UC Berkeley
UC Berkeley Electronic Theses and Dissertations

Title
Oxidative Measurement of Perfluoroalkyl Acid Precursors: Implications for Urban Runoff Management and Remediation of AFFF-Contaminated Groundwater and Soil

Permalink
https://escholarship.org/uc/item/4jq0v5qp

Author
Houtz, Erika Frances

Publication Date
2013

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

Oxidative Measurement of Perfluoroalkyl Acid Precursors: Implications for urban runoff management and remediation of AFFF-contaminated groundwater and soil

By

Erika Frances Houtz

A dissertation submitted in partial satisfaction of

the requirements for the degree of

Doctor of Philosophy

in

Engineering - Civil and Environmental Engineering

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor David L. Sedlak, Chair
Professor James R. Hunt
Professor S. Katharine Hammond

Fall 2013

Oxidative Measurement of Perfluoroalkyl Acid Precursors: Implications for urban runoff management and remediation of AFFF-contaminated groundwater and soil

© 2013

Erika Frances Houtz

Abstract

Oxidative Measurement of Perfluoroalkyl Acid Precursors: Implications for urban runoff management and remediation of AFFF-contaminated groundwater and soil

by

Erika Frances Houtz

Doctor of Philosophy in Engineering - Civil and Environmental Engineering

University of California, Berkeley

Professor David L. Sedlak, Chair

Perfluoroalkyl acids (PFAAs) are used to impart oil- and water-repellant and surfactant properties to numerous products, and they are among the most persistent chemicals to ever enter commerce. PFAAs are detected in the blood of humans all over the world. The two 8-carbon PFAAs, perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), have been associated with a number of adverse health outcomes. Compounds capable of transforming to PFAAs, known as PFAA precursors, are a potentially important but poorly-understood source of indirect PFAA exposure. Unlike the PFAAs, there are many types of PFAA precursors, some of which are not publically known, and analytical standards for their measurement are often unavailable. Thus, a central challenge to characterizing PFAA precursor occurrence is a lack of available analytical tools to measure them. In this research, a new oxidation-based technique of PFAA precursor measurement was developed for aqueous and solid samples. Along with other analytical tools, this precursor assay was used to gain insight into the occurrence and fate of PFAA precursors in two sources: urban runoff and soil and groundwater impacted by firefighting materials known as aqueous film forming foams (AFFF).

The occurrence of PFAA precursors in urban runoff was investigated to determine the extent to which runoff could serve as a source of PFAAs to drinking water supplies during storage in aquifers or reservoirs. An indirect technique of measuring PFAA precursors was developed for urban runoff samples by exposing samples to a high concentration of hydroxyl radicals and converting precursors to measureable perfluorinated carboxylate products (Chapter 2). By comparing perfluorinated carboxylate concentrations before and after oxidation, the total concentration of PFAA precursors was inferred. Analysis of thirty-three urban runoff samples collected from locations around the San Francisco Bay, CA indicated that C_8 forms of PFAAs and C_6 forms of PFAA precursors were predominant in runoff. The assay demonstrated that commonly measured PFAA precursors represented only a small fraction (<25%) of the total concentration of precursors present in runoff, confirming the utility of the precursor assay.

To assess the persistence of AFFF-derived PFAA precursors, groundwater, soil, and aquifer solids were obtained in 2011 from an unlined firefighter training area at a U.S. Air Force Base where AFFF was regularly used between 1970 and 1990 (Chapter 3). To measure the total

concentration of PFAA precursors in archived AFFF formulations and AFFF-impacted environmental samples, the oxidation-based assay developed in Chapter 2 was adapted for these media. This precursor assay was employed along with direct measurement of twenty-two precursors found in AFFF and a suite of other poly- and perfluoroalkyl substances (PFASs). On a molar basis, precursors accounted for 41% to 100% of the total concentration of PFASs in archived AFFF formulations. In the training area, precursors measured by the precursor assay accounted for an average of 23% and 28% of total PFASs in groundwater and solids samples, respectively. Thus, much of the mass of precursors released at the site appeared to be converted to perfluorinated carboxylates and sulfonates over a residence time of twenty years or more. One precursor in AFFF, perfluorohexane sulfonamide amine, was detected at low concentrations on several highly contaminated soil and aquifer solids samples, but no other precursors also measured in AFFF formulations were detected in any samples at this field site. Suspected intermediate transformation products of precursors in AFFF that were directly measured accounted for approximately half of the total precursor concentration in samples from the training site.

In order to elucidate the conditions most amenable to AFFF-derived PFAA precursor transformation, microcosms were constructed with soil and sediment inocula and were incubated under different redox conditions with two different types of AFFF (Chapter 4). Live microcosms amended with AFFF manufactured by 3M demonstrated an ability to utilize the carbon in AFFF, but no changes in PFAS concentrations were observed over 60- to 90-day incubation periods. The main precursor in AFFF manufactured by Ansul, 6:2 fluorotelomer thioamido sulfonate (6:2 FtTAoS), was transformed in both aerobic and anaerobic live incubations. Under aerobic conditions, three amendments of 6:2 FtTAoS were completely transformed over a 90-day incubation and 8% of the 6:2 FtTAoS loss was accounted for as perfluorinated carboxylate and fluorotelomer sulfonate transformation products. Two additional aerobic transformation products containing one or two oxygen additions to 6:2 FtTAoS were also identified. Transformation was much slower under all anaerobic conditions, with complete transformation of 6:2 FtTAoS under live nitrate-reducing conditions after 200 days of incubation and 45% to 71% transformation of 6:2 FtTAoS after 320 days of incubation under sulfate-reducing, iron-reducing, and methanogenic conditions. A transformation product not observed under aerobic conditions, a carboxylate hydrolysis product of the 6:2 FtTAoS amide group, was identified under all anaerobic conditions. Application of the precursor assay to microcosm slurries suggested that all unquantifiable biological transformation products under aerobic conditions were partitioned to the microcosm slurry. In the anaerobic microcosms, the precursor assay indicated that unidentified biological transformation products were either sufficiently volatile to leave the slurry or unable to be oxidized to perfluorinated carboxylates. Transformation products reported in soil and groundwater beneath many U.S. military firefighter training areas (Chapter 3) are consistent with the aerobic transformation products observed in Ansul AFFF-amended microcosms

This dissertation is dedicated to my parents.

TABLE OF CONTENTS

LIST OF FIGURES ..... **IV**

LIST OF TABLES ..... **VI**

ACKNOWLEDGMENTS ..... **VII**

CHAPTER 1. INTRODUCTION **1**

1.1 MOTIVATION 2

1.2 BACKGROUND ON FLUOROCHEMICALS 3

1.2.1 History of Fluorochemical Production *3*

1.2.2 PFAA Transformation *7*

1.2.3 PFAA Environmental Releases and Occurrence *7*

1.2.4 Human Exposure Routes *12*

1.2.5 Bioaccumulation and Toxicological Studies *12*

1.3 PERFLUOROALKYL ACID PRECURSORS 14

1.3.1 Importance *14*

1.3.2 Types of PFAA Precursors *14*

1.3.3 PFAA Precursor Occurrence *16*

1.3.4 Abiotic Transformation of PFAA Precursors *18*

1.3.5 Biological Transformation of PFAA Precursors *18*

1.3.6 Analytical Limitations of PFAA Precursor Measurement *22*

1.4 RESEARCH OBJECTIVES 22

1.4.1 Objective 1 – Assess the occurrence of PFAA precursors in urban runoff samples. .. *22*

1.4.2 Objective 2 – Evaluate the persistence of AFFF-derived PFAA precursors at an AFFF-impacted firefighter training area. *23*

1.4.3 Objective 3 – Assess the transformation of AFFF formulations in soil and sediment microcosms under aerobic and anaerobic conditions. *23*

CHAPTER 2. OXIDATIVE CONVERSION AS A MEANS OF DETECTING PRECURSORS TO PERFLUOROALKYL ACIDS IN URBAN RUNOFF **24**

2.1 INTRODUCTION 25

2.2 MATERIALS AND METHODS 26

2.2.1 Materials *26*

2.2.2 Stormwater collection *26*

2.2.3 PFAA - Precursor Oxidation *28*

2.2.4 Analysis *28*

2.3 METHOD VALIDATION 30

2.4 RESULTS AND DISCUSSION 30

2.4.1 Oxidation Control Experiments *30*

2.4.2 Direct Measurement of PFASs in Urban Runoff *36*

2.4.3 PFAA Precursors Indicated by Oxidation in Urban Runoff *38*

2.4.4 Estimation of PFAA Precursor Concentrations by Chain Length *40*

2.5 ENVIRONMENTAL IMPLICATIONS 42

CHAPTER 3. PERSISTENCE OF PERFLUOROALKYL ACID PRECURSORS IN AFFF-IMPACTED GROUNDWATER AND SOIL **44**

3.1 INTRODUCTION 45

3.2 MATERIALS AND METHODS 47
3.2.1 Materials *47*
3.2.2 Site Information *48*
3.2.3 Groundwater, Soil, and Aquifer Solids Collection *48*
3.2.4 Sample Storage and Preparation for Direct Analysis *51*
3.2.5 Sample Preparation for Precursor Oxidation Assay *52*
3.2.6 Analytical Methods *52*
3.2.7 Analyte Quantification *52*
3.2.8 Total Precursor Concentration and Total PFAS Concentration *53*
3.3 RESULTS 53
3.3.1 Analysis of Pure AFFF Samples *53*
3.3.2 Analysis of AFFF-Contaminated Groundwater and Soil Samples *57*
3.4 DISCUSSION 62
3.4.1 Identifying Likely Sources of AFFF *62*
3.4.2 AFFF Precursor Transformation *63*
3.5 ENVIRONMENTAL IMPLICATIONS 68

CHAPTER 4. BIOTRANSFORMATION OF AFFF-DERIVED PFAA PRECURSORS IN SOIL MICROCOSMS UNDER AEROBIC AND ANAEROBIC CONDITIONS..... 69
4.1 INTRODUCTION 70
4.2 MATERIALS AND METHODS 71
4.2.1 Materials *71*
4.2.2 Inocula Sources *73*
4.2.3 Microcosm Preparation and Maintenance *73*
4.2.4 Sampling and Preparation for Analysis *74*
4.2.5 HPLC-MS/MS Analysis *74*
4.3 RESULTS 74
4.3.1 3M AFFF-Amended Aerobic and Anaerobic Microcosms *74*
4.3.2 Transformation of Ansul AFFF PFASs Under Aerobic Conditions *75*
4.3.3 Transformation of Ansul AFFF PFASs Under Anaerobic Conditions *81*
4.4 DISCUSSION 85
4.4.1 Mass Balance of PFASs in Ansul AFFF Aerobic Microcosms *85*
4.4.2 Aerobic Biotransformation Pathway of 6:2 FtTAoS *86*
4.4.3 Mass Balance of PFASs in Ansul AFFF Anaerobic Microcosms *88*
4.4.4 Co-metabolic Transformation *88*
4.4.5 Implications for the Fate of AFFF in Contaminated Groundwater and Soil *89*

CHAPTER 5. CONCLUSIONS..... 91
5.1 SUMMARY 92
5.2 FLUOROCHEMICALS IN URBAN RUNOFF 92
5.3 THE FATE OF FLUOROCHEMICALS AT AFFF-IMPACTED FIREFIGHTER TRAINING AREAS 93
5.4 FUTURE RESEARCH AND RECOMMENDATIONS 95

APPENDIX: CHAPTER 2 111

APPENDIX: CHAPTER 3 123

LIST OF FIGURES

Figure 1.1: Structures of the protonated C8 perfluoroalkyl acids. 2
Figure 1.2: Example of a fluorotelomer-based fluoropolymer. 5
Figure 1.3: Mean concentrations of PFOS and PFOA in human serum collected after 1988, in (A) North America, (B) Asia, (C) Europe, and (D) other countries. 10
Figure 1.4: PFOS concentrations in the livers of marine mammals reported between 2006 and 2010. 11
Figure 1.5: Generic n:2 fluorotelomer and C_n sulfonamide-containing precursors. 14
Figure 1.6: Proposed biotransformation pathway of ^{14}C-8:2 FtOH in activated sludge. 21

Figure 2.1a-b: Concentrations of C8 perfluorooctane sulfonamide-containing precursors and PFOS and PFOA in individual HPLC-grade water reactors before (a) and after (b) oxidation. 31
Figure 2.2a-b: Conversion of *N*-MeFOSAA (a) and FOSA (b) in HPLC-grade water in the presence of 5 mM persulfate and 50 mM NaOH over time at 85°C. 31
Figure 2.3: PFAS concentrations before and after oxidation of a stormwater sample. 32
Figure 2.4a-b: Concentrations of 6:2 FtS, 6:2 diPAP, 8:2 FtS, and 8:2 diPAP and their PFCA products in individual HPLC-grade water reactors before (a) and after (b) oxidation with 20 mM persulfate and 50 mM NaOH. 35
Figure 2.5a-b: Conversion of 6:2 FtS (a) and 8:2 FtS (b) in HPLC –grade water in the presence of 5 mM persulfate and 50 mM NaOH over time at 85°C. 35
Figure 2.6: Range of concentrations of PFAS analytes in all urban runoff samples. 36
Figure 2.7: Range of concentrations of PFCAs generated upon oxidation in each sample. 39

Figure 3.1: Structures of PFAA precursors found in AFFF formulations. 46
Figure 3.2: Map of sampling area and groundwater sampling locations. 49
Figure 3.3: Map of sampling area and soil and aquifer solids sampling locations. 50
Figure 3.4: Average concentrations of perfluorinated sulfonates and carboxylates in AFFF formulations analyzed before (a) and after oxidation (b). 54
Figure 3.5: The average molar fraction of different classes of PFASs in groundwater, aquifer solids, soils and AFFF formulations. 58
Figure 3.6: The measured PFAA precursor compounds in a contaminated soil sample extract compared with the expected and measured perfluorinated carboxylate products in the oxidized extract. 65
Figure 3.7: The average composition of perfluorinated carboxylates generated by precursor compounds in groundwater, aquifer solids, and soils from the firefighter training area. 66

Figure 4.1: Structures of (A) PFHxSAm, (B) PFHxSAmA, (C) 6:2 FtTAoS, (D) 6:2 FtS, and (E) 6:2 FtTCA 72
Figure 4.2: Time course of (A) 6:2 FtTAoS, (B) fluorotelomer sulfonates, and (C) perfluorinated carboxylates in live culture and autoclaved culture aerobic AFFF-acclimated soil microcosms. 76
Figure 4.3: Time course of average molecular ion response of (A) 6:2 FtTAoS, (B) m/z 602, and (C) m/z 618 during the 2nd growth cycle in aerobic acclimated live culture, autoclaved culture, and medium control microcosms. 78

Figure 4.4: Chromatograph of m/z 586, m/z 602, and m/z 618 molecular ions in an aerobic live microcosm on Day 29 of incubation. 78
Figure 4.5: Product ion scans of m/z 602 and 618 and proposed structures of daughter ions.....79
Figure 4.6: Average concentration of PFAA precursors and perfluorinated carboxylates measured in live culture microcosm slurries before and after oxidation at the beginning and end of the third growth cycle. 80
Figure 4.7: Time-course transformation of 6:2 FtTAoS and 6:2 FtTCA in UC Berkeley creek sediment live culture and autoclaved culture microcosms under (A) nitrate-reducing, (B) iron (III)-reducing, (C) sulfate-reducing, and (D) methanogenic conditions. 82
Figure 4.8: Average production of perfluorinated carboxylates measured from the oxidation of aqueous precursors in live culture and autoclaved culture anaerobic slurries on Day 339. 83
Figure 4.9: Average concentration of PFAA precursors and perfluorinated carboxylates measured before and after oxidation in triplicate live sub-cultured, low-solids, nitrate-reducing microcosms on Days 0, 45, and 118 of a 118-day incubation. 84
Figure 4.10: Proposed transformation pathway of 6:2 FtTAoS in the presence of aerobic soil microbes. 87

Figure A3.1: Fractional recovery of PFASs added to an uncontaminated soil sample. 129
Figure A3.2: Comparison of whole soil sample oxidation and soil extract oxidation approach for a single sample. 132
Figure A3.3: Effect of ENVI-CARB on precursor recovery in soil extracts. 133

LIST OF TABLES

Table 1.1: Global historical perfluorinated carboxylate production and emissions summary 4
Table 1.2: Global historical POSF- and PFOS production, use, and emissions summary 6
Table 1.3: Global background occurrence of PFAAs and PFAA precursors. 9
Table 1.4: Examples of PFAA Precursors. 15
Table 1.5: Production of perfluorinated carboxylates from the microbial transformation of fluorotelomer PFAA precursors. 20

Table 2.1: Watershed and storm characteristics for each watershed sampled. 27
Table 2.2: Molar yields of PFCAs from precursors thermolyzed in the presence of persulfate..34
Table 2.3: Average mass ratios for analytes in samples from each site. 37

Table 3.1: Concentrations of PFASs in all AFFF formulations measured. 55
Table 3.2: Percentage recovery of measured precursors in AFFF formulations as perfluorinated carboxylate products. 56
Table 3.3: Concentrations of individual PFASs and perfluorinated carboxylates generated upon oxidation in groundwater samples. 59
Table 3.4: Concentrations of individual PFASs and perfluorinated carboxylates generated upon oxidation in surficial soil and aquifer solids samples. 61

Table 4.1 Typical composition of AFFF formulations 72
Table 4.2: Molar precursor concentrations in AFFF formulations amended to microcosms. 72
Table 4.3: Measured mass, theoretical mass, and mass accuracy of intermediate products identified by high resolution mass spectrometry. 79

Table A2.1: Perfluoroalkyl and polyfluoroalkyl substances (PFASs) and stable isotope surrogate standards. 112
Table A2.2: Method and instrument detection limits and limits of quantification (LOQ) for each PFAS analyte. 113
Table A2.3: Recovery of perfluorinated carboxylates, perfluorinated sulfonates, and fluorotelomer sulfonates after WAX-SPE extraction. 114
Table A2.4: Recovery of perfluorooctane sulfonamide-containing precursors after WAX-SPE extraction.....114
Table A2.5: Monitored Ion Transitions and MS Conditions. 115
Table A2.6: The minimum and maximum concentrations of C8 PFAA precursors in stormwater samples.....119
Table A2.7: The minimum and maximum concentrations of C6 PFAA precursors in stormwater samples.....122

Table A3.1: Monitored ion transitions, MS Conditions, and internal standard used for quantification of each analyte. 124
Table A3.2: Recovery, precision, and whole method LOD of PFASs in groundwater.....127
Table A3.3: Recovery, precision, and whole method LODs of PFASs in soil and aquifer solids samples.....130
Table A3.4: Coefficients used to compute concentrations of precursors in 3M formulations without quantitative standards. 136

ACKNOWLEDGMENTS

This dissertation was completed with the indispensable guidance of my advisor David Sedlak. I am immensely thankful that he gave me the best of both advising worlds: ideas, input, and critique as well as time and space to work independently. In addition to his excellent scientific input, I am grateful for David's efforts in connecting me with key collaborators and securing research funding for my work. Finally, I am very appreciative of David's mentorship, both individually and through the discussions and retreat he thoughtfully developed for our research group.

I am grateful to my dissertation committee members, Katharine Hammond and James Hunt, who helpfully critiqued the progress of my research throughout my preliminary exam, qualifying exam, and dissertation. I'd also like to thank my former exam committee members, Kara Nelson, Jennifer Field, and Lisa Alvarez-Cohen. In hindsight, I think of those exams as among the most fortunate opportunities I had to get constructive advice on my research. I used many of the insights my committee members provided to improve my research.

Chris Higgins, our collaborator at Colorado School of Mines, was akin to a secondary advisor to me. I am tremendously grateful for his early advice on fluorochemical analysis, his generosity in providing field samples, and the opportunity to work with him on two manuscripts. I'd also like to thank Chris' group members Jenn Guelfo, Meg McGuire, and Kaneen Christensen for their help in sampling and their advice along the way.

Jennifer Field, our collaborator at Oregon State University, was an important role model and mentor to me in graduate school. Her scientific guidance and advice helped me to effectively analyze and communicate my research findings. I am also grateful to Jennifer's students, Will Backe and Ben Place, for lending their expertise on AFFF.

O'Brien Hall was a fun place to spend six years among passionate and intelligent peers. Katie Harding became my most indispensable friend, collaborator, and daily morale boost. Katie and Shan Yi taught me nearly everything I know about microbiology and made significant contributions to the work in my 4th chapter. I am grateful for my Sedlak groupmates, in particular Eva Agus, Justin Jasper, Anh Pham, and Jorge Loyo-Rosales, who provided technical and intellectual support, as well as my considerate neighbors at the lab bench, Scott Mansell and Jannis Wenk, my early grad student role models, Christina Keenan Remucal and Patrick Ulrich, my favorite addition to our lab, Samantha Beardsley, my office neighbors Ekrem Karpuzcu and Tom Bruton, and Changha Lee, Haizhou Liu, Janel Grebel, Tom Hennebel, Sun Bohit, James Barazesh, Jean Van Buren, Joe Charbonnet, and Amanda Greenleaf. I am grateful to Himani Nadgauda for letting me pay it forward and mentor her as an undergraduate researcher. Her enthusiasm for lab reinvigorated my own. Ian Tse, Daniella Rempe, Lindsey Nolan Kerkez, Tiffany Louie, Gabriele Bellani, and Chris Sales were among the best friends and coffee dates that ever took the edge off a failed lab experiment.

San Francisco Estuary Institute kindly brought me into their Regional Monitoring Program sampling campaigns and helped me collect stormwater in water years 2009 and 2010. I'd like to thank Meg Sedlak in particular for coordinating my involvement in sampling, among

other kind things she's done for me over the years. I'd also like to thank Rachel Allen, Lester McKee, Alicia Gilbreath, and Jen Hunt, who were all a lot of fun to hang out with while waiting for it to rain in the middle of the night.

From my former research group at Ohio State, I acknowledge and thank my advisors, Linda Weavers and Yo Chin, my grad student mentor Laura Helsabeck, and my labmates and friends Ale Hakala and Collin Ward. These people introduced me to research, made me excited about life in the lab, and ultimately encouraged me to come to Berkeley for graduate school.

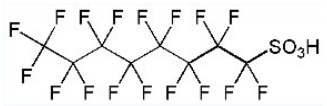
I thank my family in Ohio for their support from afar. Both my parents and my grandparents emphasized the paramount importance of education at every stage of my life and made sacrifices to help me attain my education. Their encouragement and moreover their apparent trust in me as an individual helped me develop the confidence I needed to finish graduate school and everything that preceded it. I thank my parents, sisters, and my brother-in-law for visiting me in Berkeley and taking a firsthand interest in the life that I built here.

Finally, I'd like to thank my partner James for his counsel, his patience, and his joie de vivre. Notably, this dissertation and James have been in my life for roughly equal amounts of time. I am grateful to part ways with one and not the other.

CHAPTER 1. Introduction

1.1 Motivation

Since the 1940's, perfluoroalkyl acids have been manufactured and used in both industrial applications and consumer products. The most abundant of the perfluoroalkyl acids are two, eight-carbon (C_8) anionic surfactants known as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) (Figure 1.1). A range of shorter and longer perfluoroalkyl acids containing between 4 and 18 carbons have also been manufactured. The perfluoroalkyl acids have been detected in surface waters [Huset *et al.* 2008, Ahrens *et al.* 2009b, Zushi and Masunaga 2009b, Meyer *et al.* 2011], humans [Kato *et al.* 2011, Yeung *et al.* 2013a, Yeung *et al.* 2013b], and biota [Giesy and Kannan 2001, Houde *et al.* 2011] in areas far from known point sources. In addition to this widespread occurrence, these compounds have caused concern because of their potential to cause adverse health effects in humans and biota [Lau *et al.* 2007, Gallo *et al.* 2012] and their resistance to transformation [Vecitis *et al.* 2009].



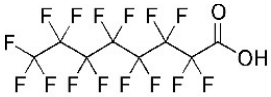


Figure 1.1: Structures of the protonated C8 perfluoroalkyl acids: (left) perfluorooctane sulfonic acid (PFOS) and (right) perfluorooctanoic acid (PFOA).

Many partially fluorinated compounds that contain one or more perfluorinated moieties were manufactured in addition to the perfluoroalkyl acids. Typically, these compounds contained either an ethyl linkage or a sulfonamide linkage between the perfluorinated group and the rest of the molecule. Previous research has shown that these linkages are more readily broken than the C-C and C-F bonds in the perfluorinated group [Ahrens 2011, Martin *et al.* 2010]. Because these polyfluorinated compounds can be transformed either to the perfluorinated sulfonates or perfluorinated carboxylates under conditions typically encountered in the environment [Ahrens 2011, Martin *et al.* 2010], they are often referred to as precursors to the perfluoroalkyl acids. According to most reports, the mass of perfluoroalkyl acid precursor compounds manufactured was two to twenty times greater than that of the perfluoroalkyl acids [Prevedouros *et al.* 2006, Paul *et al.* 2009].

