in Wang et al.22 but not the rates of PFOA production. 8:2 FTOH in the Wang et al.22 was all applied at day 0 (2–20 mg 8:2 FTOH/kg) unlike the current study where 8:2 FTOH is generated as 8:2 FTS is degraded. Not all FTOH degradation pathways lead to PFOA, and each pathway has its own rate, which is likely dependent on concentration, the types of microbial communities, and other soil factors. In some cases, certain reactions could be reversible (Figure SI-1 of the Supporting Information).22 In addition, the irreversible binding of degradation products to soil (presumably soil organic matter) over time will also affect PFOA production. The irreversible binding process of FTOH and acid degradation products are still not well understood, but it can easily be hypothesized that the magnitude of the process will be affected by the rate of FTOH production and subsequent degradation to other products including PFOA. Additional degradation studies with fluorotelomer monomers that reflect the variety of chemical linkages or hydrocarbon backbones that may be used commercially are needed to improve our assessment of indirect PFOA sources in the soil environment.

ASSOCIATED CONTENT
Supporting Information
Details of the analytical methods, list of analytes, information on chemicals used, details on experimental methods and extraction efficiencies, matrix recovery, recoveries of degradation products over time, degradation products in Raub-33, degradation products in autoclaved controls of Raub-42P, and stearic acid and palmitic acid levels over time. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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