This dissertation addresses the hypothesis that the transformation of partially fluorinated precursors represents a significant, ongoing source of perfluoroalkyl acids. To characterize the occurrence and fate of precursors to perfluoroalkyl acids in locations where they pose the greatest potential risks to surface water and groundwater, research was focused on two potential sources. Precursors to the perfluoroalkyl acids were investigated in urban stormwater because long residence times in reservoirs or aquifers could lead to future releases of perfluoroalkyl acids to potable water supplies. Precursors to the perfluoroalkyl acids are also a concern at locations where fluorochemical-containing firefighting foams have been used. The long-term fate of perfluoroalkyl acid precursors in firefighting foams was investigated at a firefighter training area and in laboratory microcosm experiments inoculated with soils and sediments. Efforts to assess the occurrence and fate of perfluoroalkyl acid precursors required the development of new techniques for measuring precursors and application of these methods under conditions encountered in urban stormwater, soil, and groundwater.

1.2 Background on Fluorochemicals

1.2.1 History of Fluorochemical Production

Perfluorinated carboxylates and perfluorinated sulfonates, collectively referred to as perfluoroalkyl acids (PFAAs), were first manufactured in the 1940's. PFAAs contain a common structural element, a fully-fluorinated (*i.e.,* perfluorinated) carbon chain, which can vary in length from three (C_3) to eighteen (C_{18}) carbons. The C_8-based PFAAs, PFOA and PFOS (Figure 1.1), and other compounds containing a C_8 perfluorinated backbone were manufactured in the largest annual quantities prior to the early 2000's [Prevedouros *et al.* 2006, Paul *et al.* 2009]. In 2002, the main manufacturer of PFOS, the 3M Company, phased-out production of PFOS and other C_8-based fluorochemicals. The U.S. EPA PFOA Stewardship Council subsequently partnered with eight major fluorochemical companies in a plan to reduce PFOA production by 95% by 2010 and completely eliminate it by 2015 [U.S. EPA 2006]. Compounds containing perfluorinated moieties with six or fewer carbons are reported to have replaced C_8-based fluorochemicals in the U.S. [Ehresman *et al.* 2007, Telomer Research Program Update 2002]; however, production of C_8 fluorochemicals chemicals reportedly continues in other parts of the world.

In 2009, the U.S. EPA issued Provisional Health Advisories for PFOS and PFOA in drinking water, 200 ng/L and 400 ng/L, respectively [U.S. EPA 2009]. In 2012, the C_4, C_6, and C_8 perfluorinated sulfonates and the C_7, C_8, and C_9 perfluorinated carboxylates were added to the U.S. EPA Unregulated Contaminant Monitoring Rule (UCMR 3) for Public Water Systems [U.S. EPA 2012]. In conjunction with this Rule, Public Water Systems must monitor for these PFAAs in their drinking water and report their occurrence at thresholds ranging from 10 ng/L to 90 ng/L; reporting thresholds for PFOS and PFOA are 40 ng/L and 20 ng/L, respectively [U.S. EPA 2012]. PFOS was added to the Stockholm Convention as an Annex B restricted persistent organic pollutant in 2009 and was restricted under the European Union's chemical regulation, REACH, in 2009 [Commission Regulation No 552/2009 2009].

PFAAs are used directly in some consumer and industrial products, such as some formulations of aqueous film forming foam (AFFF) used for firefighting [Prevedouros *et al.* 2006, Paul *et al.* 2009]. However, most PFAA production is related to the manufacture of other fluorochemical products in which PFAAs are residuals or byproducts of the manufacturing process (Table 1.1, Table 1.2).

The C_8 and C_9 perfluorinated carboxylates, PFOA and PFNA, have been used in ammonium salt form (APFO, APFN) as non-reactive processing aids to solubilize fluoromonomers in the production of fluoropolymers such as polytetrafluoroethylene, also known as Teflon®. Between 4,200 and 8,000 tonnes of these ammonium salts were produced prior to 2005 (Table 1.1). An average of 16% of the initial mass of perfluorinated carboxylates remained in the finished fluoropolymer products [Prevedouros *et al.* 2006]. Another 60% of the perfluorinated carboxylates used in fluoropolymer manufacture are believed to have been emitted locally to air, land, and water [Prevedouros *et al.* 2006]. These fluoropolymer-related emissions, estimated to be between 2,600 and 5,700 tonnes, were the largest historical direct release of perfluorinated carboxylates to the environment (Table 1.1) [Prevedouros *et al.* 2006]. Releases of perfluorinated carboxylates at these fluoropolymer manufacturing facilities were

dramatically reduced starting around 2000 through addition of systems that captured and recycled perfluorinated carboxylates at manufacturing facilities.

Table 1.1: Global historical perfluorinated carboxylate production and emissions summary (adapted from Prevedouros *et al.* 2006).

	Area of Manufacture or Use	Historical time period	Estimated total global production of perfluorinated carboxylates (tonnes)	Estimated total global perfluorinated carboxylate emissions (tonnes)
APFO/APFN Perfluorinated Carboxylate Manufacture and Use	**Manufacture**			
	PFO/APFO	1951-2004	3600-5700	400-700
	PFN/APFN	1975-2004	800-2300	70-200
	Industrial and Consumer Uses of APFO and APFN			
	Fluoropolymer manufacture (APFO)	1951-2004	Included in PFO/APFO	2000-4000
	Fluoropolymer dispersion processing (APFO)	1951-2004	Included in PFO/APFO	200-300
	Fluoropolymer manufacture (APFN)	1975-2004	Included in PFN/APFN	400-1400
	Fluoropolymer processing (APFN)	1975-2004	Included in PFN/APFN	10-20
	Aqueous film forming foams (AFFF)	1965-1974	Inc. in PFO/APFO, PFN, APFN	50-100
	Consumer and industrial products	1960-2000	Inc. in PFO/APFO, PFN, APFN	40-200
	Total		**4400-8000**	**3200-6900**
Residual Perfluorinated Carboxylate Sources	**Perfluorooctanesulfonyl-based products**			
	Perfluorinated carboxylate impurities	1960-2000	Not available	20-130
	POSF-based precursor degradation*	1960-2000	Not available	1-30
	POSF-based AFFF	1970-2002	Not available	3-30
	Fluorotelomer-based products			
	Perfluorinated carboxylate impurities	1974-2004	Not available	0.3-30
	Fluorotelomer-based precursor degradation*	1974-2004	Not available	6-130
	Fluorotelomer-based AFFF	1975-2004	Not available	<1
	Total			**30-350**
*Estimate made by Prevedouros et al. 2006	**Emissions among all historical sources**	**Total**		**3200-7300**
	Production among all historical sources	**Total**	**4400-8000**	

Perfluorinated carboxylates have also been unintentionally generated during the manufacture of fluoropolymers and other fluorochemical products. Perfluorinated carboxylates were created as residuals (0.1% - 1% by weight) in products that employed a perfluorooctanesulfonyl (POSF) starting material, which was commonly used to make PFOS and related products [Prevedouros *et al.* 2006]. Perfluorinated carboxylates are also trace residuals (*i.e.,* <1 – 100 ppm) in fluorochemical products that use a fluorotelomer starting material [Prevedouros *et al.* 2006]. Fluorotelomer compounds resemble perfluorinated compounds, but they contain a perfluorinated group bound to two or more non-fluorinated carbons. Fluorotelomer alcohols (0.1% - 0.5%, unreacted) and fluorotelomer olefins (3% - 8%, byproduct) also remain as impurities in fluorotelomer-based polymers [Prevedouros *et al.* 2006], (Figure 1.2). These compounds can serve as precursors to perfluorinated carboxylates [Russell *et al.* 2008]. Total perfluorinated carboxylate releases from POSF- and fluorotelomer products, including secondary release from fluorotelomer precursor transformation, were estimated to be between 30 and 350 tonnes before 2004 (Table 1.1).

Fluorotelomer-based polymers have been used in many oil and water-repellent applications, including food contact paper. C_8-based chemistry was reportedly preferred for fluorotelomer-based polymers, while C_6-based chemistry was preferred for the manufacture of fluorotelomer-based surfactants [Telomer Research Program Update 2002]. Fluorotelomer production has increased since its introduction, reaching 12,000 tonnes annually in 2004 [DuPont 2005]. In 2004, roughly 80% of fluorotelomers were used in the production of polymers and 20% were used as surfactants [Telomer Research Program Update 2002].

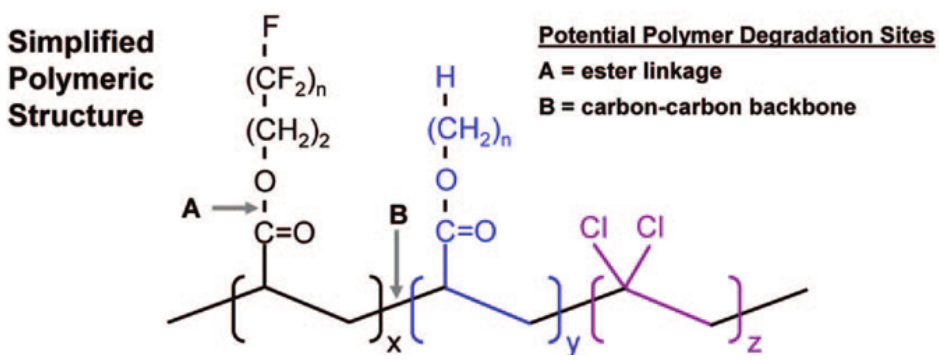


Figure 1.2: Example of a fluorotelomer-based fluoropolymer [Russell *et al.* 2008].

Like the perfluorinated carboxylates, perfluorinated sulfonates were also produced as unintended products during the manufacture of fluoropolymer products. POSF, a product that can hydrolyze to PFOS, was the starting material of many fluoropolymers used in coatings for fabric, paper, and carpet. Between 0.1% and 5% of the starting amount of POSF typically was converted to PFOS and remained as a residual in these products [Paul *et al.* 2009]. POSF was also used in smaller quantities to produce PFOS for AFFF and other non-polymerized fluorochemical products such as pesticides.

Table 1.2: Global historical POSF- and PFOS production, use, and emissions summary (adapted from Paul *et al.* 2009).

		Historical time period	Estimated total global POSF production/use (tonnes)	Estimated total global historical POSF emissions (tonnes) to water/air	Estimated total global historical PFOS emissions (tonnes) to water/air
POSF manufacture					
POSF production		1970-2002	96,000		
PFOS residual		1970-2002	470		
Manufacturing wastes		1970-2002	26,500	650-2600	6.5 to 130
	Total		**122,500**	**650-2600**	**6.5 to 130**
Secondary industrial application		1970-2002	Inc. in POSF manufacture	2600-10,000	26 to 500
Estimated consumer use and disposal			Inc. in POSF manufacture but broken down here		
Carpets		1970-2002	*48,000*	20,500	205 - 1000
Paper and Packaging		1970-2002	*24,000*	350	3.5 - 17
Apparel		1970-2002	*12,500*	12,000	120 - 600
Performance chemicals		1970-2002	*6000*	45	<0.1 - 2.2
AFFFs		1970-2002	*10,000*	9150	91 - 460
Total	**Total**		***96,000***	**4200 - 42,000**	**420 - 2100**
Source Emissions	**Total**			**6800 - 45,000**	**450 - 2700**
Production	**Total**		**122,500**		

The total estimate of POSF production between 1970 and 2002 is 122,500 tonnes, 20% of which was lost as industrial waste (Table 1.2). According to Paul *et al.,* between 450 and 2,700 tonnes of PFOS were emitted to water and air from the manufacture, use, and disposal of POSF-based products (Table 1.2) [2009]. During that same period, an estimated 6,800 to 45,000 tonnes of POSF were emitted from the use and disposal of other C_8-based fluorochemical products (Table 1.2). POSF- and perfluorohexane sulfonyl-based product lines are reported to have been replaced with perfluorobutane analogs [Ehresman *et al.* 2007]. However, estimates of production volumes of these products are not available.

1.2.2 PFAA Transformation

PFAAs are resistant to most transformation processes that occur in the environment. Because their perfluorinated chain precludes the abstraction of hydrogen atoms and they do not have double bonds, PFAAs do not react with hydroxyl radical or other reactive oxygen species at appreciable rates [Schröder and Meesters 2005, Hori *et al.* 2004]. Perfluorinated carboxylates can be oxidized by sulfate radical [Hori *et al.* 2005] or light with wavelengths less than 280 nm [Vecitis *et al.* 2009, Hori *et al.* 2004], but neither of these conditions is typically encountered in sunlit waters or subsurface environments. Neither the perfluorinated sulfonates nor the perfluorinated carboxylates have been observed to undergo biotransformation under conditions typically encountered in soil and groundwater [Parsons *et al.* 2008].

Transformation of PFAAs has occurred through processes involving the usage of biomolecules. Biomimetic reductive defluorination of PFOS has been observed in laboratory experiments where aqueous solutions of PFOS were exposed to Vitamin B_{12} and Ti(III)-citrate under anoxic conditions [Ochoa Herrera *et al.* 2008]. Defluorination of PFOA was observed in an enzyme-catalyzed oxidative coupling reactive system: horseradish peroxidase enzyme catalyzed oxidation of a phenolic co-substrate, leading to secondary phenolic radical reactions with PFOA [Colosi *et al.* 2009]. Enzymes such as peroxidases and oxidases thus could be capable of catalyzing the transformation of PFAAs in waters containing phenolic-rich natural organic matter.

Engineered treatment technologies can transform PFAAs. Sonochemistry [Moriwaki *et al.* 2005, Vecitis *et al.* 2008] and incineration [Vecitis *et al.* 2009] can both lead to thermal decomposition of PFAAs. Both Ti/SnO_2-Sb-Bi anodes and Ce-doped modified porous nanocrystalline PbO_2 film electrodes can mineralize PFOA [Zhuo *et al.* 2011, Niu *et al.* 2012]. Finally, both perfluorinated sulfonates and perfluorinated carboxylates are susceptible to reduction and subsequent mineralization by solvated electrons [Park *et al.* 2009, Qu *et al.* 2010]. Most of these chemical treatment technologies were tested in aqueous systems in which PFAAs were the primary organic constituent; the efficacy of these treatment processes under conditions encountered in full-scale systems has yet to be demonstrated.

1.2.3 PFAA Environmental Releases and Occurrence

The disposal of fluorochemical manufacturing waste has contributed large quantities of PFAAs and other fluorochemicals to the environment. The majority of fluorochemical manufacturing waste is in solid forms (*e.g.,* 90% of waste from 3M's fluorochemical production before 2002 were solids), and was historically disposed of through land farming or landfilling [Paul *et al.* 2009, Prevedouros *et al.* 2006]. Since 1998, incineration and hazardous and non-hazardous landfilling have been used to dispose of 3M's manufacturing solid wastes [Paul *et al.* 2009]. The remainder of 3M and other companies' industrial wastes has been released to air and wastewater treatment plants. Soils treated with sludge obtained from municipal wastewater treatment plants receiving fluorochemical manufacturing waste contained up to 1 mg/kg of individual fluorochemicals and 5 mg/kg total fluorochemicals [Washington *et al.* 2010b, Yoo *et al.* 2010]. Nearby drinking water wells contained PFOS and PFOA at levels exceeding their U.S. EPA Provisional Health Advisories [Washington *et al.* 2010b].

In addition to the disposal of manufacturing waste, the use and disposal of consumer and industrial products containing fluorochemicals is a significant pathway through which fluorochemicals are released to the environment. The worst-case estimates of POSF and PFOS emitted to air and water from the use and disposal of carpets, apparel, and firefighting foams are approximately four times the amount of POSF and PFOS released to air and water from manufacturing and secondary industrial applications (Table 1.2).

The use of fluorochemical-containing consumer products has resulted in the detection of fluorochemicals in effluent from wastewater treatment plants [Schultz *et al.* 2006, Boulanger *et al.* 2005, Ahrens *et al.* 2009b, Guo *et al.* 2010] (Table 1.3), biosolids-treated soils [Sepulvado *et al.* 2011], and landfill leachate [Huset *et al.* 2011] collected from areas that are not close to industrial fluorochemical sources. In areas without fluorochemical manufacturing, municipal wastewater treatment plant effluent contained total PFAA concentrations ranging from tens of ng/L to a few hundred ng/L (Table 1.3) [Schultz *et al.* 2006, Boulanger *et al.* 2005, Ahrens *et al.* 2009b, Guo *et al.* 2010]. Additionally, a correlation between biosolids loading rates and soil PFAA concentrations has been observed in biosolids-treated agricultural soils [Sepulvado *et al.* 2011]. Notably, PFOS and PFOA were still the main forms of PFAAs in wastewater effluent [Schultz *et al.* 2006, Boulanger *et al.* 2005, Ahrens *et al.* 2009b, Guo *et al.* 2010] and sludge [Higgins *et al.* 2005] from domestic sources five to ten years after the phase-out of C_8-based fluorochemical products, suggesting a continuing source associated with consumer products. PFAA concentrations are much higher in landfill leachate than wastewater treatment plant effluent: between 100 and 2000 ng/L of individual C_4 to C_8 perfluorinated carboxylates and C_4, C_6, and C_8 perfluorinated sulfonates were detected in leachate from five landfills in the U.S. [Huset *et al.* 2011].

The global distribution of PFAAs has been documented by their detection in rivers, lakes, marine waters, and urban runoff in regions where a fluorochemical point source was not present (Table 1.3). Individual PFAAs in marine waters have been detected at concentrations ranging between 0.001 and 0.3 ng/L, with C_6 to C_8 perfluorinated carboxylates and PFOS predominating [Ahrens *et al.* 2009a, Benskin *et al.* 2012]. Concentrations of PFAAs that are two to three orders of magnitude higher than those observed in marine waters were detected in rivers and lakes [Huset *et al.* 2008, Zushi and Masunaga 2009b, Meyer *et al.* 2011, Plumlee *et al.* 2009] and urban runoff [Kim and Kannan 2007, Murakami *et al.* 2009a, Houtz and Sedlak 2012]. Generally PFOA and PFOS were the dominant PFAAs in surface water and runoff, but PFNA was also found at relatively high concentrations in Japan [Zushi and Masunaga 2009b, Murakami *et al.* 2009a] due to the historical manufacture of ammonium PFNA (APFN) that occurred in Japan [Prevedouros *et al.* 2006].

Table 1.3: Global background occurrence of PFAAs and PFAA precursors.

			Perfluorinated carboxylates					Perfluorinated Sulfonates			PFAA Precursors		
Location	**Year of Sampling**	**Reference**	**PFHxA**	**PFHpA**	**PFOA**	**PFNA**	**PFDA**	**PFHxS**	**PFOS**	**PFDS**	**FOSA**	**N-EtFOSAA**	**6:2 FtS**
Marine Waters (ng/L)													
Atlantic Ocean	2007	Ahrens *et al.* 2009a	<0.006 – 0.127	<0.006 – 0.104	<0.004 – 0.229	<0.005 – 0.107		ND	<0.001 - 0.291		<0.0017 – 0.307		
Canadian Arctic	2005, 2008	Benskin et al. 2012	ND – 0.065	0.011 – 0.084	0.0065 – 0.054	ND – 0.013	ND – 0.033	ND – 0.019	ND – 0.039		ND – 0.044		
Fresh Surface Waters (ng/L)													
Hayabuchi River, Japan	2007	Zushi and Masunaga 2009b		4.-7	18 - 40	50 - 270	2.-4	9.- 20	40 - 60				
Toronto, Canada	2010	Meyer *et al.* 2011	4.- 14	1.2 – 7.9	2.2 – 7.9	0.33-1.6		0.24-1.7	2.1 – 6.5	0.07 – 0.44	0.005 to 0.32		
San Jose, U.S.	2006-2007	Plumlee *et al.* 2008		<4	<4 – 15		<21	<4	9.3 -38	<21 – 44	<21		
Glatt Valley, Switzerland	2006	Huset *et al.* 2008	ND	0.7 – 2.7	7.0 – 7.6	ND	ND	8.9 - 14	43 - 60	ND	ND		ND
Stormwater (ng/L)													
San Francisco Bay Area	2010-2011	Houtz and Sedlak 2012	0.9 – 9.7	<0.2 – 5.7	2.3 – 15.7	0.3 – 3.8	<0.2 – 4.0	<0.2 – 6.5	2.6 – 26.3	<0.2 – 0.7	<0.2 -1.8		
Albany, NY	2006	Kim and Kannan 2007		1.13 median	3.8	0.71		0.35	0.81	ND	ND		
Tokyo	2006-2007	Murakami *et al.* 2009a			40 - 105	5 – 60	1 – 50		2.-20		0.1-2		
Municipal Wastewater (ng/L)													
Pacific Northwest, U.S.	2004	Schultz *et al.* 2006	6.4		11	3.4	2.3	1.2	24	8.2		4.6	9.8
Iowa, U.S.	2004	Boulanger *et al.* 2005			22				26		<2	3.6	
River Elbe, Germany	2007	Ahrens *et al.* 2009b	3.7 - 57	1.6 - 15.7	12.3 - 77.6	1 - 18.6	0.9 - 34.5	<0.3-6.3	<0.06-86		0.3-1.1		<0.2 - 38
Korea	2008	Guo *et al.* 2010	1.1 - 14.8	<0.8 - 16.1	3.4 - 49	<0.7 - 15.8	<0.5 - 4.1	<0.5 - 10.5	1.1 - 8.9				

PFAAs have also been detected in humans (Figure 1.3) [Houde *et al.* 2006, Kato *et al.* 2011, Olsen *et al.* 2012, Glynn *et al.* 2012, Yeung *et al.* 2013a, Yeung *et al.* 2013b] and biota (Figure 1.4) [Giesy and Kannan 2001, Houde *et al.* 2011] that have not been directly exposed to an industrial source. The relatively high concentrations of PFAAs such as PFOS in Arctic marine animals (Figure 1.4) are particularly striking because of the distance between the organisms and the major sources of the chemicals. PFOS and PFOA are the fluorochemicals typically detected in the highest concentrations in all biota, reflecting the predominance of C_8 chemistry in fluorochemical manufacturing [Houde *et al.* 2011].

PFAA temporal concentrations in human sera have been widely documented around the world in recent years [Kato *et al.* 2011, Glynn *et al.* 2012, Olsen *et al.* 2012, Yeung *et al.* 2013a-b]. Since 2000, concentrations of PFOS have declined globally in human sera [Kato *et al.* 2011, Glynn *et al.* 2012, Olsen *et al.* 2012, Yeung *et al.* 2013b], while concentrations of C_4 and C_6 perfluorinated sulfonates (PFBS, PFHxS) have increased in some locations [Glynn *et al.* 2012, Kato *et al.* 2011] and remained constant or decreased in other locations [Yeung *et al.* 2013b, Olsen *et al.* 2012]. PFOA concentrations in human sera have declined globally since 2000, but at a much slower rate than the decline of PFOS [Kato *et al.* 2011, Glynn *et al.* 2012, Olsen *et al.* 2012, Yeung *et al.* 2013a]. Concentrations of C_9 and other longer perfluorinated carboxylates have increased in human sera since 2000 [Kato *et al.* 2011, Glynn *et al.* 2012, Yeung *et al.* 2013a]. The C_6 perfluorinated carboxylate (PFHxA) has not routinely been detected above method detection limits in any human sera samples [Glynn *et al.* 2012, Olsen *et al.* 2012, Yeung *et al.* 2013a], and the C_4 perfluorinated carboxylate (PFBA) has not been measured in a temporal study.

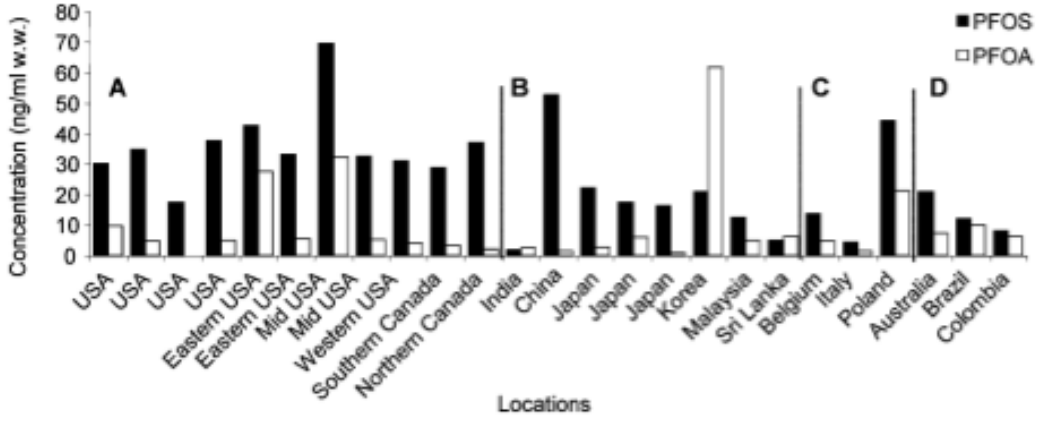


Figure 1.3: Mean concentrations of PFOS and PFOA (ng/mL ww) in human serum collected after 1988, in (A) North America, (B) Asia, (C) Europe, and (D) other countries. Blood concentrations were adjusted to serum concentrations by multiplying by 2 [Houde *et al.* 2006].

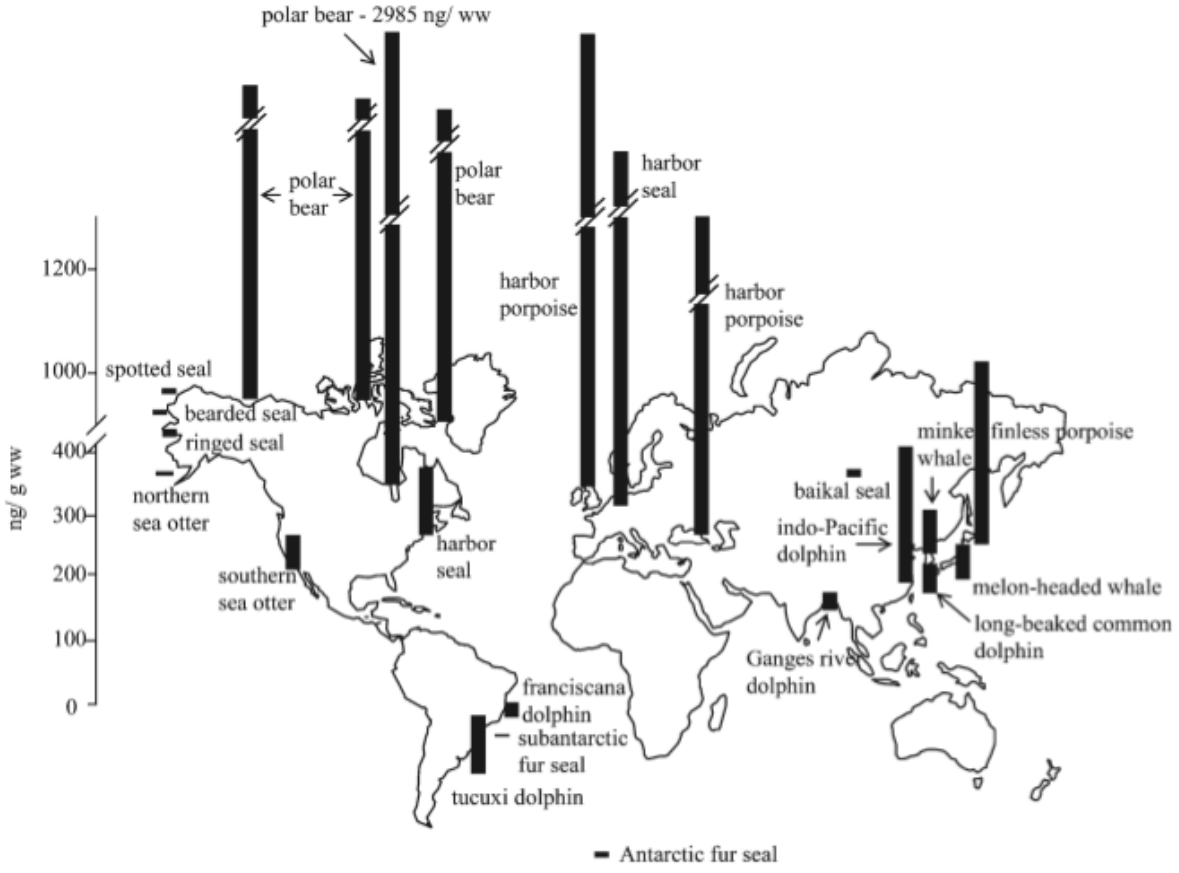


Figure 1.4: PFOS concentrations (ng/g-ww) in the livers of marine mammals reported between 2006 and 2010 [Houde *et al.* 2010].

The detection of PFAAs in remote, non-populated regions has been attributed to two mechanisms. First, oceanic transport is believed to be the primary mechanism for transport of PFOS and perfluorinated carboxylates shorter than C_{12} to the Arctic [Armitage *et al.* 2009a-b]. Second, volatile precursors to PFAAs, including fluorotelomer alcohols and POSF-related compounds, may be released in the troposphere where they undergo long range atmospheric transport and transformation into the C_8 (PFOA), C_{10} and C_{12} perfluorinated carboxylates and PFOS in the Arctic [Ellis *et al*. 2004, Armitage *et al.* 2009a]. Because the PFAAs are negatively charged at the pH of seawater, their potential for tropospheric vapor phase transport is minimal [Armitage *et al*. 2009a-b].

1.2.4 Human Exposure Routes

For most people, the main exposure route to PFAAs and other fluorochemicals is believed to be ingestion of food [Vestergren and Cousins 2009, Haug *et al.* 2011, Björklund *et al.* 2009, Fromme *et al.* 2007]. The C_6 to C_{14} perfluorinated carboxylates and the C_6 and C_8 perfluorinated sulfonates are present at their highest concentrations in fish (*e.g.*, up to 1300 pg/g PFOS, 9.2 pg/g PFHxS, and 50 pg/g PFOA in fish from Sweden in 2010). The C_6 and C_8 perfluorinated sulfonates also occur at relatively high concentrations in eggs (2.5 pg/g PFHxS, 39 pg/g PFOS in 2010) and meat (4.5 pg/g PFHxS, 25 pg/g PFOS in 2010) [Vestergren *et al.* 2012]. Compared to the perfluorinated sulfonates, PFOA is detected in many more types of food, including dairy products, fruit, vegetables, and cereals in addition to fish, meat, and eggs at concentrations ranging from 10 to 60 pg/g [Vestergren *et al.* 2012]. Temporal trends in PFAA concentrations in food vary by type of food and PFAA homolog and do not always reflect changes in manufacturing processes [Vestergren *et al.* 2012]. For infants, breast milk is thought to be the primary PFAA exposure source [Haug *et al.* 2011].

Food packaging materials treated with fluorochemicals may contribute to the importance of food as a PFAA exposure source. Both PFAAs [Begley *et al.* 2005] and precursors to the PFAAs are capable of migrating from food contact paper into oily foods [Begley *et al.* 2008]. Furthermore, enhanced migration of fluorochemicals into food from fluorochemical-containing contact paper was particularly important for fatty foods containing an emulsifier [Begley *et al.* 2008]. For example, in five different brands of microwave popcorn,1400 to 3900 pg/g of fluorochemicals that are likely precursors to the PFAAs were detected in popcorn after preparation according to package instructions [Begley *et al.* 2008]. Some researchers have argued that it is the exposure to PFAA precursors in food packaging materials rather than the food itself that is primarily responsible for the PFAAs found in humans [D'Eon and Mabury 2011].

In addition to exposure to PFAAs through ingestion of food, other exposure pathways are important for some groups of people. Hand-to-mouth contact with fluorochemical-embedded dust and carpets is estimated to be a more significant source of exposure than food for toddlers and young children [Trudel *et al.* 2008, Björklund *et al.* 2009]. Drinking water is an important exposure pathway to fluorochemicals when the drinking water source is located near an industrial fluorochemical production facility [Vestergren and Cousins 2009, Emmett *et al.* 2006].

1.2.5 Bioaccumulation and Toxicological Studies

Longer chain PFAAs bioaccumulate in humans and wildlife through mechanisms that are unlike most organic contaminants. Perfluorinated sulfonates and perfluorinated carboxylates with eight or more fluorinated carbons (*i.e.,* PFOS and the C_9 perfluorinated carboxylate, PFNA) tend to bioaccumulate, while those with fewer than eight fluorinated carbons do not [Conder *et al.* 2008]. The ionized sulfonate and carboxylate functional groups cause the PFAAs to be oleophobic, so unlike most organic contaminants, the bioaccumulation of PFAAs does not involve passive partitioning into lipids. Instead, PFAAs have a strong affinity for proteins [Bischel *et al.* 2010] and tend to accumulate in protein-rich tissues including the liver (Figure 1.4), kidneys, and blood [Olsen *et al.* 2005, Kannan *et al.* 2004, Giesy and Kannan 2001, Martin *et al.* 2004]. PFAAs bind to proteins at least partially through site-specific interactions [Bischel *et al.* 2010, Liu *et al.* 2011]. As a result of the finite number of potential interaction sites, the

bioaccumulation factor for individual PFAAs decreases as PFAA exposure increases [Liu *et al.* 2011].

Human serum elimination rates of PFAA compounds are related to the human sex and to PFAA chain length and ionic head group type. Elimination of PFHxS, PFOA, and PFOS in human serum tends to follow first-order kinetics [Olsen *et al.* 2007]. In fluorochemical workers, PFHxS had the longest observed elimination half-life (8.5 years), followed by PFOS (5.4 years), and PFOA (3.8 years) [Olsen *et al.* 2007]. Women tend to have lower concentrations of the C_6 and C_8 PFAAs than men [Kato *et al.* 2011, Yeung *et al.* 2013a, Yeung *et al.* 2013b], a phenomenon that has been attributed to accelerated elimination of PFAAs through menses [Harada *et al.* 2004].

Adverse human health effects have been associated with blood concentrations of PFAAs in studies of industrially exposed populations and the general population. Adults living near a Teflon manufacturing facility demonstrated positive associations between the highest PFOA blood levels and testicular (adjusted odd ratio = 2.8, 95% CI: 0.8, 9.2, n=6) and kidney (adjusted odds ratio = 2.0, 95% CI: 1.0, 3.9, n=9) cancers [Vieira *et al.* 2013]. Positive associations between serum PFOS and PFOA and a serum liver function biomarker, ALT, which is related to hepatocellular damage were also observed in this population [Gallo *et al*. 2012]. Children living in the same manufacturing-exposed region demonstrated an increased susceptibility to hypothyroidism in relation to serum PFOA concentrations [Lopez-Espinosa *et al.* 2012]. PFOA concentrations in blood and total cholesterol are positively associated in both fluorochemical manufacturing workers [Sakr *et al.* 2007a, Sakr *et al.* 2007b, Costa *et al.* 2009] and the general U.S. population [Nelson *et al.* 2009]. In prospective studies of the general population, prenatal exposure concentrations of PFOS or PFOA have been negatively associated with abdominal circumference and birth length of infants [Fei *et al.* 2007], negatively associated with concentrations of vaccine antibodies in vaccinated children [Grandjean *et al.* 2012], and positively associated with obesogenic effects in women at age 20 [Halldorsson *et al.* 2012]. Health effects associated with non-C_8 PFAAs have generally not been studied.

In response to concerns about human exposure to PFAAs, the U.S. EPA developed Provisional Health Advisories (PHAs) for PFOS and PFOA for drinking water in 2009 [U.S. EPA 2009] using critical animal studies with mice [Lau *et al.* 2006] and Cynomolgus monkeys [Seacat *et al.* 2002] for toxicological guidance. The PHA for PFOA, 400 ng/L, was developed from a study that fed CD-1 mice ammonium PFOA salt at different dosages by oral gavage [Lau *et al.* 2006]. A number of non-fatal toxicity endpoints were identified, the most sensitive of which was increased maternal liver weight at term. On the basis of this toxicity endpoint, a PFOA benchmark dose (BMD_{10}) of 0.46 mg/kg/day was established. The PHA for PFOS, 200 ng/L, was developed from a study that fed Cynomolgus monkeys potassium PFOS salt at different dosages by oral gavage [Seacat *et al.* 2002]. Toxicity endpoints observed in this study included mortality. Increased levels of thyroid-stimulating hormone in males, reduced total T3 levels in males and females, and reduced levels of high-density lipoproteins in females were each observed at the second to lowest dosage of PFOS fed to the monkeys, 0.15 mg/kg/day [Seacat *et al.* 2002]. The No Adverse Effect Level (NOAEL) for PFOS was set at 0.03 mg/kg/day. The Public Health Advisories were then computed from the BMD_{10} for PFOA and the NOAEL for PFOS on the basis of a 10 kg child drinking 1 L of water per day with 20% additional exposure from other sources, a bodily clearance rate corrected for species, and a safety factor of 10 [U.S.

EPA 2009]. Additionally, PFOS was added to the Stockholm Convention as an Annex B restricted persistent organic pollutant in 2009 and was restricted under the European Union's chemical regulation, REACH, in 2009 [Commission Regulation No 552/2009 2009] due to its toxicity to mammals and aquatic organisms.

1.3 Perfluoroalkyl Acid Precursors

1.3.1 Importance

Most fluorochemical research has focused on the PFAAs; however, as indicated in Section 1.2.2, the PFAAs account for a small percentage of the mass of fluorochemicals produced [Prevedouros *et al.* 2006, Paul *et al.* 2009] (Table 1.2). Many other fluorochemicals that contain perfluorinated moieties were and continue to be used in industrial processes and consumer and industrial products. These partially fluorinated compounds have the potential to undergo transformation processes that convert them into PFAAs. Because of this potential to produce PFAAs, these compounds, as well their partially fluorinated intermediate transformation products, will be referred to throughout this dissertation as PFAA precursors.

The PFAA precursors are a topic of interest for a number of reasons related to efforts to minimize exposure to PFAAs. First, the slow transformation of PFAA precursors represents a long-term source of PFAAs. Simply eliminating the production of specific PFAAs and PFAA precursors (*e.g.,* the elimination of C_8 product lines) may not result in the near term disappearance of those homologs from common exposure sources like food. Second, removing PFAAs from products may be ineffective at preventing PFAA exposure if PFAA precursors are still in commerce. Finally, efforts targeted at cleaning up point sources of PFAAs may be insufficient or even harmful with respect to reducing PFAA exposure if those remediation efforts do not consider the co-occurrence of PFAA precursors.

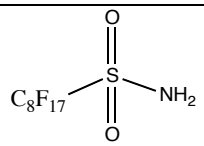
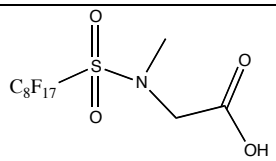
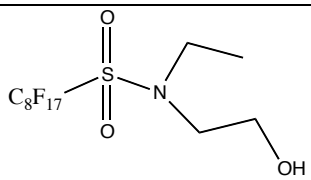
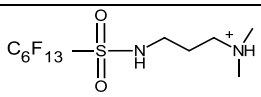
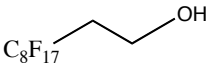
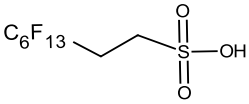
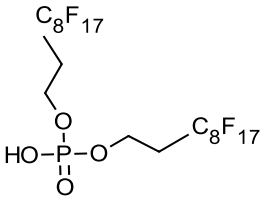
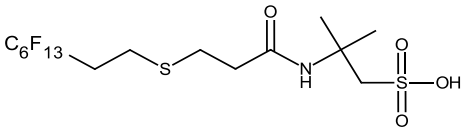
1.3.2 Types of PFAA Precursors

PFAA precursors consist of a perfluorinated carbon backbone with different alkyl moieties attached to the terminal carbon. Like the PFAAs, the perfluoroalkyl group can vary in length; the number n of carbons contained in the perfluorinated group is denoted by "C_n" or "n:2".

PFAA precursors are categorized by the chemical linkage connecting the perfluorinated group to the rest of the molecule. The two main categories of PFAA precursors are sulfonamide-containing precursors, which contain a $–SO_2N$ group bound to the perfluorinated chain and fluorotelomer precursors, which contain an ethyl group bound to the perfluorinated chain (Figure 1.5). Some common sulfonamide-containing and fluorotelomer PFAA precursors are shown in Table 1.4.

Figure 1.5: Generic n:2 fluorotelomer (left) and C_n sulfonamide-containing precursors (right). R is an alkyl group of non-specific structure.

Table 1.4: Examples of PFAA Precursors.

Sulfonamide-containing Precursors		
Name	Structure	Major Use
Perfluoro-1-octanesulfonamide (FOSA)		Transformation product of fluorinated surfactants
N-Methyl-perfluoro-1-octanesulfonamideoacetic acid (*N*-MeFOSAA)		Residual found in polymer products
2-*N*-Ethyl-perfluoro-1-octanesulfonamido ethanol (*N*-EtFOSE)		Polymer building block, residual found in polymer products
Perfluorohexane sulfonamido amine (PFHxSAm)		Firefighting foams
Fluorotelomer Precursors		
8:2 Fluorotelomer alcohol (8:2 FtOH)		Residual found in polymer products
6:2 Fluorotelomer sulfonate (6:2 FtS)		Intermediate transformation product of other surfactants
8:2 polyfluoroalkyl phosphate diester (8:2 diPAP)		Food contact paper
6:2 fluorotelomer thioamido sulfonate (6:2 FtTAoS)		Firefighting foams

Sulfonamide-containing precursors are manufactured mainly by the 3M Company. The perfluorinated part of the molecule is generated by electrolyzing octanesulfonyl chloride, or another alkyl sulfonyl chloride compound, in a hydrogen fluoride bath. This process consistently results in 30% branched and 70% linear perfluorinated products (*e.g.*, POSF and other homologs), an isomer distribution that is maintained in the subsequent production of sulfonamide-containing precursors [3M Company 1998]. Historically, POSF was further reacted with methyl or ethyl amines to form alkyl substituted amines, followed by reaction with ethylene carbonate to form alkyl-substituted sulfonamidoethanols. The methyl and ethyl-substituted perfluorooctane sulfonamidoethanols, *N*-MeFOSE and *N*-EtFOSE, were reportedly the building blocks of the 3M Company's C_8-based fluorochemical product lines [Martin *et al.* 2010]. Within a few years of its phase-out of C_8-based products in 2002, 3M reportedly reformulated their product lines to use C_4-perfluorinated chains with methyl and ethyl-substituted perfluorobutane sulfonamidoethanols as building blocks [Ehresman *et al.* 2007].

Unlike the sulfonamide-containing precursors, which are mainly manufactured by a single company, the fluorotelomer precursors are manufactured by a handful of Japanese and American companies. DuPont is one important U.S. manufacturer of fluorotelomer compounds, although its exact market share is unknown. In the telomerization process, C_2F_4 groups (taxogens) are added sequentially to C_2F_5I (the telogen), followed by the addition of an ethene-based terminal group [D'Eon and Mabury 2011]. Even numbered, linear perfluorinated groups are exclusively formed. The 6:2 and 8:2 fluorotelomer alcohols are used as starting materials to form many fluorotelomer-based polymers and surfactants.

PFAA precursor compounds as defined in this dissertation include manufactured chemical products (8:2 diPAP, 6:2 FtTAoS, PFHxSAm), residual starting materials (*N*-EtFOSE, 8:2 FtOH), and unintentional manufacturing byproducts and transformation products (*N*-MeFOSAA, FOSA, 6:2 FtS) (Table 1.4). Because all of these partially fluorinated compounds have the potential to form PFAAs, they are grouped together regardless of their purpose or lack thereof in commerce.

Within both categories of PFAA precursors, a variety of structural properties are observed. The primary fluorochemical building blocks of both precursor categories are volatile, neutral alcohols. However, sulfonamide-based and fluorotelomer PFAA precursors can contain anionic, cationic, and zwitterionic functional groups (Table 1.4). Some types of precursors, such as the polyfluoroalkyl phosphonates (PAPs) (Table 1.4), contain two perfluorinated moieties in a single molecule.

1.3.3 PFAA Precursor Occurrence

PFAA precursors have been detected in serum and aqueous samples by two different techniques. In the simplest technique, individual PFAA precursors have been measured in whole or extracted samples by LC-MS/MS. The second technique of PFAA precursor detection combines measurement of PFAAs by LC-MS/MS with the measurement of total organic fluorine in samples. Fluoride is measured before and after sample combustion using ion chromatography, and the total organic fluorine concentration is measured by the difference in fluoride concentration [Miyake *et al.* 2007a-b, Yeung *et al.* 2008]. The PFAA precursor concentration as fluoride can then be estimated by the concentration of total organic fluorine less the concentration of PFAAs that were separately measured. While the total organic fluorine method

yields no information about the identity of PFAA precursor compounds, it provides a better estimate of the total concentration of PFAA precursors in a sample, especially if the sample contains a high concentration of precursors that are not routinely measured by LC-MS/MS.

Analyses of human sera by both PFAA precursor detection methods suggest that PFAA precursors are present. For example, in human sera samples collected from five different cities in China in 2004, between 15% and 70% of the total extractable organic fluorine was not attributable to PFAAs [Yeung *et al.* 2008]. The remainder was attributed to PFAA precursors. In a more recent study, fifty human sera samples collected in the U.S. in 2009 were analyzed for PFAAs and a suite of 40 common precursors [Lee and Mabury 2011]. Both fluorotelomer and sulfonamide-containing precursors were detected in sera, but the average total concentration of PFAA precursors was between one and two orders of magnitude less than the average total concentration of PFAAs.

The exposure of humans to PFAA precursors can also be evaluated indirectly on the basis of PFAA temporal trends in sera. PFOS concentrations declined by more than 75% from 2000 to 2010 in sera samples from Germany and the U.S. [Yeung *et al.* 2013b, Olsen *et al.* 2012]. These PFOS declines coincide with reported human half-lives of PFOS [Olsen *et al.* 2007] and suggest that precursors either did not contribute significantly to PFOS concentrations in serum or declined at a similar rate. Stagnation and one to three-fold increases in the concentrations of C_9 to C_{12} perfluorinated carboxylates have been observed in German, American, and Scandinavian sera samples in the 2000's [D'Eon and Mabury 2011, Yeung *et al.* 2013a, Glynn *et al.* 2012]. Combined with manufacturing trends, these data suggest that the transformation of fluorotelomer compounds might be responsible for the concentrations of long-chain perfluorinated carboxylates. Similarly, PFOA concentration in sera have declined two- to three-fold from 2000 to 2010 in most countries [Jin *et al.* 2007, Olsen *et al.* 2012, Glynn *et al.* 2012, Yeung *et al.* 2013a-b], but these declines are approximately 50% slower than expected if all exposure to PFOA had been removed. Transformation of fluorotelomer compounds is believed to be responsible for the slower-than-expected decline in PFOA concentrations.

Individual PFAA precursors have typically been detected at less than 10% of the concentration of individual PFAAs in wastewater, urban runoff, and surface water (Table 1.3). Nonetheless, the total concentration of unmeasured PFAA precursors may be significant in these media. In a wastewater treatment plant in the U.S., the masses of both PFOS and the C_{10} perfluorinated sulfonate doubled from influent to effluent, a phenomenon that could not be explained by the transformation of four common C_8-sulfonamide-containing precursors that were concurrently measured [Schultz *et al.* 2006]. Production of these two perfluorinated sulfonates was most significant in the activated sludge and anaerobic digester processes, suggesting that PFAA precursors were transformed through biological processes to the perfluorinated sulfonates. A similar observation was made in groundwater, where PFOS concentrations were consistently higher than PFOS concentrations in the two most likely sources of contamination, wastewater and urban runoff [Murakami *et al.* 2009a]. A companion study suggested the presence of significant concentrations of PFOS precursors in urban runoff, demonstrated by an increase in the concentration of PFOS by approximately 60% upon passage of runoff through a saturated soil column [Murakami *et al.* 2008]. Finally, the concentration of PFOA measured in a lake near Albany, NY was higher than predicted by estimates based on PFOA concentrations in the known sources, suggesting an unmeasured PFOA precursor source [Kim and Kannan 2007].

1.3.4 Abiotic Transformation of PFAA Precursors

Select PFAA precursors are amenable to acid- and base- catalyzed hydrolysis. Over a pH range of 1.5 to 11, hydrolysis half-lives on the order of months were observed for perfluorooctane sulfonamido ethanol acrylates [Martin *et al.* 2010]; hydrolysis was proposed to occur at the ester moieties, yielding smaller sulfonamide-containing precursors. Production of perfluorinated sulfonates from hydrolysis of sulfonamide-containing precursors is not expected to occur because the sulfonamide moiety is unlikely to undergo abiotic hydrolysis [Martin *et al.* 2010]. Fluorotelomer phosphate mono and di esters were not susceptible to hydrolysis reactions at pH 9 and 50°C over a two-week period [D'Eon and Mabury 2007], and sulfonamide-based phosphate esters are not expected to readily hydrolyze either [Martin *et al.* 2010].

In the gas phase, both C_n fluorotelomer and sulfonamide-based precursors react with chlorine radicals and hydroxyl radicals, forming a mixture of smaller precursor compounds and a homologous series of C_{n+1} and shorter perfluorinated carboxylates [Ellis *et al*. 2004, Martin *et al.* 2005, D'Eon *et al.* 2006, Jackson *et al.* 2013]. The C_4 perfluorinated sulfonate was generated by reaction of *N*-ethyl perfluorobutane sulfonamido ethanol with hydroxyl radicals, albeit at one tenth of the yield of C_4 and C_5 perfluorinated carboxylates [D'Eon *et al.* 2006]. The formation of perfluorinated sulfonates was not observed in other smog chamber experiments with sulfonamide-containing precursors such as N-ethyl perfluorobutane sulfonamide [Martin *et al.* 2005]. The transport and transformation of fluorotelomer alcohols in the troposphere is thought to be partially responsible for the presence of C_8 (PFOA), C_{10} and C_{12} perfluorinated carboxylates in the Arctic [Ellis *et al*. 2004, Armitage *et al.* 2009].

Aqueous reactions of C_n fluorotelomer and sulfonamide-containing precursors with hydroxyl radical generated polyfluorinated products and perfluorinated C_{n+1} and C_n carboxylates [Plumlee *et al.* 2009, Gauthier and Mabury 2005]. For reactions with hydroxyl radical in natural waters, half-lives of fluorotelomer compounds were estimated to be on the order of days to weeks [Gauthier and Mabury 2005] while half-lives of sulfonamide-containing precursors were estimated to be slower, on the order of weeks to months [Plumlee *et al.* 2009].

As precursors are more susceptible to oxidative and reductive reactions than PFAAs, subjecting precursors to engineered processes capable of degrading PFAAs would also likely lead to the transformation of precursors.

1.3.5 Biological Transformation of PFAA Precursors

The oxidative microbial transformation of fluorotelomer precursors has been observed in the presence of microbes from activated sludge reactors [Wang *et al.* 2005, Lee *et al.* 2010, Wang *et al.* 2011], aerobic soils [Wang *et al.* 2009, Liu *et al.* 2010, Dasu *et al.* 2012], and enrichments of ethanol-degrading bacteria [Dinglasan *et al.* 2004]. Within days to weeks, the aerobic transformation of n:2 fluorotelomer precursors consistently results in production of C_n perfluorinated carboxylates (Table 1.5), n:2 fluorotelomer unsaturated and saturated carboxylates, and (n-1):3 fluorotelomer carboxylates (Figure 1.6) [Dinglasan *et al.* 2004, Wang *et al.* 2005, Wang *et al.* 2009, Lee *et al.* 2010, Lee *et al.* 2010, Liu *et al.* 2010, Wang *et al.* 2011, Dasu *et al.* 2012]. The C_{n+1}, $C_{n-1,}$ and C_{n-2} perfluorinated carboxylates (Table 1.5) have also been observed as products of microbial transformation, demonstrating that partial defluorination of the

perfluorinated chain also occurs (Figure 1.6) [Wang *et al.* 2005, Wang *et al.* 2009, Lee *et al.* 2010, Lee *et al.* 2010, Wang *et al.* 2011, Dasu *et al.* 2012]. Because the potential for PFAA bioaccumulation increases with the length of the perfluorinated chain [Conder *et al.* 2008], microbial defluorination of PFAA precursors could diminish the bioaccumulation of their environmental transformation products.

Recently, the transformation of a fluorotelomer PFAA precursor under anaerobic conditions was reported. Anaerobic digester sludge amended with 6:2 and 8:2 fluorotelomer alcohols and incubated under methanogenic conditions for two to five months [Zhang *et al.* 2013] yielded n:2 fluorotelomer saturated and unsaturated acids and trace amounts of perfluorinated carboxylates (Table 1.5) [Zhang *et al.* 2013].

Although perfluorinated carboxylates were produced by microbial transformation of fluorotelomer compounds, they never accounted for more than 10% of the mass of the transformed precursor, per perfluorinated moiety (Table 1.5). Therefore, it is likely that precursors were formed that could not be detected with existing analytical methods. Most studies indicated that the fluorotelomer acid transformation products had significantly longer half-lives than their parent compound and that their slow transformation limited the rate of formation of the perfluorinated carboxylates.

Only one study has addressed the microbial transformation of sulfonamide-containing precursors [Rhoads *et al.* 2008]. PFOS was a terminal product of five different perfluorooctane sulfonamide precursors in ten-day activated sludge microcosm experiments [Rhoads *et al.* 2008]. Individual PFOS yields ranged from 5% to 40% of the moles of precursor transformed. These results indicate a possible pathway for the production of PFOS in wastewater treatment plants [Schultz *et al.* 2006].

In addition to microbial biotransformation of PFAA precursors, biotransformation of PFAA precursors occurs in fish and rats dosed with PFAA precursors. The 8:2 fluorotelomer unsaturated and saturated carboxylates and the 7:3 fluorotelomer saturated carboxylate were converted to PFOA and the C_7 carboxylate in Rainbow trout fed commercial fish food amended with these compounds [Butt *et al.* 2010]. Fluorotelomer alcohols and fluorotelomer polyfluoroalkyl phosphates were transformed to perfluorinated carboxylates in rats [Martin *et al.* 2005, D'Eon and Mabury 2007]. In *vivo* rat studies documented the conversion of perfluorooctane sulfonamides to PFOS [Martin *et al.* 2010].

Fluoropolymer materials might also act as precursors to PFAAs. The slow transformation of fluoropolymers has been studied in aerobic soil systems [Russell *et al.* 2008, Russell *et al.* 2010a, Washington *et al.* 2009]. Based on PFOA formation, biodegradation half-lives varying over a wide range, from 10-17 years [Washington *et al.* 2009] up to 1200-1700 years [Russell *et al.* 2008, Washington *et al.* 2009, Russell *et al.* 2010a] were observed for several fluorotelomer-based polymers. In one study, disappearance of PFOA was also reported [Washington *et al.* 2010a], but this finding was called into question by the other major research group publishing on the biodegradation of fluoropolymers [Russell *et al.* 2010b]. Despite their low reported reactivity, fluoropolymers could be a very important source of PFOA and PFOS due to the relatively large quantities produced [Prevedouros *et al.* 2006, Paul *et al.* 2009].

Table 1.5: Production of perfluorinated carboxylates (PFCAs) from the microbial transformation of fluorotelomer PFAA precursors.

Type of PFAA Precursor	Inoculum	Reference	Duration	Product Percentage Relative to Initial PFAA Precursor Concentration						
				PFBA (C4)	PFPeA (C5)	PFHxA (C6)	PFHpA (C7)	PFOA (C8)	PFNA (C9)	Total PFCAs
8:2 FtOH	Ethanol-degrading mixed microbial enrichment	Dinglasan *et al.* 2004	90 days					3%		3%
8:2 FtOH	Activated sludge	Wang et *al.* 2005	90 days			1%		6%	<0.2%	7%
6:2 mono-PAP	Wastewater mixed liquor	Lee *et al.* 2010	90 days		<1%	2%	8%			10%
6:2 di-PAP	Wastewater mixed liquor	Lee *et al.* 2010	90 days		<1%	6%	7%			13% (6.5%)
6:2 FtOH	Soil	Liu *et al.* 2010	84 days	0.8%	4.2%	4.5%				9.5%
6:2 FtS	Activated sludge	Wang *et al.* 2011	90 days	0.14%	1.5%	1.1%				2.7%
8:2 Ft stearate monoester	Soil	Dasu *et al.* 2012	80 days			0.16%	0.4%	1.7%		2.3%
6:2 FtOH	Anaerobic digester sludge	Zhang *et al.* 2013	200 days			<0.4%				<0.4%
8:2 FtOH	Anaerobic digester sludge	Zhang *et al.* 2013	200 days					0.3%		0.3%

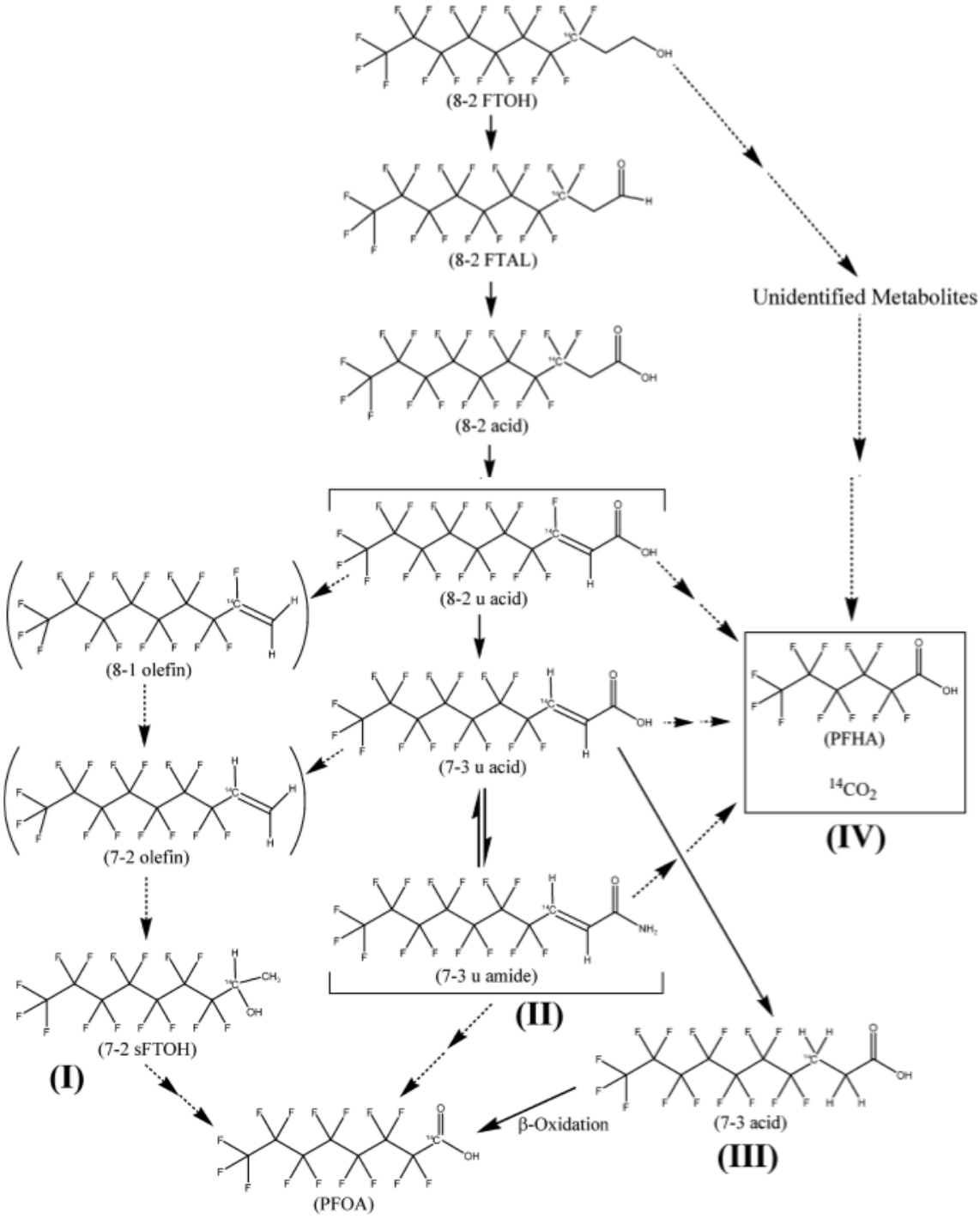


Figure 1.6: Proposed biotransformation pathway of ^{14}C-8:2 FtOH in activated sludge [Wang *et al.* 2005].

1.3.6 Analytical Limitations of PFAA Precursor Measurement

Analytical standards are commercially available for nine classes of PFAA precursors. C_6 and C_8 homologs of six classes of fluorotelomer precursors are available as analytical standards, as well as C_4 and C_{10} homologs for some of those classes. Standards are available for three classes of C_8 sulfonamide-containing precursors, while no shorter or longer precursor homologs are available. Isotopically-labeled versions of at least one homolog of all of these types of PFAA precursors are available. Most of these standards have become available since 2008. Due to the high price and low quantities of individual standards that are for sale, it is not yet possible to conduct high concentration (*i.e.*, >100 µg/L) experiments.

While the commercially available standards encompass many of the reported PFAA precursor compounds in commerce and the environment, they are not exhaustive. For example, no PFAA precursors identified in AFFF formulations [Place and Field 2012] are commercially available. Sulfonamide-based precursors that resemble the fluorotelomer di-PAP compounds (Table 1.4), *N*-EtFOSE-based phosphate di-esters, are also not available. Many intermediate, polyfluorinated molecules reported in the transformation of fluorotelomer and sulfonamide-containing precursors do not have standards commercially available. The measurement of PFAA compounds that do not have commercial analytical standards has been conducted with standards synthesized by chemists at fluorochemical manufacturing companies, or by using commercial reference materials that are not quantitatively certified. Finally, there are very limited numbers of standards available for the PFAA precursors containing shorter perfluorinated chain lengths that are reported to have replaced their C_8 analogs. It is not possible to determine how many PFAA precursors are in commerce or in the environment that do not have commercially available analytical standards, but it is likely that standards are only available for a small fraction of the possible precursors.

Combustion ion chromatography has been used to measure total organic fluorine in human sera and seawater samples [Miyake *et al.* 2007a-b, Yeung *et al.* 2008]. Coupled with direct measurement of PFAAs, the total organic fluorine concentration can be used to estimate an upper limit for the concentration of PFAA precursors in a sample. However, this approach has several drawbacks. First, it cannot distinguish between fluorinated compounds that do not contain a perfluorinated moiety. Second, it provides no information about the homologs or types of precursors present. Finally, this method is limited by the IC detection limit of fluoride, which is typically not higher than 1 µg F/L, or 1.3 µg/L PFOA equivalents [Miyake *et al.* 2007a-b].

1.4 Research Objectives

1.4.1 Objective 1 – Assess the occurrence of PFAA precursors in urban runoff samples.

Previous research suggests that PFAA precursors are present at concentrations similar to PFAAs in urban runoff [Murakami *et al.* 2008, Murakami *et al.* 2009a, Kim and Kannan 2005], but identifying the types of PFAA precursors present in runoff was difficult due to the limited availability of analytical standards. To assess the potential importance of PFAA precursors, a new oxidation-based method was developed to indirectly measure the total concentration of PFAA precursors in aqueous samples. The method involves the use of hydroxyl radical to convert PFAA precursors to readily measured perfluorinated carboxylates.

The oxidation-based precursor measurement technique was employed in the quantification of PFAA precursors in urban runoff collected around the San Francisco Bay Area. Samples were obtained from ten watersheds with varying amounts of residential, industrial, and commercial activities. The samples were also analyzed for a suite of fluorochemicals. The results of the assay and direct analysis of fluorochemicals were used to identify the most prevalent types of PFAAs and PFAA precursors present in runoff and to determine the extent to which runoff could serve as a source of fluorochemical contamination in aquifers and reservoirs.

1.4.2 Objective 2 – Evaluate the persistence of AFFF-derived PFAA precursors at an AFFF-impacted firefighter training area.

In groundwater below unlined firefighter training areas where AFFF was repeatedly applied, some of the highest aqueous concentrations of PFAAs and PFAA precursors have been reported [Moody *et al.* 2003, Schultz *et al.* 2004]. However, information about the types of PFAA precursors in AFFF was not available until recently. The persistence of these compounds in the subsurface was also largely unknown.

To gain a better understanding of the long-term fate of PFAAs and PFAA precursors in AFFF, contaminated groundwater, soil, and aquifer solids were collected from an area near an unlined firefighter training area at a U.S. Air Force Base where AFFF was used from 1970 to 1990. These samples were analyzed for a suite of fluorochemicals, including probable transformation products, and were subjected to a precursor assay modified from the one used for runoff samples developed in Objective 1. Additionally, AFFF formulations from five AFFF manufacturers spanning three decades of production were analyzed for PFAAs and PFAA precursors. Transformation of AFFF-derived PFAA precursors to terminal PFAA products in the training area was estimated by comparing the AFFF samples to the environmental samples.

1.4.3 Objective 3 – Assess the transformation of AFFF formulations in soil and sediment microcosms under aerobic and anaerobic conditions.

To gain a better understanding of the microbially-mediated transformation of AFFF under different redox conditions, laboratory microcosms were prepared using soil and sediment inocula. Bottles containing mineral salt medium amended with AFFF formulations were incubated with live inocula and appropriate electron acceptors to create aerobic, nitrate-reducing, sulfate-reducing, iron-reducing, and methanogenic conditions. Autoclaved and medium controls were also prepared to distinguish between abiotic and biological processes. Consumption of dissolved organic carbon and electron acceptors was monitored. Production of fluorochemical transformation products from PFAA precursors was measured using LC-MS/MS for compounds for which analytical standards were available. The structures of transformation products in microcosms slurries for which standards were not available were identified using LC-MS/MS and were confirmed with high resolution mass spectrometry. A modified precursor assay was used to track the concentration of PFAA precursors in microcosm slurries over the incubation. Results from microcosm experiments were used to predict the fluorochemical transformation products in the subsurface at AFFF-impacted sites and to determine the conditions that may have led to the production of previously observed transformation products.

CHAPTER 2. Oxidative Conversion as a Means of Detecting Precursors to Perfluoroalkyl Acids in Urban Runoff

Reproduced with permission from Houtz, E.H.; Sedlak, D.L. Oxidative conversion as a means of detecting precursors to perfluoroalkyl acids in urban runoff. *Environ. Sci. Technol.* **2012**, *46*, 9342-9349.

© 2012 American Chemical Society.

2.1 Introduction

For more than fifty years, perfluoroalkyl and polyfluoroalkyl substances (PFASs) have been manufactured and used as surfactants, processing aids, and oil and water repellent coatings in consumer products and industrial applications [Prevedouros *et al.* 2006]. Two classes of recalcitrant PFASs, the perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs), have been widely detected in human sera [Kato *et al.* 2011, Kannan *et al.* 2004, Lee and Mabury 2011], wildlife [Houde *et al.* 2011], municipal wastewater [Schultz *et al.* 2006, Boulanger *et al.* 2005, Ahrens *et al.* 2009], and surface waters [Ahrens *et al.* 2009, Huset *et al.* 2008, Zushi and Masunaga 2009b, Zushi *et al.* 2008]. PFCAs and PFSAs enter the environment from direct emission and through transformation of precursor compounds [Prevedouros *et al.* 2006]. PFASs containing 8-carbon (C8) perfluoroalkyl chains were historically produced in the largest quantities, leading to widespread distribution of the C8 perfluoroalkyl acids, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) [Kato *et al.* 2011, Kannan *et al.* 2004, Lee and Mabury 2011, Houde *et al.* 2011, Schultz *et al.* 2006, Boulanger *et al.* 2005, Ahrens *et al.* 2009, Huset *et al.* 2008, Zushi and Masunaga 2009b, Zushi *et al.* 2008]. Concern over the potential health effects of PFOS and PFOA on humans and wildlife resulted in a manufacturing shift in the early 2000s towards PFASs containing shorter perfluoroalkyl chains that have a lower potential for bioaccumulation [Conder *et al.* 2008].

Urban runoff is a significant source of PFASs in surface waters. For example, urban runoff and wastewater effluent were estimated to contribute approximately equal masses of PFASs to rivers in urbanized regions of Japan [Zushi and Masunaga 2009b]. PFASs also have been detected in urban runoff and runoff-receiving waters in Zürich [Müller *et al.* 2011], Albany, New York [Kim and Kannan 2007], Toronto [Meyer *et al.* 2011], and Singapore [Nguyen *et al.* 2011].

Most prior efforts to quantify PFASs in runoff and runoff-receiving waters have focused on the PFSAs and PFCAs and a small number of C8- perfluoroalkyl sulfonamide-containing fluorochemicals, such as perfluorooctanesulfonamide (FOSA) and 2-(*N*-methylperfluorooctanesulfonamido) acetic acid (*N*-MeFOSAA). Measured concentrations of C8 perfluoroalkyl sulfonamides in runoff and surface waters were typically one to two orders of magnitude lower than those of PFOS and PFOA [Kim and Kannan 2007, Meyer *et al.* 2011, Nguyen *et al.* 2011]. In Singaporean surface waters receiving wet weather discharge, similar concentrations of 6:2 fluorotelomer sulfonate (6:2 FtS) and individual PFCA congeners were detected [Nguyen *et al.* 2011]. To date, 8:2 and shorter fluorotelomer compounds have rarely been measured in runoff.

Despite low concentrations of C8 perfluoroalkyl sulfonamides in runoff, results from several studies have suggested that polyfluorinated substances that can be converted to PFCAs and PFSAs might be present in runoff at appreciable concentrations [Kim and Kannan 2007, Murakami *et al.* 2009a, Murakami *et al.* 2008]. For example, PFOS concentrations in Tokyo groundwater were consistently higher than PFOS concentrations measured in the two most likely sources of contamination, wastewater and urban runoff [Murakami *et al.* 2009a]. Supporting the hypothesis that compounds in runoff were converted into PFOS during groundwater infiltration, the concentration of PFOS in street runoff increased when the runoff was infiltrated through a soil column [Murakami *et al.* 2008]. Similarly, concentrations of PFOA detected in a lake near

Albany, NY could not be explained by mass balance calculations that accounted for the contributing sources of the compound [Kim and Kannan 2007].

Polyfluorinated substances present in the environment that are transformed to the perfluoroalkyl acids (PFAAs, *i.e.,* PFCAs and PFSAs) by natural processes are referred to as PFAA precursors. While sulfonamide-containing PFAA precursors (*e.g.,* FOSA and *N*-MeFOSAA) are often monitored, many types of potential precursors [Lee and Mabury 2011, Ahrens 2011], including polyfluoroalkyl phosphate mono and di esters (mono- and diPAPs), fluorotelomer sulfonates and alcohols, and fluorinated polymers, have not been routinely measured in runoff due to a lack of analytical standards or challenges associated with available analytical techniques. Furthermore, the PFAS composition of some products that likely contain an array of PFAA precursors are proprietary and require advanced mass spectrometry techniques to identify (*e.g.* newly-identified PFAA precursors in aqueous-film forming foams [Place and Field 2012]).

The objective of this study was to quantify the total concentrations of PFAA precursors containing specific perfluorinated chain lengths in urban runoff. The method employed to measure PFAA precursors used hydroxyl radical to oxidize precursors to PFCA products. By comparing PFCA concentrations before and after oxidation, the concentrations of chain length-specific PFAA precursors were inferred. The method developed in this study offers several advantages over existing approaches for measuring the total concentration of fluorine-containing compounds by combustion ion chromatography [Miyake *et al.* 2007a, Miyake *et al.* 2007b, Yeung *et al.* 2008]. It is specific for compounds containing a perfluorinated functional group, it is extremely sensitive, and it does not require specialized equipment other than an HPLC-MS/MS system. The PFAA precursor technique was applied to urban runoff samples collected from the San Francisco Bay Area to provide insight into the potential for urban runoff to serve as a source of PFCAs and PFSAs in receiving systems.

2.2 Materials and Methods

2.2.1 Materials

All analytical standards of PFSAs, PFCAs, PFAA precursors, and stable-isotope surrogates, were purchased from Wellington Laboratories (Table A2.1). HPLC-grade water, methanol and 99% purity potassium persulfate were purchased from Fisher Scientific. All other chemicals and solvents were of highest possible purity and were purchased from Fisher Scientific or Sigma Aldrich.

2.2.2 Stormwater collection

Urban runoff samples were collected between November 2010 and March 2011 at sites around the San Francisco Bay (SF Bay), California. Information on watershed characteristics associated with each sampling site and the amount of precipitation for each sampling event are included in Table 2.1. A total of thirty-three samples from twelve storms and ten sites were analyzed. Samples were collected during the rise, peak, and fall of the storm hydrograph. Sampling locations were situated in storm drains and streams that received runoff from a range

of watershed types, including low-density suburban housing developments, high-density urban mixed-use areas, commercial shopping districts, and areas zoned for industrial activity.

Samples were collected from the center of the runoff channel with a stainless steel bailer. Samples were decanted into methanol-rinsed 1-L HDPE bottles and kept on ice for no more than twelve hours. Upon their return to the laboratory, samples were stored at 4°C until analysis, which occurred within three months. Care was taken to avoid the use of any PTFE-coated materials. At each site, a field reagent blank was prepared from HPLC-grade water that was transferred to the sampling bailer and poured into a clean sampling bottle.

Table 2.1: Watershed and storm characteristics for each watershed sampled.

Site	Drainage Area (km^2)	Imperv-ious Surfaces	Land Use				Rainfall over Sampling Period (cm)	Type of Sampling Site	Approx. Latitude and Longitude
			Open	Residential	Industrial	Commercial			
1	7.2	27%	10%	53%	23%	14%	2.49	Creek	37.52° N 122.27° W
2	50.1	44%	15%	46%	18%	20%	2.08	Creek	37.42° N 121.99° W
2	4.2	75%	10%	28%	34%	28%	2.13	Storm Drain	37.83° N 122.29° W
4	5.4	39%	30%	39%	21%	10%	2.84	Creek	37.81° N 122.26° W
5	73	11%	74%	16%	7%	3%	2.21	Creek	37.95° N 121.69° W
6	125	13%	66%	25%	5%	3%	0.78, 3.4	Creek	37.67° N 122.16° W
7	8	33%	36%	31%	11%	21%	0.30, 2.44	Channel	37.63° N 122.09° W
8	30.5	38%	30%	44%	19%	7%	0.69	Creek	37.32° N 121.97° W
9	108	33%	28%	45%	16%	11%	2.08	Creek	37.39° N 121.97° W
10	319	17%	43%	41%	9%	6%	3.28	Creek	37.90° N 122.06° W

2.2.3 PFAA - Precursor Oxidation

Several common PFAA precursors containing a C8-perfluorinated chain, including several C8 sulfonamide compounds and 8:2 fluorotelomer alcohol, have been shown to partially transform to PFOA via hydroxyl radical-mediated reactions in aqueous solutions [Plumlee *et al.* 2009, Gauthier and Mabury 2005]. These experiments have also demonstrated that PFOA is not oxidized at an appreciable rate by hydroxyl radical (•OH). In the PFAA precursor method described herein, an excess of hydroxyl radical was generated in samples to fully convert PFAA precursors to PFCAs. Although some PFAA precursors (*e.g.* the perfluorinated sulfonamides) are transformed to PFSAs by microbial and biological processes, reactions with •OH under the conditions used here result in formation of PFCAs.

Hydroxyl radical was produced by thermolysis of persulfate ($S_2O_8^{2-}$) under basic pH conditions. This approach offered practical advantages over other approaches (*e.g.,* Fenton's Reagent, UV/H_2O_2) because it was simple, did not require specialized equipment, and had minimal impact on subsequent analysis. At pH values above 12, thermolysis rapidly converts persulfate into sulfate radical (SO_4^{-}•), which is then quickly converted into •OH [Tsao and Wilmarth 1959]:

$$S_2O_8^{2-} + \text{heat} \rightarrow 2\ SO_4^{-}\bullet \qquad \text{Reaction 1}$$

$$SO_4^{-}\bullet + OH^{-} \rightarrow SO_4^{2-} + \bullet OH \qquad \text{Reaction 2}$$

Unfiltered stormwater samples were inverted several times before transferring 125-mL aliquots to 125-mL HDPE bottles. After filling, the bottles had less than 2 mL of headspace. For each sample, one aliquot was used as a control (*i.e.,* it was heated without persulfate or NaOH addition) and at least one aliquot was amended with 2 g (60 mM) potassium persulfate and 1.9 mL of 10 N NaOH (150 mM). One sample from each site was sub-sampled in triplicate bottles amended with potassium persulfate and NaOH. While sulfate radical can react directly with PFOA [Hori *et al.* 2005], its conversion to •OH is much faster than its reaction with PFOA at elevated pH values. The HDPE bottles were placed in a temperature-controlled water bath at 85°C for six hours, which resulted in a reduction in concentration of persulfate of approximately 95% [Johnson *et al*. 2008]. Samples were cooled to room temperature in an ice bath prior to analysis.

2.2.4 Analysis

Concentrations of PFASs were quantified before and after oxidation by HPLC/MS-MS. Three sets of bottles (*i.e.,* untreated samples, thermolyzed controls and persulfate-treated samples) were amended with 50 µL of 20 µg/L isotopically-labeled surrogate standards in a methanol stock solution. Concentrated HCl was used to adjust the pH of the samples that had been subjected to persulfate treatment to a value between 5 and 9 prior to extraction.

Samples were subjected to solid phase extraction (SPE) (Oasis WAX SPE cartridges, 6 cm^3, 150 mg, 30 µm; Waters, Milford, MA) as described by Taniyasu *et al*. [2005]. Cartridges were pre-conditioned with 4 mL each of 0.1% NH_4OH in methanol, methanol, and HPLC-grade water. Samples were pulled through the SPE cartridges under vacuum using stainless steel tubing

connected to a Supelco SPE manifold with all PTFE components removed. After extraction, cartridges were rinsed with 4 mL HPLC-grade water and dried under vacuum for approximately one hour.

Following the drying step, samples were eluted into 12-mL glass vials with 2 mL methanol followed by 2 mL 0.1% NH_4OH in methanol. Samples were evaporated to dryness under nitrogen and resuspended in 500 µL of 1:1 water: methanol before analysis. An extraction blank consisting of HPLC-grade water amended with the isotope-labeled surrogate standards was included for every 24 samples in addition to field blanks and reaction blanks. Contamination above the method detection limits (Table A2.2) was not detected in any blanks. Total method detection limits ranged between 0.1 and 0.5 ng/L (Table A2.2).

Spiking experiments in stormwater and HPLC-grade water yielded surrogate standard-relative recoveries ranging between 90% and 105% for all compounds except PFPeA, PFDoA, PFTeA, PFBS, PFDS, and FOSA whose recoveries were between 60% and 80%, and 8:2 FtS, whose recoveries ranged from 133% - 146% (Tables A2.3, A2.4). Recovery of 6:2 fluorotelomer sulfonate (6:2 FtS) was between 200% and 300%. All concentrations are reported without correction for recovery and the results of 6:2 FtS analysis are only discussed in the text in a qualitative fashion since 6:2 FtS recovery was unacceptably high.

All samples were analyzed on an Agilent HPLC coupled with an Agilent 6410 triple quadrupole mass spectrometer operating in negative electrospray ionization mode. The HPLC was modified to minimize background contamination by replacing accessible PTFE lines with PEEK tubing. The solvent degasser was bypassed and solvents were degassed offline. A Zorbax SB C-18 pre-column was attached to the aqueous mobile phase and placed before the aqueous and organic phase mixing point to remove background perfluorinated compounds. The pre-column was eluted approximately every fifty samples to prevent breakthrough of retained perfluorinated compounds.

A gradient solvent program was operated with 10 mM ammonium acetate in methanol and 10 mM ammonium acetate in HPLC-grade water. Solvent was delivered at a rate of 0.4 mL/min. Methanol was ramped from 10% to 90% over 6 minutes, held at 90% for 6 minutes, and brought back to 10% over one minute and held for three minutes. A slightly modified solvent program (*i.e.*, methanol ramped to 95% rather than 90%) was used when diPAP compounds were analyzed. Chromatographic separation was achieved on a Zorbax Eclipse XCB-C18 4.6 x 50 mm, 1.8 µm column placed after a 2-µm inline filter and a 4.6 x 12.5 mm, 5 µm Eclipse XDB-C18 guard column.

One or two multiple-reaction-monitoring (MRM) transitions were monitored per analyte and most analytes were quantified using isotope dilution (Table A2.5). Analytes possessing multiple isomers were quantified as a single analyte with a single isomer standard. Extracted stormwater samples and samples from high concentration (>1 µg/L) PFAA precursor oxidation experiments were measured using an injection volume of 100 µL while samples from low concentration (20 to 100 ng/L) PFAA precursor oxidation experiments employed 750 µL injections. To achieve the larger injection volume, an additional 700 µL of stainless steel and PEEK tubing was added to the needle seat.

2.3 Method Validation

The PFAA precursor oxidation method was validated with experiments using representative C8 and C6 PFAA precursors: FOSA, *N*-EtFOSAA, *N*-MeFOSAA, 8:2 FtS, 8:2 diPAP, 6:2 FtS, and 6:2 diPAP. The concentrations of precursors used in control experiments ranged from 25 ng/L to 25 µg/L (Table 2.2). This range was selected to test the performance of the method over a range of environmentally-relevant concentrations. An aliquot of a concentrated stock solution of each precursor in methanol was added to each HDPE bottle and dried under a gentle stream of nitrogen to remove the methanol. PFAA precursors were resuspended in 125 mL of HPLC-grade water or stormwater and pre-heated for 30 minutes at 60°C to enhance dissolution of the evaporated precursor before removing a 750-µL aliquot from each bottle. The aliquot was analyzed to determine the initial concentration of the precursor. Bottles were then amended with varying concentrations of potassium persulfate and enough sodium hydroxide to maintain a pH above 12. The samples were placed in a temperature-controlled water bath at 85°C for six hours. After cooling to room temperature and neutralizing the excess base with concentrated HCl, concentrations of PFAA precursors and PFAA products were quantified.

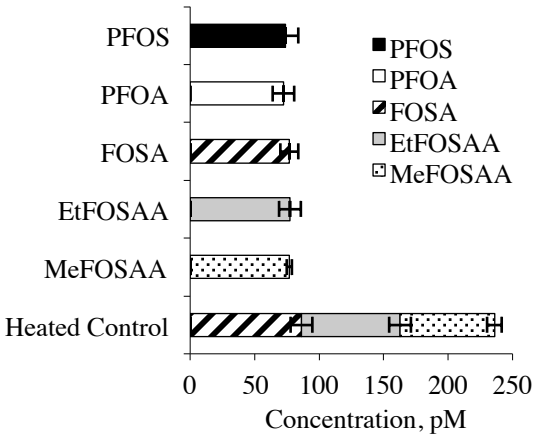
The diPAPs did not dissolve in solution after addition of water and pre-heating. To reliably measure the initial concentration of diPAP compounds, a duplicate set of bottles was filled with a 50% methanol and 50% water mixture after the evaporation step and an aliquot was removed from each bottle for LC/MS-MS analysis. Measured initial concentrations in the methanol-water mixture had a relative standard deviation of less than 10% across replicates. The average concentration was used for mass balance calculations.

2.4 Results and Discussion

2.4.1 Oxidation Control Experiments

Quantitative conversion of C8 sulfonamide-containing precursors (FOSA, *N*-EtFOSAA, *N*-MeFOSAA) to PFOA was observed in HPLC-grade water (Figures 2.1-2.2, Table 2.2) and urban runoff (Figure 2.3). Controls consisting of PFOA and PFOS demonstrated that the perfluoroalkyl acids were stable under the conditions used to oxidize the PFAA precursors (Figure 2.1). Heated controls containing a suite of sulfonamide precursors without added persulfate indicated no loss of precursors upon heating (Figure 2.1). The presence of NaOH without added persulfate did not affect the concentrations of precursors during heating and NaOH addition was not used in subsequent heated controls. Several stormwater samples were subjected to treatment with sequential 10 mM aliquots of persulfate. Addition of a second or third 10 mM aliquot of persulfate did not affect the concentration of PFOA or PFOS, indicating that all of the precursors were converted to PFOA after addition of the first aliquot. To ensure complete conversion of precursors in stormwater samples, a total of 60 mM of persulfate was used in subsequent runoff samples.

a



b

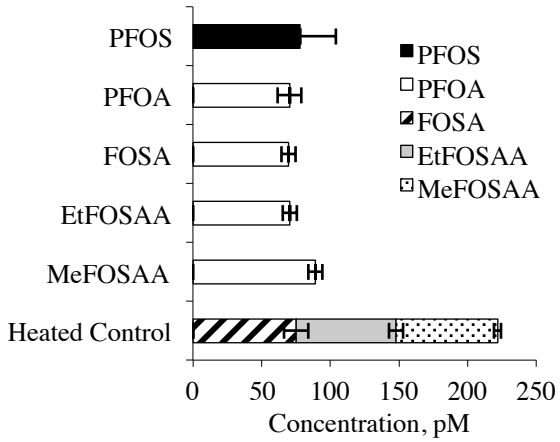
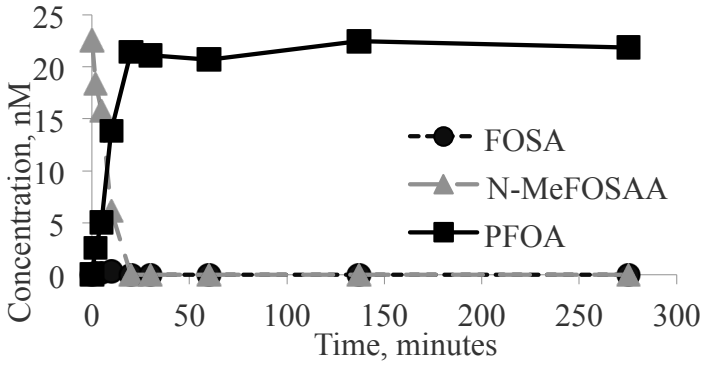


Figure 2.1 a-b: Concentrations of C8 perfluorooctane sulfonamide-containing precursors and PFOS and PFOA in individual HPLC-grade water reactors (n=4 per compound) before (a) and after (b) oxidation with 20 mM persulfate and 50 mM NaOH; heated precursor controls did not contain persulfate or NaOH addition.

a



b

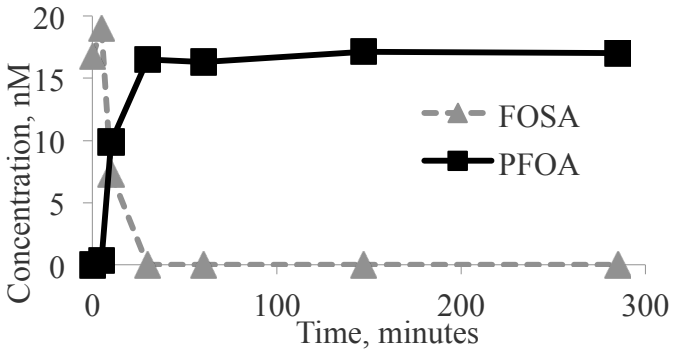


Figure 2.2 a-b: Conversion of *N*-MeFOSAA (a) and FOSA (b) in HPLC –grade water in the presence of 5 mM persulfate and 50 mM NaOH over time at 85°C.

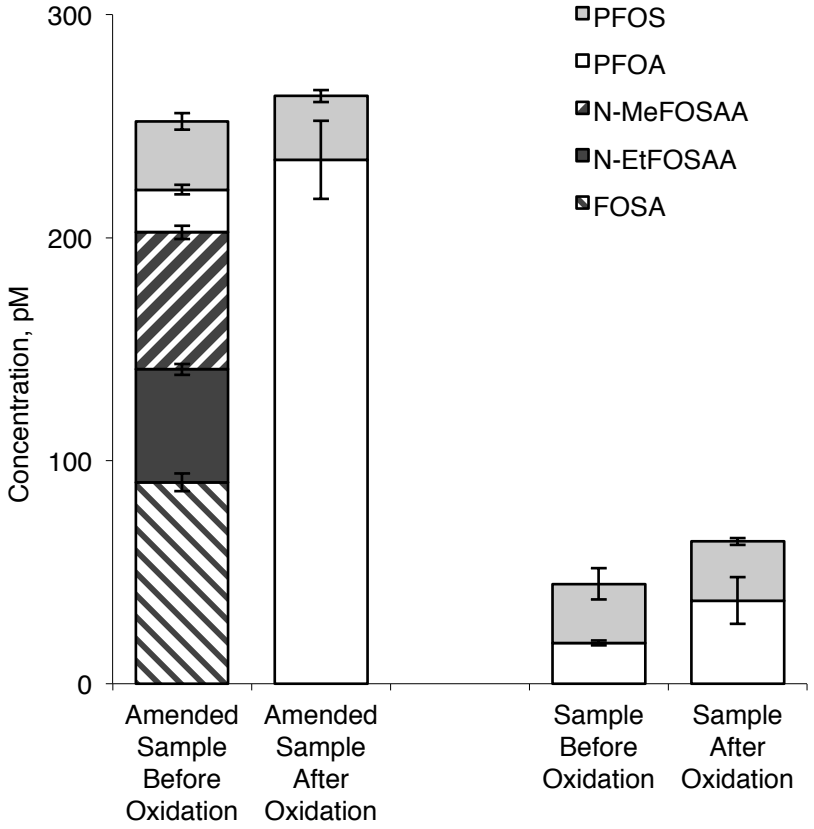


Figure 2.3: PFAS concentrations before and after oxidation of a stormwater sample, amended and unamended, with 30 mM persulfate. The amended sample (left) was spiked with *N*-MeFOSAA, *N*-EtFOSAA, and FOSA prior to oxidation. The stormwater sample was collected from a stream receiving urban runoff in San Jose, CA in January 2010.

Oxidation of solutions of the fluorotelomer precursors, 6:2 and 8:2 FtS and 6:2 and 8:2 diPAP, each produced a suite of PFCAs of varying chain lengths (Figures 2.4-2.5). The sum of the PFCAs detected after complete oxidation of each precursor compound accounted for 95% ± 9% (n = 9) of the $[8:2\ FtS]_0$, 73% ± 5% (n=8) of the $[6:2\ FtS]_0$, 146% ± 6% (n = 6) of the $[8:2\ diPAP]_0$ and 122% ± 6% (n = 6) of the $[6:2\ diPAP]_0$, where $[PFAS]_0$ is the initial concentration of the precursor (Table 2.2). Yields of PFCAs for diPAPs may be up to 200% since each diPAP molecule contains two perfluorinated alkyl groups. It is possible that the products of 6:2 FtS and the diPAPs that could not be accounted for consisted of PFCAs with fewer than four carbons and neutral or volatile compounds.

Across the range of initial persulfate concentrations examined (1 mM, 5 mM, 20 mM, and 60 mM persulfate), the 8:2 fluorotelomer sulfonate exhibited a molar yield of 21% ± 2% (n=9) for PFOA and the 6:2 fluorotelomer sulfonate exhibited a molar yield of 22 ± 2% (n=8) for PFHxA (Table 2.2). The 8:2 diPAP exhibited a molar yield of 38%± 2% for PFOA (n=6) and the 6:2 diPAP exhibited a molar yield of 33%± 2% (n=6) for PFHxA when initial persulfate concentrations of 20 mM and 60 mM were used (Table 2.2). Thus, the fluorotelomer compounds did not exhibit the same one-to-one conversion to a PFCA of the same perfluorinated chain length that the C8-sulfonamide compounds exhibited.

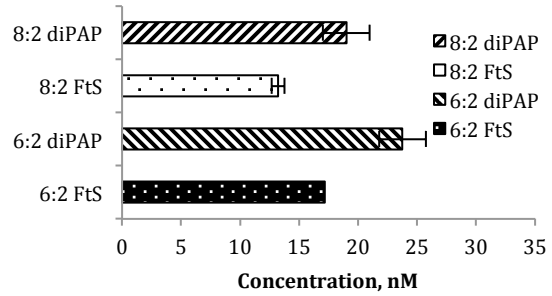
Both the fluorotelomer sulfonates and the diPAPs yielded a similar distribution of PFCA products upon oxidation despite different chemical moieties bound to each fluorotelomer group. For all four n:2 fluorotelomer precursors tested, the PFCA product formed in greatest yield was the C(n-1) PFCA and the product formed in the second greatest yield was the C(n) PFCA. Extrapolating these results to other fluorotelomer precursors, it is expected that a mixture of C4 to C(n+1) PFCAs will be produced when n:2 fluorotelomer precursors are oxidized with hydroxyl radical.

Table 2.2: Molar yields of PFCAs from precursors thermolyzed in the presence of persulfate. Initial conditions ranged from 1 mM persulfate to 60 mM persulfate and 25 ng/L to 25 μg/L precursor. Complete disappearance of precursors was observed under all conditions reported. $[\text{Precursor}]_0$ denotes the initial molar concentration of the precursor.

Starting Precursor Compound	$[\text{Persulfate}]_0$	$[\text{Precursor}]_0$	Δ[PFBA]/ $[\text{Precursor}]_0$	Δ[PFPeA]/ $[\text{Precursor}]_0$	Δ[PFHxA]/ $[\text{Precursor}]_0$	Δ[PFHpA]/ $[\text{Precursor}]_0$	Δ[PFOA]/ $[\text{Precursor}]_0$	Δ[PFNA]/ $[\text{Precursor}]_0$
N-EtFOSAA (n=7)	20 mM	25 ng/L, 250 ng/L					92%±4%	
N-MeFOSAA (n=8)	20 mM	25 ng/L, 250 ng/L					110%±8%	
	5 mM	10 μg/L						
FOSA (n=8)	20 mM	25 ng/L					97%±3%	
	5 mM	10 μg/L						
6:2 FtS (n=8)	5 to 60 mM	5 to 10 μg/L	22%± 5%	27%±2%	22%±2%	2%±1%		
8:2 FtS (n=9)	1 to 60 mM	5 to 10 μg/L	11%± 4%	12%± 4%	19%±3%	27%±3%	21%±2%	3%±0.1%
6:2 diPAP[a] (n=6)	20 to 60 mM	25 μg/L	27%± 3%	47%± 3%	33%± 2%	15%± 3%		
8:2 diPAP[a] (n=6)	20 to 60 mM	25 μg/L	10%± 2%	17%± 1%	24%± 1%	43%±2%	38%± 2%	13%± 1%

[a]Each mole of n:2 diPAP contains two perfluorinated alkyl chains; the maximum yield of PFCAs is 200%.

a



b

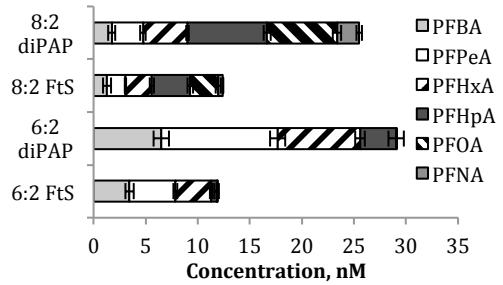
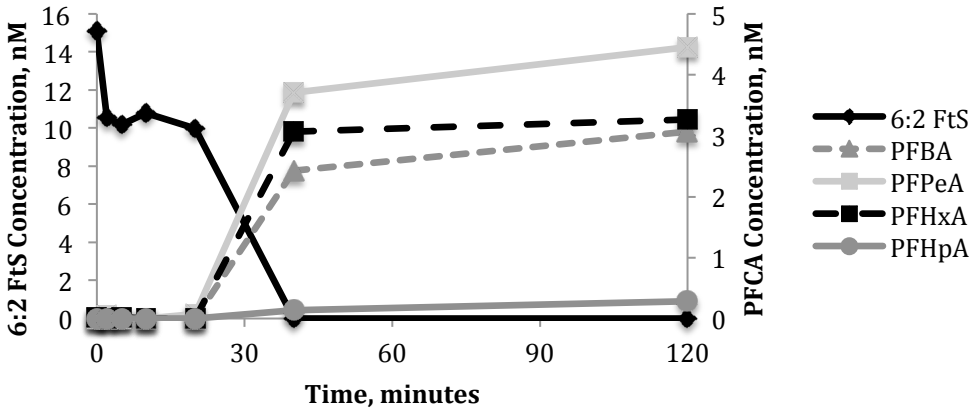


Figure 2.4 a-b: Concentrations of 6:2 FtS, 6:2 diPAP, 8:2 FtS, and 8:2 diPAP and their PFCA products in individual HPLC-grade water reactors (n=4) before (a) and after (b) oxidation with 20 mM persulfate and 50 mM NaOH.

a



b

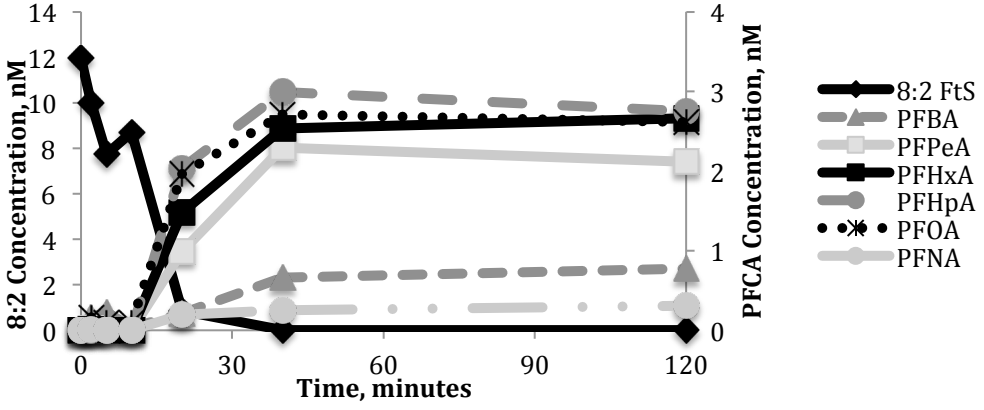


Figure 2.5 a-b: Conversion of 6:2 FtS (a) and 8:2 FtS (b) in HPLC –grade water in the presence of 5 mM persulfate and 50 mM NaOH over time at 85°C.

2.4.2 Direct Measurement of PFASs in Urban Runoff

At least seven perfluorinated acids were detected in each of the thirty-three runoff samples (Figure 2.6). The most prevalent perfluorinated acids were PFOS (median concentration = 15 ng/L), PFOA (median concentration = 7.3 ng/L), and PFHxA (median concentration = 4.5 ng/L). Total PFCA concentrations in stormwater samples ranged from 6.0 to 42 ng/L and total PFSA concentrations ranged from 2.6 to 35 ng/L.

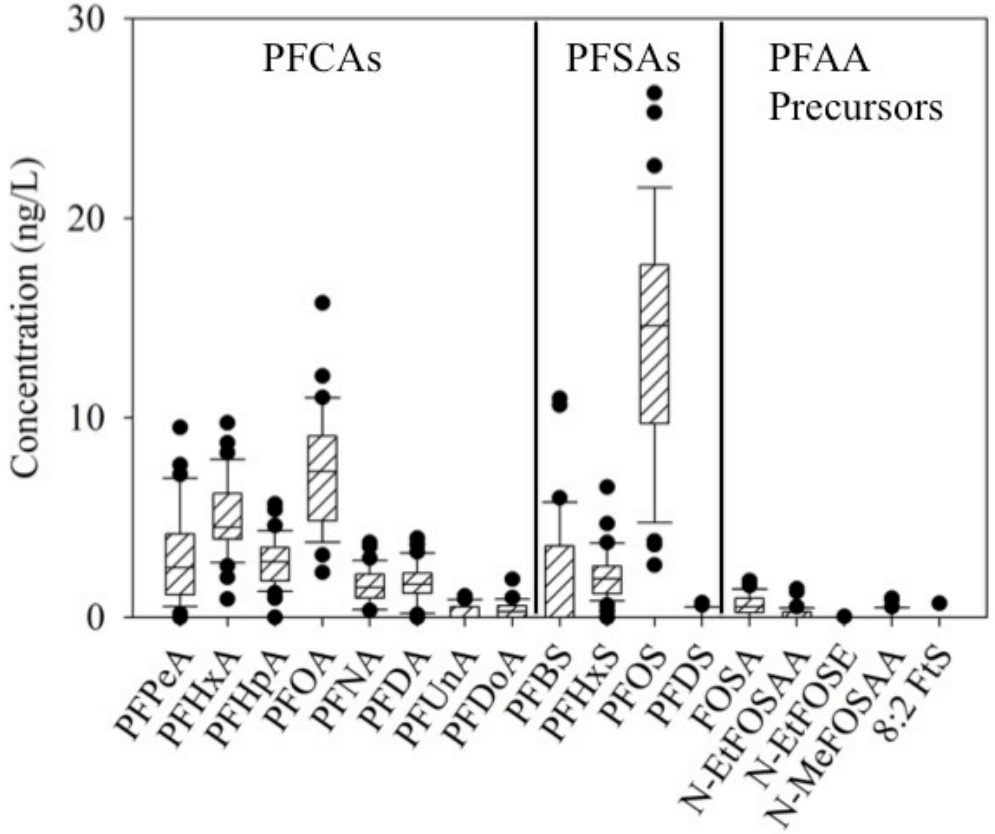


Figure 2.6: Range of concentrations of PFAS analytes in all urban runoff samples (n=33). The lower and upper ends of the boxes depict the 25th and 75th percentiles of the data. The whiskers define the 10th and 90th percentiles. PFTrA and PFTeA (not shown) and *N*-EtFOSE were not detected in any samples. Number of non-detects for each analyte: PFPeA (n=3), PFDA (n=2), PFUnA (n=22), PFDoA (n=13), PFBS (n=17), PFHxS (n=1), PFDS (n=25), FOSA (n=5), *N*-EtFOSAA (n=23), *N*-MeFOSAA (n=26), 8:2 FtS (n=32).

Concentrations of PFAS analytes in San Francisco Bay Area runoff samples were comparable to those previously reported in urban runoff and surface waters that did not receive substantial discharge of wastewater effluent [Müller *et al.* 2011, Kim *et al.* 2007, Meyer *et al.* 2011, Murakami *et al.* 2009b, Plumlee *et al.* 2008, Zushi and Masunaga 2009a]. Runoff samples collected in Zürich [Müller *et al.* 2011], Albany, New York [Kim and Kannan 2007], Toronto [Meyer *et al.* 2011], and Tokyo [Murakami *et al.* 2009b] exhibited higher concentrations of PFOA than PFOS, but higher concentrations of PFOS than PFOA have been observed previously in the San Francisco Bay region [Plumlee *et al.* 2008]. While high PFOS to PFOA ratios are sometimes caused by municipal wastewater discharge, there were no wastewater discharges upstream of our sample collection sites and no evidence that significant volumes of wastewater were released through sanitary sewer overflows. The presence of relatively high concentrations of PFHxA suggests that reformulation of consumer products might have caused a shift to shorter chain-length PFCAs and PFCA precursors. The relatively low concentrations of PFHxS and PFBS detected in these samples suggests that PFOS has not been replaced with shorter chain PFSAs, although it is possible that short-chain PFSAs in commerce have not migrated into stormwater.

The relative concentration ratios of PFCAs and PFSAs among sites did not exhibit significant spatial variability attributable to distinct watershed features (Table 2.3). The average mass ratios of PFHxA, PFHpA, PFNA, and PFOS to PFOA in samples from a single site varied by less than a factor of two among all sites (Table 2.3). The average ratios of PFPeA to PFOA and PFHxA to PFHxS varied by factors up to 4.5 and 3.8, respectively. No consistent watershed-related reasons for the greater variability in these two ratios were observed.

Table 2.3: Average mass ratios for analytes in samples from each site.

	[PFPeA]/ [PFOA]	**[PFHxA]/ [PFOA]**	**[PFHpA]/ [PFOA]**	**[PFNA]/ [PFOA]**	**[PFHxA]/ [PFHxS]**	**[PFOA]/ [PFOS]**
Site 1	0.26	0.63	0.41	0.18	1.97	0.66
Site 2	0.17	0.67	0.35	0.24	3.54	0.54
Site 3	0.76	0.70	0.39	0.33	1.72	0.43
Site 4	0.68	0.70	0.32	0.26	2.59	0.47
Site 5	0.30	0.57	0.36	0.20	6.55	0.89
Site 6	0.43	0.62	0.34	0.21	2.41	0.52
Site 7	0.35	0.60	0.33	0.26	3.77	0.61
Site 8	0.45	0.79	0.37	0.18	2.34	0.77
Site 9	0.54	0.67	0.29	0.20	3.37	0.53
Site 10	0.40	0.91	0.55	0.23	2.35	0.54
Minimum	0.17 (S2)	0.57 (S5)	0.29 (S9)	0.18 (S1,	1.72 (S3)	0.43 (S3)
Maximum	0.76 (S3)	0.91 (S10)	0.55 (S10)	0.33 (S3)	6.55 (S5)	0.89 (S5)

In general, concentrations of C8 PFAA precursors directly quantified by HPLC-MS/MS were lower than those of PFCAs and PFSAs (Figure 2.6). Among the measured C8 perfluorinated acid precursors, FOSA was the most commonly detected compound, with measured concentrations as high as 1.8 ng/L. *N*-EtFOSAA and *N*-MeFOSAA were detected at concentrations up to 1.4 and 1.0 ng/L, respectively. The presence of these C8 sulfonamide-containing precursors was consistent with the widespread presence of PFOS, all of which are produced by the same electrochemical fluorination process [Prevedouros *et al.* 2006]. The 8:2 fluorotelomer sulfonate, the only fluorotelomer-based C8 precursor analyzed, was detected in one sample below its limit of quantification (estimated concentration approximately 0.3 ng/L). The total concentration of C8 perfluorinated acid precursors measured in all samples ranged from <0.2 ng/L to 2.9 ng/L (<0.2 ng/L to 2.2 ng/L expressed as PFOA), with a median concentration of 0.7 ng/L (0.55 ng/L as PFOA). On a molar basis, precursors measured by LC/MS/MS amounted to less than 18% (median = 3%) of the combined concentration of PFOS and PFOA in individual samples.

2.4.3 PFAA Precursors Indicated by Oxidation in Urban Runoff

Oxidative treatment of stormwater samples produced considerable amounts of perfluorinated carboxylic acids (Figure 2.7). PFCAs with 5 to 12 membered perfluoroalkyl chains increased by a total concentration of 2.8 to 56 ng/L (median concentration increase = 14 ng/L). A median increase of 2.3 ng/L of PFOA was generated upon oxidation, while the median concentration of PFPeA and PFHxA increased by 3.8 ng/L and 3.0 ng/L, respectively.

The increase in PFOA concentration cannot be explained by the PFAA precursors measured by HPLC/MS-MS in most samples. If all of the directly-measured C8-precursors were converted into PFOA with the efficiency observed in the control experiments (Table 2.2), the concentration of PFOA would have increased by a median value of 0.55 ng/L, or 23% of the median measured increase in PFOA concentration. The concentration of PFOA generated upon oxidation ranged from below detection to 145% of the concentration of PFOA measured prior to oxidation (median = 37%).

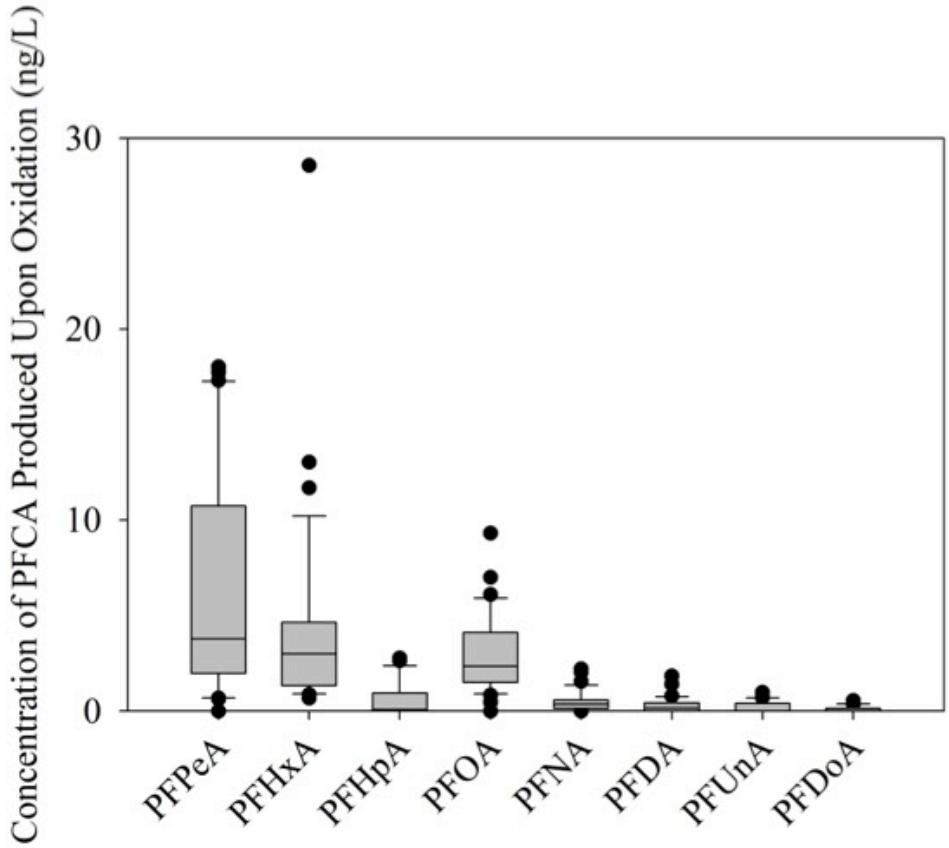


Figure 2.7: Range of concentrations of PFCAs generated upon oxidation in each sample (n=33). The lower and upper ends of the boxes depict the 25th and 75th percentiles of the data. The whiskers define the 10th and 90th percentiles. Number of samples with no measureable change for each analyte (*i.e.* Δ[PFnA]<0.1 ng/L): PFHpA (n=15), PFOA (n=1), PFNA (n= 6), PFDA (n=15), PFUnA (n=18), PFDoA (n=21).

2.4.4 Estimation of PFAA Precursor Concentrations by Chain Length

The oxidation method was developed to quantify the total concentration of PFAA precursors with a specific perfluorinated chain length (*e.g.,* oxidation can be used to infer the concentration of C8-containing PFAA precursors). The production of a C_n acid indicates the presence of a C_n precursor, but the concentration of the C_n acid produced is not necessarily equivalent to the total concentration of C_n precursors because oxidation reduces the perfluorinated chain length of some compounds.

All potential precursor compounds have one of two linkages to the perfluorinated alkyl chain: an ethyl linkage produced during telomerization or a sulfonamide linkage produced during electrochemical fluorination. As demonstrated in the control experiments with pure compounds, sulfonamide-containing C8 precursor compounds are quantitatively converted into PFOA while 8:2 fluorotelomer-containing precursor compounds react to form a mixture of PFCAs, where PFOA accounts for about 20% of the product yield for each perfluorinated group in the molecule (Table 2.2). The concentration of C8-containing PFAA precursors in a sample can be estimated by examining the concentrations of PFOA and other PFCAs generated upon oxidation.

The production of the C7 perfluorinated carboxylate, PFHpA, observed after oxidation may indicate the presence of C8 and higher fluorotelomer compounds; the oxidation of 8:2 diPAP and 8:2 FtS resulted in relative PFHpA to PFOA product yields of 1.1 and 1.3, respectively (Table 2.2). The production of detectable concentrations of PFHpA after oxidation was limited, occurring in seventeen out of thirty-three samples with a maximum production of 2.8 ng/L PFHpA (3.2 ng/L as PFOA) and a median production of 0.1 ng/L PFHpA. Because PFHpA production can also occur via oxidation of C7 PFAA precursors, using Δ[PFHpA] to estimate a C8- fluorotelomer precursor concentration establishes an upper-bound for the potential C8 PFAA precursor concentration in a sample. However, no C7 PFAA precursors have been studied or are believed to be in wide usage, so attributing PFHpA production to C8 PFAA fluorotelomer precursors is reasonable.

In addition to PFOA production from C8 precursors, PFOA can be produced from the oxidation of fluorotelomer precursors that contain *more* than eight fluorocarbons. Median concentrations of 0.4 ng/L PFNA and 0.2 ng/L PFDA were observed in samples after oxidation with hydroxyl radical, while detectable increases in PFUnA and PFDoA were observed in fewer than half of all samples measured. Attributing part of the increase in PFOA concentrations to the oxidation of higher chain fluorotelomer precursors creates a lower bound estimate for the C8 PFAA precursor concentration; if increases in C9 and C10 PFCAs upon oxidation are attributable to non-fluorotelomer based precursors, then no contribution to PFOA production would be expected.

Measurements of C7 through C9 PFCA production were used to estimate the minimum and maximum total C8 precursor concentration in samples (see discussion and calculations in the Appendix). For most samples, the concentration of PFOA generated upon oxidation was a reasonable approximation (*i.e.* within a factor of 3) of the estimated possible range of concentrations of C8-containing precursors. Eighty percent of samples contained an estimated total C8 precursor concentration that ranged between 50% and 300% of the concentration of PFOA generated upon oxidation (Table A2.6). Total concentrations of C8 PFAA precursors were

estimated to range between <0.1 ng/L and 16 ng/L in samples, with a median low-bound estimate of 1.8 ng/L and a median high-bound estimate of 4.5 ng/L (Table A2.6). By comparison, Δ[PFOA] ranged from <0.1 to 9.6 ng/L (median = 2.3 ng/L). The closer the upper bound estimate of [C8 PFAA precursors] is to Δ[PFOA], the greater the proportion of likely PFOS precursors. Since the median value of the upper estimate of [C8 PFAA precursors] is 111%, the majority of C8 precursors in most samples are likely precursors to PFOS.

The perfluorooctane sulfonamide precursors and 8:2 fluorotelomer sulfonate measured in this study account for a small fraction of the C8-based PFAA precursors in commerce. Mono- and diPAPs, perfluorophosphates (PFPAs), perfluorophosphinates (PFPiAs) and sulfonamidoethanol-based polyfluoroalkyl phosphate diesters (SAmPAPs) have all been detected in human sera [Lee and Mabury 2011]. The PAPs are not likely to be found at high concentrations in these samples because they contain a fluorotelomer linkage and would produce PFHpA upon oxidation. It is unknown if the PFPAs and PFPiAs are converted into PFOA by hydroxyl radical. The C8-based PFPA compound has been measured at concentrations up to 3.4 ng/L in a Canadian river that receives urban runoff and up to 2.5 ng/L in municipal wastewater effluent [D'Eon *et al.* 2009]. C8 PFAA precursor compounds containing sulfonamide linkages like the SAmPAPs and related compounds are potential candidates for the unidentified C8 precursors. PFOA might also be released from oxidation of fluoropolymers in runoff. Fluoropolymers often contain fluorotelomer linkages [Russell *et al*. 2008, Washington *et al.* 2009, Russell *et al.* 2010], which would make them poor candidates for unidentified PFOA production if they react similarly to the fluorotelomer sulfonates and diPAPs. Additional research is needed to quantify the presence of these and other compounds in urban runoff.

Despite the relatively low concentrations of the C5 and C6 perfluoroalkyl acids in runoff, relatively high concentrations of PFHxA and PFPeA were produced upon oxidation (Figure 2.7). The median concentrations of PFPeA and PFHxA evolved in oxidized samples were 3.8 ng/L and 3.0 ng/L, respectively. On a molar basis, the median increase in concentration of PFPeA was 1.7 times that of PFHxA. The concurrent production of PFPeA and PFHxA is consistent with the presence of fluorotelomer-based C6 precursors. The significantly higher increase in PFPeA relative to PFHxA compared to control experiments also indicates that the runoff may contain C5 precursors or C4 telomer-based precursors that produce PFPeA upon oxidation.

Due to the limited availability of suitable standards, only one C6 precursor was measured by HPLC/MS/MS in runoff samples. 6:2 FtS was detected at two sites. In three samples from Site 3, the site with the most industrialized land use, 6:2 FtS was detected at low concentrations. Because of uncertainties associated with the 6:2 FtS data (*i.e.* recoveries above 200%) we can only estimate that the concentrations were less than 4.1 ng/L. Concentrations of PFHxA produced in samples from Site 3 were between 3.1 and 6.6 ng/L, suggesting the presence of C6 precursors unrelated to 6:2 FtS. Both samples collected from Site 4, a site that was also industrialized, contained less than 0.9 ng/L of 6:2 FtS. At all other sites, the 6:2 FtS concentrations were <0.2 ng/L. The absence of 6:2 FtS in samples from the other sites suggests the presence of other C6 PFAA precursors.

Measurements of C5 through C7 PFCA production were used to estimate the minimum and maximum total C6 precursor concentrations in samples using a similar approach to the one

used to estimate C8 precursor concentrations (see discussion in the Appendix). Total concentrations of C6 PFAA precursors were estimated to range between <0.1 ng/L and 87 ng/L in samples, with a median low-bound estimate of 2.3 ng/L and a median high-bound estimate of 14 ng/L (Table A2.7). These calculations indicate that C6-precursors in these samples are likely to be fluorotelomer-based. The closer the upper bound estimate of [C6 PFAA precursors] is to Δ[PFHxA], the greater the proportion of likely PFHxS precursors; conversely, the closer the upper bound estimate of [C6 PFAA precursors] is to Δ[PFHxA]/0.19, or 5.3 times Δ[PFHxA], the greater the proportion of likely C6 fluorotelomer-based (*i.e.* PFHxA) precursors. For more than 90% of samples, the upper estimate of [C6 PFAA precursors] is at least 2.7 times Δ[PFHxA] (Table A2.7), indicating that a majority of C6 precursors in the runoff samples may be fluorotelomer-based precursors. This prediction is consistent with the higher relative concentrations of PFHxA compared to PFHxS measured in samples (Figure 2.6, Table 2.2).

2.5 Environmental Implications

The use of hydroxyl radical to convert difficult-to-measure and unidentified PFAA precursors into stable, easily measured PFCAs provides a basis for estimating the concentration and chemical structure of PFAA precursor compounds that are not routinely measured in stormwater samples. Data from these ten sites indicates that PFAA precursor compounds are present in stormwater samples at levels comparable to their routinely monitored PFAA counterparts. Failure to monitor or control these compounds could mean that chemical and biological processes in reservoirs, aquifers, or drinking water treatment plants could result in exposure to higher-than-expected concentrations of PFAAs.

There is still a need to identify the sources and structures of the PFAA precursor compounds in runoff that are not routinely measured. Elucidating the structure of unknown PFAA precursors has implications for predicting the mobility of these compounds in heterogeneous systems [Higgins and Luthy 2006], their susceptibility to biotransformation [Wang *et al.* 2011, Martin *et al.* 2005, Dinglasan *et al.* 2004, Martin *et al.* 2010, Rhoads *et al.* 2008] and abiotic transformation, and their toxicity. Tools such as fast atom bombardment mass spectrometry, time-of-flight mass spectrometry (QTOF/MS), and high resolution mass spectrometry may be helpful in future efforts to identify the unknown PFAA precursor compounds [Place and Field 2012].

Other methods of indirectly quantifying the potential formation of undesirable reaction products formed through complex mechanisms have proven to be useful to water quality researchers. The trihalomethane formation potential (THMFP) method was described in 1977 [Stevens and Symons 1977] and became a standard method used by practitioners in 1998 [Eaton *et al.* 1998]. A similar approach for measuring precursors to nitrosamines formed during chloramination has been used by numerous researchers interested in identifying and controlling precursor sources [Mitch *et al*. 2003]. The PFAA precursor oxidation method developed in this study may similarly be used to characterize the sources and fate of difficult-to-measure PFAA precursors in environmental systems other than urban runoff. For example, the precursor oxidation method may be used to evaluate the removal of PFAA precursors during treatment of municipal wastewater effluent by reverse osmosis or advanced oxidation processes. If PFAA compounds such as PFOS and PFOA are subject to further regulation in drinking water or

wastewater effluent, this inexpensive and efficient method for quantifying a suite of PFAA precursors may be particularly relevant to monitoring programs.

CHAPTER 3. Persistence of Perfluoroalkyl Acid Precursors in AFFF-Impacted Groundwater and Soil

Reprinted with permission from 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.

© 2013 American Chemical Society.

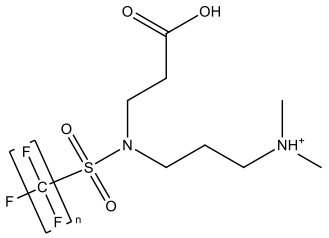
3.1 Introduction

Aqueous film-forming foams (AFFFs) have been used since the 1960's to extinguish hydrocarbon-based fuel fires at military bases, airports, oil refineries, and municipal firefighter training facilities [Moody and Field 2000]. AFFF formulations contain both hydrocarbon and fluorocarbon surfactants to enhance the spreading of AFFF along hydrocarbon-water and air-water interfaces [Moody and Field 2000]. Where these foams have been deployed accidentally, in response to fires, or in unlined firefighter training areas, high concentrations of poly- and perfluoroalkyl substances (PFASs) have been detected in biota [Moody *et al.* 2002, Oakes *et al.* 2010, Awad *et al.* 2011, Kärrman *et al.* 2011], surface water [Moody *et al.* 2002, Oakes *et al.* 2010, Awad *et al.* 2011, Kärrman *et al.* 2011], and groundwater [Moody *et al.* 2003, Weiss *et al.* 2012, Schultz *et al.* 2004, Backe *et al.* 2013]. For example, nine years after 22,000 liters of AFFF was released at the Toronto airport, perfluorooctane sulfonate (PFOS) concentrations in fish and surface waters were 2 to 10 times higher than those observed in locations upstream of the release [Awad *et al.* 2011].

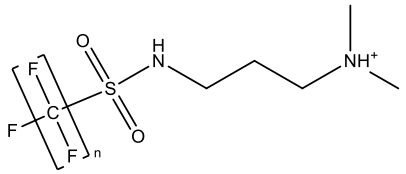
The types of PFASs in AFFF vary by year of production and manufacturer. AFFF manufactured by the 3M Company reportedly contained perfluorinated carboxylates in the 1960's and early 1970's [Prevedouros *et al.* 2006, Tuve 1966, Chiesa 1974, Chiesa and Di Maio 1976] and perfluorinated sulfonates from the 1970's to 2001 [Prevedouros *et al.* 2006, Chiesa and Di Maio 1976, Paul *et al.* 2009, Place and Field 2012] when 3M ceased AFFF production. AFFF formulations manufactured by 3M between 1988 and 2001 also contained multiple polyfluorinated surfactants with a four to six carbon perfluorinated chain linked by a sulfonamide moiety to a positively charged alkyl group (Figure 3.1)[Place and Field 2012]. Notably, no polyfluorinated surfactants containing an 8-carbon perfluoroalkyl chain (*i.e.,* C_8) were observed in these 3M formulations [Place and Field 2012]. In fluorotelomer-based AFFF manufactured by six other formulators between 1984 and 2010, the only PFASs identified were polyfluorinated surfactants containing a four to ten carbon perfluorinated chain linked by two or three non-fluorinated carbons to a charged alkyl group (Figure 3.1) [Place and Field 2012].

The fate of the polyfluorinated compounds in AFFF released to soil and groundwater is not well understood, but the non-fluorinated part of the molecule attached to the perfluorinated chain may be amenable to microbial or chemical transformation [Rhoades *et al.* 2009, Wang *et al.* 2011, Wang *et al.* 2009, Dinglasan *et al.* 2004, Plumlee *et al.* 2009, Dasu *et al.* 2012]. These reactions could produce perfluorinated carboxylates or perfluorinated sulfonates. Therefore, we collectively refer to these polyfluorinated compounds in AFFF and their polyfluorinated intermediate products as perfluoroalkyl acid (PFAA) precursors, or simply precursors.

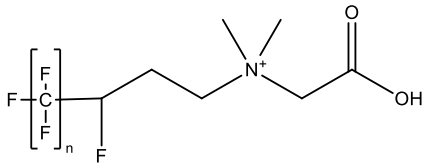
Figure 3.1: Structures of PFAA precursors found in AFFF formulations.



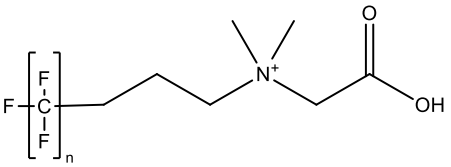
3M: Perfluoroalkyl sulfonamide amino carboxylates (PFnSAmA). n=4,5,6.



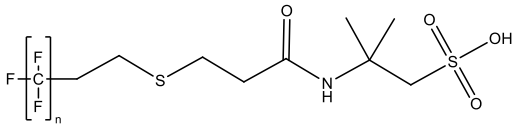
3M: Perfluoroalkyl sulfonamido amines (PFnSAm). n= 4,5,6.



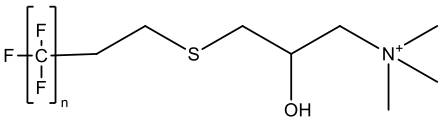
Buckeye: n:1:2 Fluorotelomer betaine. n=5,7,9.



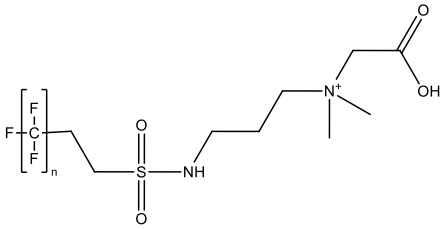
Buckeye: n:3 Fluorotelomer betaine. n=4,7,9.



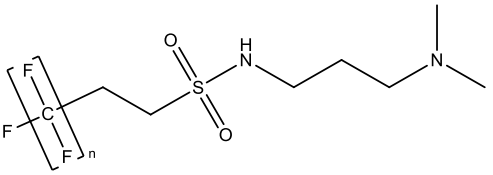
Ansul, Chemguard: Fluorotelomer thioamido Sulfonates (n:2 FtTAoS). n=4,6,8.



Ansul: Fluorotelomer thio hydroxy ammonium (n:2 FtTHN+). n=6.



National Foam (NF): Fluorotelomer Sulfonamido Betaines (n:2 FtSaB). n=6,8,10,12.



NF: Fluorotelomer Sulfonamido Amines (n:2 FtSaAm). n=6,8.

Previous studies have provided evidence of the occurrence of PFAA precursors at AFFF-impacted sites. For example, precursors in Ansul and 3M AFFF formulations were detected in groundwater from five U.S. military firefighter training areas, albeit at low concentrations relative to PFAAs [Backe *et al.* 2013]. One precursor in AFFF manufactured by National Foam, 6:2 fluorotelomer sulfonamide betaine, was recently detected on soils near an airport in Norway where AFFF was used [Moe *et al.* 2012]. The precursors may exhibit a strong affinity for soil and aquifer solids: due to the presence of anionic, cationic, and zwitterionic alkyl moieties, many of the precursors in AFFF are likely to undergo ion exchange processes. Precursors in AFFF may also partition to soil organic matter, especially those containing perfluorinated groups with more than seven carbon atoms [Higgins and Luthy 2007].

Multiple analytical tools are needed to assess the transformation of precursors in AFFF, because the transformation products may not be readily identifiable. An assay developed for detection of PFAA precursors in stormwater [Houtz and Sedlak 2012] was adapted to conditions encountered in dilute AFFF, groundwater, and surficial soil and aquifer solids extracts. The assay was used to quantify the total concentration of PFAA precursors in the samples by converting them into readily measureable perfluorinated carboxylates through reactions with hydroxyl radical. The precursor assay was applied to archived AFFF formulations and groundwater, soil, and aquifer solids from a site where AFFF was used between 1970 and 1990 in conjunction with direct measurement of twenty-two PFAA precursors found in AFFF formulations, potential intermediate transformation products for which standards were available, and perfluorinated sulfonates and carboxylates. Results were used to assess the persistence of AFFF-derived precursors and to evaluate their *in situ* transformation to PFAAs.

3.2 Materials and Methods

3.2.1 Materials

Structures of PFAA precursors in AFFF are depicted in Figure 3.1. Abbreviations for these and other PFASs are listed in Table A3.1. All isotopically-labeled standards, perfluorinated carboxylates, perfluorinated sulfonates, perfluorooctane sulfonamide (FOSA), 6:2 fluorotelomer sulfonate (6:2 FtS), and 8:2 fluorotelomer sulfonate (8:2 FtS) were purchased from Wellington Laboratories. Commercial source materials containing 6:2 fluorotelomer sulfonamido betaine, 6:2 fluorotelomer sulfonamido amine, 6:2 fluorotelomer thioamido sulfonate, 6:2 fluorotelomer thio hydroxy ammonium and 5:1:2, 7:1:2, 9:1:2, 5:3, 7:3, and 9:3 fluorotelomer betaines were provided by the Fire Fighting Foam Coalition [Backe *et al.* 2013] and were used for quantitation of those analytes. All other solvents and chemicals were purchased from Sigma Aldrich or Fisher Scientific at the highest purity available.

Archived samples of AFFF formulations manufactured by 3M, Ansul, Chemguard, National Foam, and Buckeye were obtained from U.S. military bases as described previously [Place and Field 2012]. Some of these AFFF formulations were previously used to identify the structures of PFASs in AFFF [Place and Field 2012].

3.2.2 Site Information

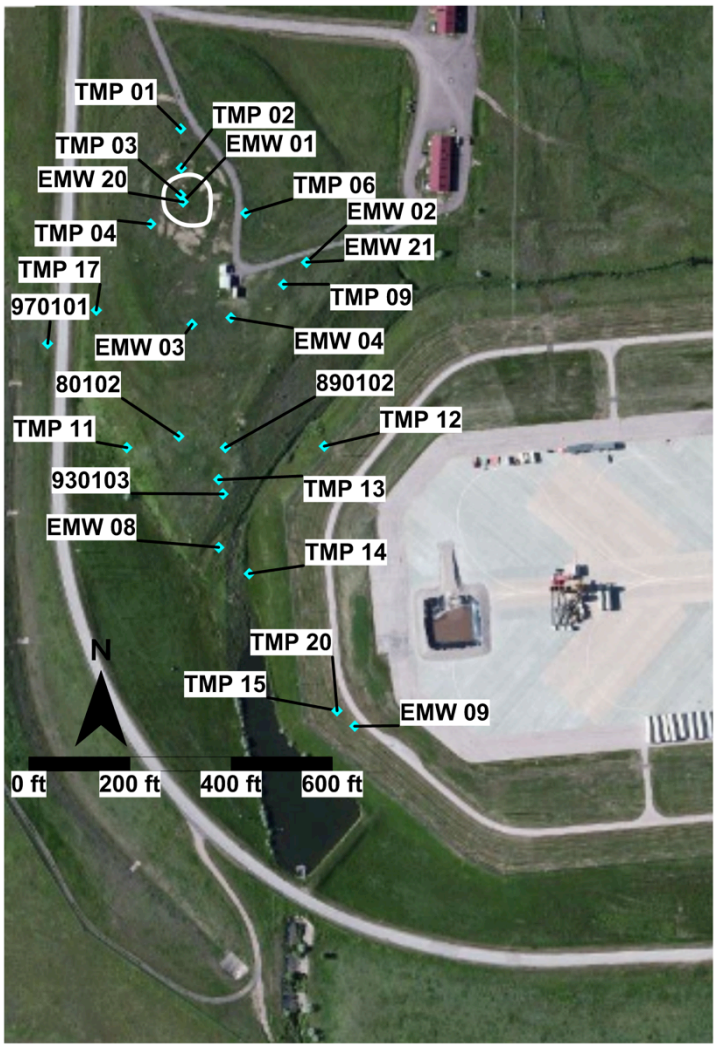
Groundwater, soil, and aquifer solids samples were collected from a location at Ellsworth Air Force Base in Piedmont, South Dakota, US. Firefighter training was conducted between 1942 and 1990. As part of these activities, waste oil, solvents and fuels were placed in an unlined pit, ignited, and subsequently extinguished using various firefighting agents, including AFFF after 1970. Remedial activities targeted at solvent contamination, including oxygen infusion, soil vapor extraction, and groundwater extraction followed by *ex situ* treatment, occurred in the area where samples were collected. The subsurface at this site consists of low-to-moderate permeability clay loam and gravely sandy loam alluvial soil, underlain by Pierre Shale bedrock. Additional details on the site and firefighter training exercises are summarized elsewhere [McGuire *et al.* 2013].

Records of the exact makeup of AFFF formulations used onsite during this period are not available. However, it is likely that AFFF manufactured by 3M, National Foam, and Ansul accounted for most of the materials used because they were approved for U.S. military use from 1976 to 1990 [MIL-F-24385 2011]. 3M reportedly supplied most of the U.S. military's AFFF prior to its cessation of AFFF production in 2001 [Darwin 2004]. As of 2004, 75% of the military's stockpiled AFFF was manufactured by 3M [Darwin 2004].

3.2.3 Groundwater, Soil, and Aquifer Solids Collection

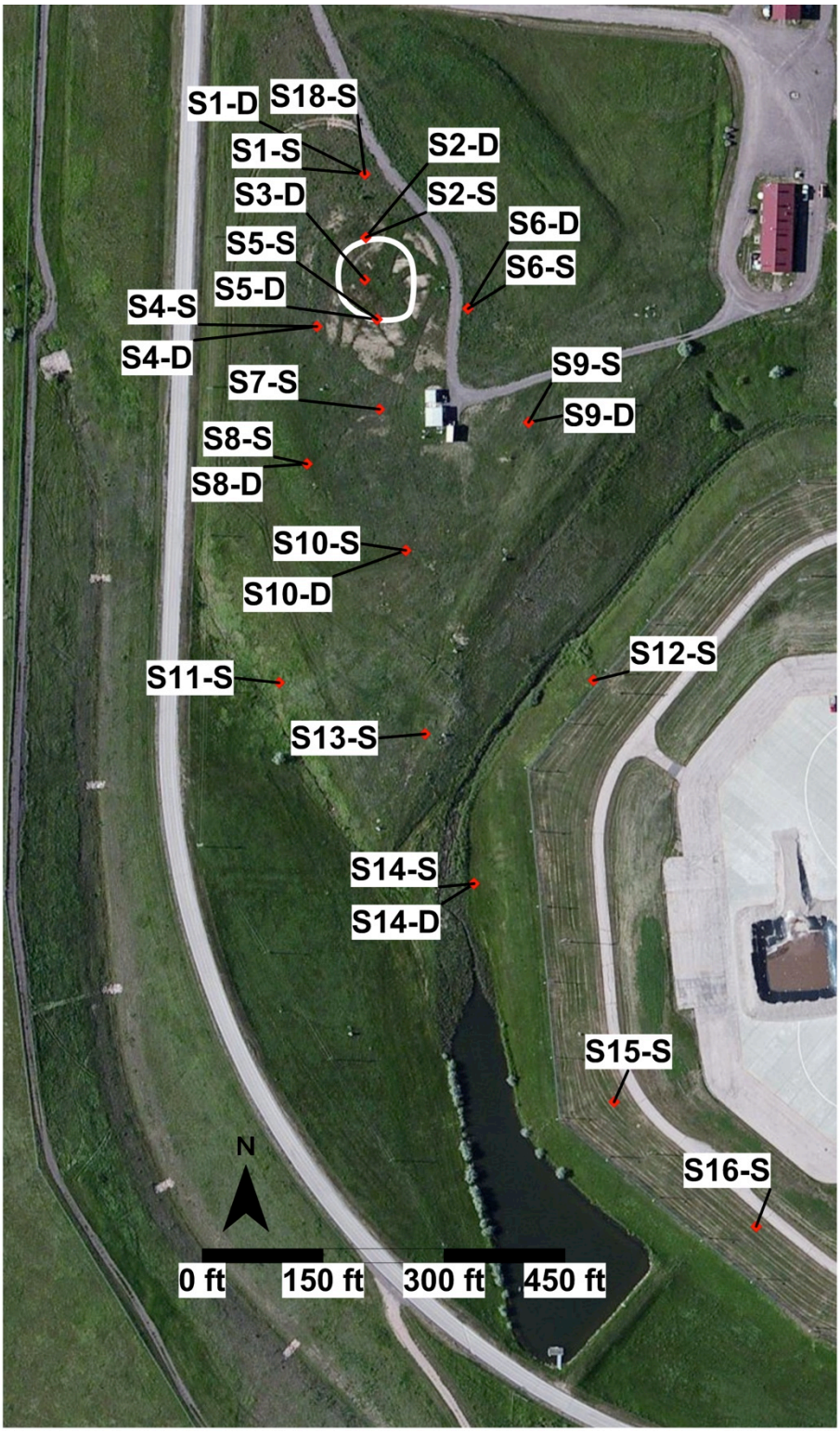
Groundwater and soil samples were collected in October 2011 from a 1200 m by 600 m area encompassing the burn pit (Figures 3.2-3.3). Groundwater was collected from permanent and temporary wells using techniques designed to minimize contamination of samples with PFASs [McGuire *et al.* 2013]. The groundwater depth was 2 to 8 m below ground surface (bgs). Soil samples were collected 0.6 m bgs and aquifer solids were collected approximately 5 to 6 m bgs [McGuire *et al.* 2013].

Figure 3.2: Map of sampling area and groundwater sampling locations. The burn pit used for fire training is circled.



0 ft 300 ft 600 ft 900 ft

Figure 3.3: Map of sampling area and soil (S) and aquifer solids (D) sampling locations. The burn pit used for fire training is circled.



3.2.4 Sample Storage and Preparation for Direct Analysis

Archived AFFF samples were stored at room temperature. The number (n) of AFFF samples analyzed from each manufacturing category was as follows: 3M 1988,1989: n=2; 3M 1993-2001: n=6; National Foam 2002-2008: n=3; Buckeye 2009: n=1; Chemguard 2008, 2010: n=2; Ansul 1984, 1987: n=2; Ansul 2009, 2010: n=2. AFFF formulations were analyzed in duplicate after two sequential thousand-fold dilutions in methanol. Internal standards were added to AFFF after the final dilution.

Groundwater samples were stored at 4°C until use and analyzed in duplicate. Twenty-two groundwater samples were analyzed. Groundwater samples were inverted five times to ensure adequate mixing before subsampling. 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 minutes to remove suspended particles. Each groundwater sample was initially analyzed in 50:50 methanol: water at 2.5 times dilution. Samples were diluted further as necessary to achieve concentrations within the working range of the calibration curve (*i.e.*, 0.1 μg/L to 24 μg/L). Internal standards were added to groundwater after the final dilution. Whole method limits of detection (LOD) ranged from 0.1 to 0.5 μg/L (Table A3.2). Spike recoveries and method precision were within the range reported in previous studies [Schultz et al. 2004, Backe et al. 2013] (Table A3.2).

Soil and aquifer solids samples were stored at 4°C until analysis. Sixteen soil samples and ten aquifer solids samples were analyzed. All samples were extracted in triplicate. The soil extraction method was similar to the approach used by Higgins *et al*. [2005]. Briefly, a 500-mg sub-sample of a homogenized soil sample was placed in a 15-mL LDPE centrifuge tube containing 2.5 mL of 0.1% ammonium hydroxide (NH_4OH) in methanol. Tubes were vortexed for twenty seconds, sonicated for 30 minutes at 30-35°C, and shaken on a rotating table at 150 RPM for two hours. The extract was separated from the soil by centrifugation at 5000 RPM for 5 minutes. The supernatant was transferred to a new 15-mL centrifuge tube with a glass pipet. Extractions were repeated two more times to ensure complete removal of all PFASs (Figure A3.1). The combined 7.5 mL extract was evaporated to dryness with nitrogen in a 45°C water bath (Organomation N-EVAP 24). Three extractions in 0.1% NH_4OH/methanol were sufficient to remove all anionic, cationic, and zwitterionic PFASs; additional extractions in acidic methanol did not improve recoveries. Extracts were reconstituted in 1.5 mL of 0.1% acetic acid in methanol and kept at 45°C for another 30 minutes to ensure dissolution of target analytes. The extract was transferred to a 2-mL microcentrifuge tube containing 25 mg of ENVI-CARB and centrifuged at 15,000 rpm for 5 minutes. The ENVI-CARB was used to remove excess organic matter that could suppress ionization of the compounds in the mass spectrometer or reduce the efficiency of the oxidative assay. Control experiments demonstrated that the ENVI-CARB did not remove any PFASs. Reconstituted soil extracts were initially diluted 5 times for analysis in a 50:50 methanol: water mixture and diluted further as necessary to achieve peak areas that were within the working range of the calibration curve. Internal standards were added after final dilution. Whole method LODs ranged from 0.4 to 3 μg/kg (Table A3.3). Spike recoveries and method precision for solid sample analysis were within the range reported in previous studies [Higgins *et al.* 2005] (Table A3.3).

3.2.5 Sample Preparation for Precursor Oxidation Assay

A previously developed method for converting PFAA precursors to perfluorinated carboxylates in urban runoff was adapted to the AFFF matrix [Houtz and Sedlak 2012]. Under the conditions of the assay, precursors to both the perfluorinated carboxylates and the perfluorinated sulfonates are converted to perfluorinated carboxylates [Houtz and Sedlak 2012]. Exposure of diluted AFFF or groundwater amended with 60 mM potassium persulfate in 0.125 M NaOH followed by heating for six hours at 85°C produced an amount of hydroxyl radical that was sufficient to convert all precursors into perfluorinated carboxylates. For soils and aquifer solids, the methanolic soil extract was evaporated to dryness with nitrogen gas and resuspended in 6 mL of 60 mM persulfate and 0.125 M NaOH before heating. The ENVI-CARB cleanup step did not remove PFAA precursors as determined by the oxidation assay (Figure A3.3). After reaction, all samples were neutralized with concentrated HCl and amended with methanol before they were sub-sampled for analysis. Detailed information on sample preparation for the oxidation assay is included in the Appendix (discussion and Figures A3.2-A3.3).

3.2.6 Analytical Methods

All samples were analyzed in 500 μL of 50:50 methanol: water containing 50 μL of a 20 μg/L internal standard stock solution. Analysis was conducted on an Agilent 6410 LC-MS/MS according to the method described previously [Houtz and Sedlak 2012]. A list of ion transitions and MS parameters is included in Table A3.1.

3.2.7 Analyte Quantification

All perfluorinated carboxylates, perfluorinated sulfonates, 6:2 FtS, 8:2 FtS, and FOSA were quantified using isotope dilution (Table A3.1) with certified analytical standards. The fluorotelomer precursors in AFFF formulations were quantified using commercial source materials; a source material was only available for the C_6 homologs (*i.e.,* compounds containing a 6-carbon perfluorinated group) of Ansul, Chemguard, and National Foam fluorotelomer precursors. For non-C_6 homologs of these compounds, the calibration curve for the C_6 compound was used to estimate the concentration of the entire family of homologs, with a correction for perfluorinated chain length, as detailed in the Appendix.

No certified standard or commercial source material was available for perfluorohexane sulfonamide (FHxSA) or any of the precursor compounds in 3M AFFF formulations. The concentration of FHxSA was estimated from the calibration curve of FOSA with a correction for perfluorinated chain length. The concentrations of the 3M compounds were estimated using the precursor oxidation data from 3M AFFF formulations (Table A3.4). Briefly, the total molar concentration of precursors for each homolog group was estimated from the concentration of the corresponding perfluorinated carboxylate homolog produced upon oxidation of the AFFF sample. Because most 3M samples contained two types of precursors per homolog, each precursor of the same chain length was assumed to yield an equal instrument response on a molar basis. A more detailed explanation and a discussion of the uncertainty associated with this approach is included in the Appendix.

3.2.8 Total Precursor Concentration and Total PFAS Concentration

The summation of the total molar concentration of perfluorinated carboxylates produced upon oxidation can be used to estimate the total concentration of PFAA precursors (*i.e.,* the total amount of precursors to both perfluorinated carboxylates and perfluorinated sulfonates) present in a sample. Because less than 75% of the 6:2 and shorter fluorotelomer compounds initially present in a sample are converted into measureable perfluorinated carboxylates [Houtz and Sedlak 2012], the total PFAA precursor concentration determined by this approach slightly underestimates the actual concentration of precursors in samples that contained a high proportion of C_6 and shorter fluorotelomer compounds. For sulfonamide-containing and 8:2 fluorotelomer compounds, this approach does not lead to a substantial error because more than 95% of these precursors are converted into measureable perfluorinated carboxylates [Houtz and Sedlak 2012]. The total PFAA precursor concentration combined with the concentrations of all perfluorinated sulfonates and perfluorinated carboxylates is referred to from this point forward as the total PFAS concentration.

3.3 Results

3.3.1 Analysis of Pure AFFF Samples

AFFF samples manufactured by 3M (1988-2001), National Foam (2002-2008), Ansul (1984-2010), Chemguard (2008-2010), and Buckeye (2009) were directly analyzed for AFFF-related PFAA precursors (Table 3.1), perfluorinated sulfonates, and perfluorinated carboxylates (Figure 3.4a, Table 3.1) in addition to perfluorinated carboxylates generated upon oxidation (Figure 3.4b, Table 3.2). The perfluorinated sulfonates were the primary PFASs in the 3M formulations, with 0.1 to 0.2 times as much PFHxS (0.5 g/L to 1.4 g/L) as PFOS (4.9 to 11.4 g/L). Relatively low concentrations of perfluorinated carboxylates (0.1 g/L to 0.5 g/L) were also detected in the 3M formulations. Neither perfluorinated carboxylates nor perfluorinated sulfonates were detected in any of the formulations from the other manufacturers.

Upon oxidative treatment with the precursor assay, between 5.9 and 10.8 g/L of perfluorinated carboxylates were generated from oxidation of the PFAA precursors in AFFF samples (Figure 3.4b). As observed previously [Houtz and Sedlak 2012], when a sample containing PFAA precursors was exposed to hydroxyl radical under the assay conditions, the C_n sulfonamide-containing precursor compounds (*i.e.,* typically precursors to the perfluorinated sulfonates) were transformed to equimolar quantities of the corresponding C_n perfluorinated carboxylates. The fluorotelomer precursor compounds (*i.e.,* typically precursors to the perfluorinated carboxylates) were transformed to a mixture of C_4 to C_{n+1} perfluorinated carboxylates [Houtz and Sedlak 2012]. For n:2 fluorotelomer sulfonates, the ratio between the C_{n+1}: C_n: C_{n-1}: C_{n-2} perfluorinated carboxylate products was approximately 0.25:1.0:1.3:1.0 [Houtz and Sedlak 2012]. Thus, C_n precursor compounds in 3M formulations produced equimolar quantities of the corresponding C_n perfluorinated carboxylate, whereas n:2 precursors in the other AFFF formulations produced a mixture of perfluorinated carboxylate products, mainly C_n and lower.

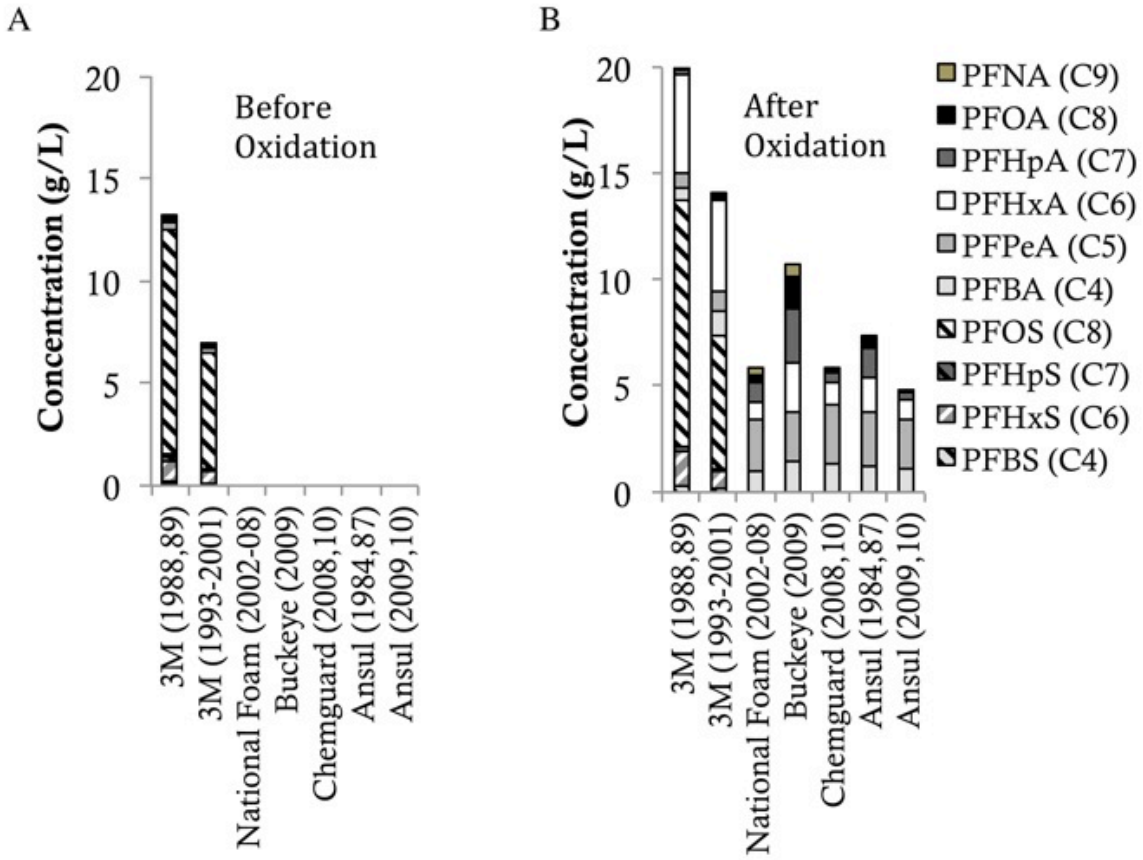


Figure 3.4: Average concentrations of perfluorinated sulfonates and carboxylates in AFFF formulations analyzed before (a) and after oxidation (b). Dates represent the years of manufacture of AFFF formulations analyzed in each category.

Table 3.1: Concentrations of PFASs (g/L) in all AFFF formulations measured. Analytes that were not detected in any samples are not shown.

	Precursors (g/L)						Perfluorinated sulfonates (g/L)				Perfluorinated Carboxylates (g/L)				
Formulation and Year	PFBSAm[a]	PFPeS-Am[a]	PFHxS-Am[a]	PFBS-AmA[a]	PFPeS-AmA[a]	PFHxS-AmA[a]	PFBS	PFHxS	PFHpS	PFOS	PFBA	PFPeA	PFHxA	PFHpA	PFOA
3M 1988			0.4				0.1	1.4	0.3	11		0.3	0.1		0.1
3M 1989	0.1		1.1			0.1	0.1	0.9	0.2	11		0.5	0.3		0.1
3M 1992	0.9	0.6	4.8	1.0	0.4	2.2	0.0	0.5	0.1	5.1		0.2	0.1		0.1
3M 1993	1.3	0.8	5.1	1.3	0.6	2.7	0.1	0.6	0.1	5.6		0.3	0.1		0.1
3M 1993	0.7	0.5	3.0	1.1	0.6	2.4	0.0	0.6	0.1	5.3		0.2	0.1		0.1
3M 1998	1.3	0.9	4.7	1.4	0.6	2.6	0.1	0.8	0.1	5.7		0.5	0.1		0.1
3M 1998	1.1	0.8	5.1	1.3	0.5	2.6	0.0	0.7	0.1	4.9		0.1	0.1		0.1
3M 1999	0.8	0.6	3.8	1.2	0.5	2.5	0.1	0.6		4.9		0.2	0.1		0.1
3M 2001	0.6	0.5	4.2	1.5	0.7	3.6	0.2	1.0	0.1	8.0		0.5	0.2		0.1
	6:2 FtTAoS[b]	8:2 FtTAoS[c]													
Chemguard 2008	17														
Chemguard 2010	21														
Ansul 1986	9	3.1													
Ansul 1987	10	2.7													
Ansul 2009	18														
Ansul 2010	10														
	5:1:2 FtB[b]	7:1:2 FtB[b]	9:1:2 FtB[b]	5:3 FtB[b]	7:3 FtB[b]	9:3 FtB[b]									
Buckeye 2009	2.3	4.3	1.4	0.6	1.0	0.3									
	6:2 FtSaB[b]	8:2 FtSaB[c]	10:2 FtSaB[c]	6:2 FtSaAm[b]	6:2 FtS	8:2 FtS									
National Foam 2005	7.0	0.5	0.1	1.8	0.2	0.0									
National Foam 2005	4.8	0.3	0.0	0.4	0.1	0.0									
National Foam 2008	7.5	0.5	0.0	1.4	0.2	0.0									

[a] These compounds were quantified using the precursor oxidation assay as described earlier.
[b] These compounds were quantified using the reference materials provided by the Fire Fighting Foam Coalition
[c] These compounds were quantified using the calibration of an alternate analyte as described earlier.

Table 3.2: Percentage recovery of measured precursors in AFFF formulations as perfluorinated carboxylate products. Analytes that were not detected in any samples are not shown. [d]Formulation chemistry not captured by measured analytes.

	Precursors Measured by LC-MS/MS, mmol/L							Perfluorinated carboxylates (PFCAs) Generated Upon Oxidation, mmol/L							
	PFBS-Am	PFPeS-Am	PFHxS-Am	PFBS-AmA	PFPeS-AmA	PFHxS-AmA	Sum	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	Sum	% Recovery of Precursors as PFCA Products
3M 1988	0.1	0.1	0.8	0.0	0.0	0.0	1.1	2.6	1.3	15	0.5	0.7		20	N/A[d]
3M 1989	0.1	0.1	2.2	0.0	0.0	0.1	2.6	3.2	0.4	14	0.3	0.6		19	N/A[d]
3M 1992	2.3	1.5	9.8	2.1	0.8	4.0	21	4.3	2.0	13	0.2	0.4		19	95%
3M 1993	1.9	1.2	6.3	2.4	1.2	4.2	17	4.8	2.5	14	0.2	0.4		21	130%
3M 1993	3.3	1.7	11	2.8	1.1	4.9	24	4.5	2.0	11	0.1	0.3		18	75%
3M 1998	2.9	1.8	11	2.8	1.0	4.7	24	4.5	3.0	13	0.3	0.4		21	87%
3M 1998	3.3	2.1	9.7	3.0	1.1	4.7	24	4.5	1.2	11	0.2	0.2		17	73%
3M 1999	2.2	1.3	7.8	2.6	1.1	4.5	19	0.0	4.2	11	0.8	0.3		16	82%
3M 2001	1.6	1.1	8.7	3.2	1.4	6.6	23	6.6	2.8	22	0.6	0.9		33	150%
	6:2 FtTAoS	8:2 FtTAoS					Sum	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	Sum	% Recovery of Precursors as PFCA Products
Chemguard 2008	29						29	6.4	11	3.2	1.1	0.3	0.4	22	76%
Chemguard 2010	37						37	5.9	11	3.5	1.2	0.3	0.4	22	61%
Ansul 1986	16	4.5					20	5.7	10	5.2	4.0	1.2	0.6	27	130%
Ansul 1987	16	4.0					20	5.6	10	5.2	3.5	1.2	0.5	25	130%
Ansul 2009	30						30	6.5	11	3.9	0.9	0.3	0.1	23	76%
Ansul 2010	18						18	3.6	6	2.3	0.6	0.1	0.1	13	74%
	5:1:2 FtB	5:3 FtB	7:1:2 FtB	7:3 FtB	9:1:2 FtB	9:3 FtB	Sum	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	Sum	% Recovery of Precursors as PFCA Products
Buckeye 2009	5.3	1.4	8.1	1.9	2.2	0.5	19	6.9	8.9	7.1	7.3	3.4	1.4	35	180%
	6:2 FtSaB	8:2 FtSaB	10:2 FtSaB	6:2 FtSaAm	6:2 FtS	8:2 FtS	Sum	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	Sum	% Recovery of Precursors as PFCA Products
National Foam 2005	10	0.8	0.1	3.4	0.6	0.0	15	4.1	8.0	2.6	1.9	0.7	0.7	18	120%
National Foam 2005	7.1	0.5	0.0	0.8	0.2	0.0	8.7	4.2	7.4	2.6	2.2	0.7	0.8	18	200%
National Foam 2008	11	0.9	0.0	2.7	0.4	0.0	15	5.9	12	3.5	3.2	0.9	1.0	26	170%

All AFFF formulations except those from Buckeye produced mainly C_5 and C_6 perfluorinated carboxylates upon oxidation: in 3M formulations, PFHxA was the predominant product whereas National Foam, Chemguard, and Ansul produced substantial amounts of both PFPeA and PFHxA upon oxidation. The C_6 PFAA precursors responsible for PFHxA production in the 3M AFFF were perfluorohexane sulfonamide amine (PFHxSAm) and perfluorohexane sulfonamide amino carboxylate (Tables 3.1, 3.2) [Place and Field 2012]. The C_6 PFAA precursors responsible for PFHxA and PFPeA in the fluorotelomer-based AFFF formulations were 6:2 fluorotelomer sulfonamide amine and 6:2 fluorotelomer sulfonamide betaine in National Foam formulations and 6:2 fluorotelomer thioamido sulfonate in Ansul and Chemguard formulations (Tables 3.1, 3.2) [Place and Field 2012]. Notably, PFOA was not the major oxidation product of any of the formulations studied. Ansul AFFF formulations from 1984 and 1987 produced approximately four times as much PFOA upon oxidation as samples from 2009 and 2010, suggesting a shift away from C_8-based compounds. AFFF manufactured by Buckeye contained a mixture of precursor compounds, all fluorotelomer betaines, with perfluorinated chains ranging in length from C_5 to C_9. With C_7 serving as the dominant homolog in Buckeye's AFFF, PFHpA and PFHxA were the predominant perfluorinated carboxylates produced upon oxidation.

3.3.2 Analysis of AFFF-Contaminated Groundwater and Soil Samples

Groundwater samples collected from the firefighter training area contained a suite of PFASs: the average PFAS composition in groundwater samples was 37% perfluorinated carboxylates, 40% perfluorinated sulfonates, and 23% PFAA precursors (Figure 3.5). PFAS concentrations decreased in groundwater as distance from the burn pit increased; the spatial significance of PFAS concentrations in groundwater are discussed in more depth in a companion paper [McGuire *et al.* 2013]. Median concentrations of PFOS (19 μg/L) and PFOA (26 μg/L) were comparable to or lower than those of their C6 analogs, PFHxS (71 μg/L) and PFHxA (36 μg/L) (Table 3.3). The measureable PFAA precursors in groundwater, which predominately consisted of C_6-based compounds, included 6:2 FtS (median concentration = 25 μg/L), FHxSA (median concentration = 4.0 μg/L), and 8:2 FtS, which was detected in fewer than half of groundwater samples (Table 3.3). 6:2 and 8:2 FtS made up 11%, on average, of PFAS content of groundwater samples, and FHxSA made up 3% (Figure 3.5). None of the PFAA precursors in AFFF formulations were detected in groundwater at concentrations above method detection limits (0.1 to 0.4 μg/L) (Table A3.2). An average of 9% of the total PFAS concentration (approximately 40% of all PFAA precursors in groundwater) consisted of PFAA precursors not detected by direct LC-MS/MS analysis (Figure 3.5).

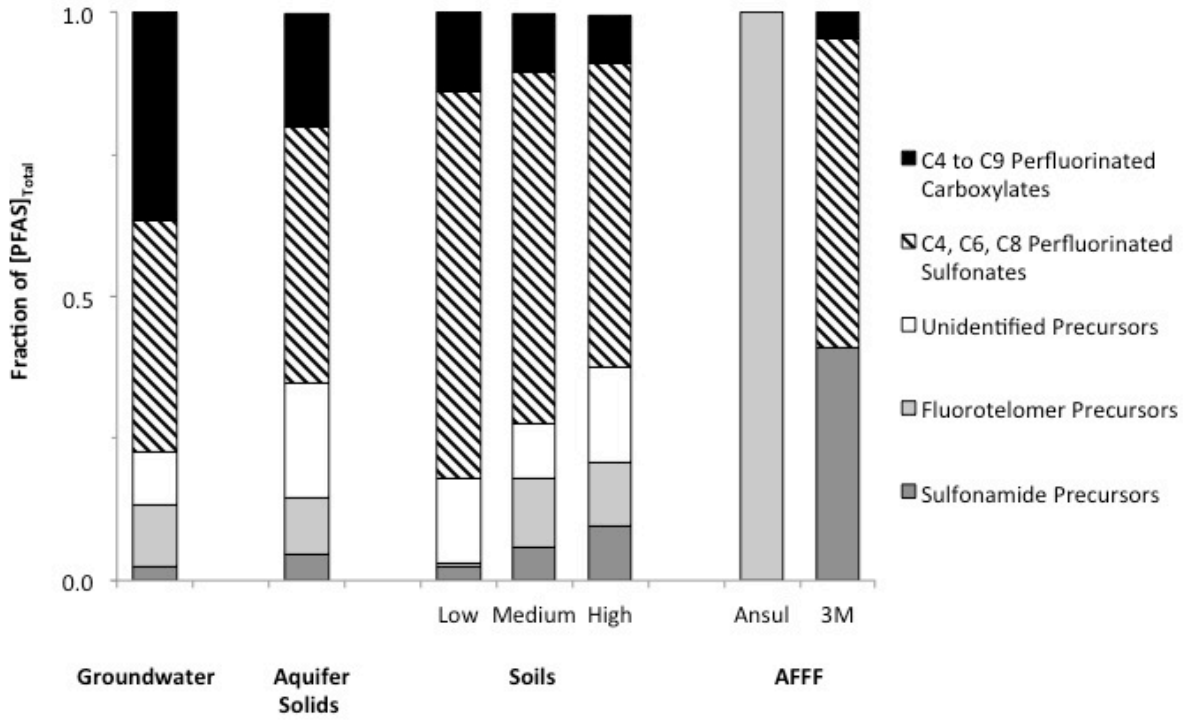


Figure 3.5: The average molar fraction of n samples of different classes of PFASs in groundwater (n=22), aquifer solids (n=10), low contamination soils (n=6), medium contamination soils (n=5), high contamination soils (n=5) and AFFF formulations (Ansul: n=2, 3M: n=2) from the 1980's. The total concentration of PFASs is equal to the summation of the concentrations of perfluorinated carboxylates, perfluorinated sulfonates, and total precursors (*i.e.,* precursors measured by LC-MS/MS plus additional precursors measured by the oxidation assay). Low, medium, and high contamination soils contained 0.1 to 1 µmol/kg, 1 to 10 µmol/kg, and 10 to 200 µmol/kg total PFASs, respectively. Fluorotelomer and Sulfonamide Precursors were measured through chemical-specific analysis. Unidentified Precursors are the portion of perfluorinated carboxylates produced in oxidized samples that could not be attributed to the fluorotelomer and sulfonamide-based precursors.

Table 3.3: Concentrations (µg/l) of individual PFASs and perfluorinated carboxylates generated upon oxidation (*i.e.* Δ[perfluorinated carboxylates]) in groundwater samples. Analytes that were not detected in any samples are not shown.

	Precursors			Perfluorinated carboxylates						Perfluorinated sulfonates				Δ [Perfluorinated carboxylates]				
	6:2 FtS	**8:2 FtS**	**FHxSA**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C4**	**C6**	**C7**	**C8**	**C4**	**C5**	**C6**	**C7**	**C8**
Sample	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
LOQ	0.06	0.1	0.1	0.5	0.3	0.1	0.2	0.1	0.2	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TMP 21	0.1	ND	ND	0.0	0.1	ND	ND	ND	ND	ND	ND	ND	ND	0.3	ND	ND	ND	1.2
TMP 12	ND	ND	ND	0.2	0.3	0.3	ND	0.2	ND	ND	ND	ND	ND	0.6	ND	ND	1.7	0.9
970101	0.5	ND	ND	ND	0.5	1.4	ND	0.2	ND	1.1	3.2	ND	ND	1.2	0.7	ND	ND	ND
TMP 11	0.5	ND	0.5	ND	1.3	3.3	ND	2.1	ND	1.6	9.9	0.2	0.2	2.7	0.6	ND	2.0	0.2
TMP 17	7.0	ND	ND	6.5	12.6	22	2.9	11	ND	8.4	32	0.1	1.6	ND	1.6	8.7	0.3	ND
TMP 03	6.4	0.4	7.2	4.0	8.0	14	2.0	8.4	ND	5.8	31	0.7	16	4.4	3.4	13	0.2	1.1
TMP 14	12	0.4	1.2	4.0	8.8	17	2.3	11	0.5	7.6	38	0.9	19	3.9	3.1	8.0	0.4	ND
TMP 15	17	ND	0.7	4.1	7.5	14	2.6	17	ND	5.6	39	2.1	14	6.5	4.5	7.9	0.2	ND
TMP 20	24	ND	1.3	4.2	7.2	14	2.3	14	ND	6.2	40	1.9	14	5.2	5.2	8.6	0.2	1.8
EMW 21	11	1.7	11	4.4	8.1	16	2.0	10	ND	5.5	37	1.4	35	4.8	5.7	19	0.9	1.6
EMW 01	13	2.1	15	3.6	8.4	16	2.4	11	0.4	5.0	37	1.0	35	7.2	5.2	21	0.6	1.6
TMP 01	38	0.1	12	6.2	8.9	31	3.2	12	ND	9.7	40	1.5	26	13	14	31	0.3	1.8
EMW 08	51	ND	ND	6.9	12	35	4.3	36	ND	14	77	2.6	8.8	10	8.7	11	0.9	ND
EMW 04	18	ND	3.5	12	22	58	6.1	27	ND	25	64	0.7	1.9	22	8.0	18	0.4	4.1
EMW 09	44	ND	ND	7.2	15	37	5.6	36	ND	18	98	2.7	7.8	13	8.3	20	ND	4.1
TMP 02	26	0.1	21	12	18	51	7.9	28	0.3	21	99	3.9	17	31	20	34	ND	3.1
EMW 20	48	0.9	33	12	26	61	6.8	26	0.5	21	93	3.8	55	23	15	49	1.0	1.3
EMW 02	40	0.6	34	12	28	68	6.7	26	ND	21	92	3.6	60	27	17	50	1.3	4.4
TMP 13	110	0.6	4.5	16	45	61	13	34	2.8	23	170	4.6	31	20	16	24	ND	0.0
TMP 06	140	13	ND	14	24	56	6.7	48	0.2	20	120	9.2	79	27	24	76	2.5	5.6
TMP 09	69	7.1	53	19	44	77	12	50	0.7	27	170	5.9	93	28	17	72	ND	ND
930103	270	4.1	7.5	14	38	53	9.3	30	2.9	17	140	18	86	29	32	54	4.3	3.5
80102	210	ND	11	27	75	130	15	52	1.4	46	300	6.3	100	53	39	49	ND	ND
890102	17	ND	ND	87	220	320	20	40	ND	120	290	3.2	19	22	ND	ND	ND	ND
EMW 03	96	ND	11	60	130	300	33	190	2.7	140	400	4.7	24	39	1.3	36	ND	ND
TMP 04	150	0.6	26	56	130	250	29	120	2.7	120	530	6.3	57	35	15	42	ND	ND
Min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Median	25	ND	4.0	7.0	14	36	4.9	26	ND	15	71	2.4	19	13	6.8	19	0.2	1.0
Max	270	13	53	87	220	320	33	190	2.9	140	530	18	100	53	39	76	4.3	5.6

In aquifer solids and soils from the firefighter training area, perfluorinated sulfonates and PFAA precursors usually accounted for a larger fraction of total PFAS concentration relative to groundwater samples (Figure 3.5). PFAA precursors accounted for an average of 35% of the total PFAS concentration in aquifer solids samples (Figure 3.5). In surficial soils, PFAA precursors increased as a proportion of total PFAS concentration as the total PFAS concentration in the soil increased; precursors accounted for an average of 26% of the total PFAS concentration across all samples and 38% on the five most contaminated surficial soils (Figure 3.5).

PFOS was the PFAS species detected at the highest concentration on nearly every soil (median = 2,400 μg/kg) and aquifer solid (median = 270 μg/kg) sample (Table 3.4). The ratio of PFHxS (median soil = 66 μg/kg, median aquifer solids = 150 μg/kg) to PFOS ranged from 0.01 to 10. The perfluorinated carboxylates were detected at significantly lower concentrations than perfluorinated sulfonates on soils and aquifer solids (Figure 3.5). Among the perfluorinated carboxylates, PFOA predominated on surficial soils (median = 21 μg/kg PFOA, 11 μg/kg PFHxA) and PFHxA predominated on aquifer solids (median = 35 μg/kg PFOA, 45 μg/kg PFHxA) (Table 3.4). A paired t-test indicated that the log transformed total concentration of PFASs was statistically greater on surficial soils than aquifer solids ($p<0.05$). As observed in groundwater samples, PFAS concentrations on both soils and aquifer solids mainly decreased with distance from the burn pit [McGuire *et al.* 2013].

PFHxSAm, a PFAA precursor present in AFFF formulations manufactured by 3M, was the only PFAA precursor present in AFFF that was detected on soils and aquifer solids. The compound was detected on fewer than 30% of the samples, with most detections on samples that contained high concentrations of PFOS and other perfluorinated sulfonates present in AFFF manufactured by 3M. In addition to PFHxSAm, the other precursors detected on solids included 6:2 FtS (median = 85 μg/kg surficial soils; 68 μg/kg aquifer solids), 8:2 FtS (median = 81 μg/kg surficial soils; 42 μg/kg aquifer solids), FHxSA (median = 12 μg/kg surficial soils; 14 μg/kg aquifer solids), and FOSA (median = 3 μg/kg surficial soils; 7 μg/kg aquifer solids). Concentrations of the PFAA precursor compounds observed by direct LC-MS/MS analysis typically increased as the concentrations of the perfluorinated sulfonates increased on both surficial soils and aquifer solids.

Approximately half of the PFAA precursors on soil and aquifer solids samples measured by the oxidation assay were attributable to PFHxSAm, 6:2 FtS, 8:2 FtS, FHxSA, or FOSA. The fluorotelomer-based PFAA precursors accounted for 8% and 10% of average molar PFAS content on surficial soils and aquifer solids, respectively, and the sulfonamide-based PFAA precursors accounted for an average of 5% of total PFASs on both types of solids (Figure 3.5). On average, more unidentified PFAA precursors were present on aquifer solids (20% of total PFASs), than in soils (13% of total PFASs).

Many samples within each type of media contained specific PFAS analytes in similar proportions to one another. However, due to the complex pattern of AFFF contamination at the site, statistically significant correlations were not observed among two or more analytes in groundwater, soil, or aquifer solids samples.

Table 3.4: Concentrations (μg/kg) of individual PFASs and perfluorinated carboxylates generated upon oxidation (*i.e.* Δ[perfluorinated carboxylates]) in surficial soil (0.6 m below surface) and aquifer solids (5 to 6 m below surface) samples. Analytes that were not detected in any samples are not shown.

	Precursors					Perfluorinated carboxylates						Perfluorinated sulfonates				Δ [Perfluorinated carboxylates]					
Sample	6:2 FtS	FHx-SA	PFHx SaAm	8:2 FtS	FO-SA	C4	C5	C6	C7	C8	C9	C4	C6	C7	C8	C4	C5	C6	C7	C8	C9
LOQ	0.4	0.6	0.8	0.6	0.6	3	1.1	0.8	1.1	0.8	1.1	1.7	0.6	0.6	0.4	1	1	1	1	1	1
	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg
S-16S	ND	ND	ND	ND	ND	ND	ND	3	ND	2	ND	ND	18	ND	11	ND	ND	ND	ND	ND	ND
S-15S	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3	ND	45	ND	ND	ND	ND	ND	ND
S-12S	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	5	ND	41	5	1	4	ND	1	ND
S-14S	ND	2	7	ND	3	ND	4	8	ND	5	ND	3	22	ND	69	3	3	15	1	5	ND
S-13S	5	2	ND	2	ND	ND	9	4	3	7	5	ND	44	ND	190	ND	3	13	ND	7	ND
S-11S	ND	ND	ND	3	ND	4	7	10	3	17	3	4	70	ND	280	4	3	3	ND	9	1
S-7S	470	12	ND	20	3	75	290	480	51	310	ND	130	830	ND	96	73	200	160	26	100	2
S-8S	110	5	ND	800	Nd	ND	10	12	16	95	20	ND	150	ND	2400	31	130	190	220	180	20
S-6S	40	12	8	130	3	ND	4	6	3	9	7	ND	37	19	3800	30	37	200	29	30	ND
S-4S	80	23	12	780	18	ND	7	9	ND	19	5	ND	58	ND	3000	43	110	420	190	360	15
S-1S	140	870	ND	150	180	ND	0	19	ND	23	8	ND	61	ND	3100	ND	71	840	45	97	ND
S-9S	350	18	ND	790	8	ND	30	33	9	82	5	ND	270	ND	2300	38	200	1000	290	260	14
S-18S	680	1700	6	32	89	4	7	15	4	61	10	ND	140	96	2400	120	210	1500	33	78	2
S-10S	90	12	ND	730	18	ND	11	16	10	35	10	ND	160	22	8300	120	200	300	280	250	22
S-2S	1900	58	120	280	82	410	820	880	88	720	5	510	5600	89	5900	630	820	1600	290	320	13
S-5S	6200	1500	1600	240	3400	380	1300	2000	320	5200	ND	610	13000	430	20000	2200	2600	11000	830	4000	53
Min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3	ND	11	ND	ND	ND	ND	ND	ND
Med.	85	12	ND	81	3	ND	7	11	3	21	5	ND	66	ND	2400	31	91	195	31	87	2
Max	6200	1700	1600	800	3400	410	1300	2000	320	5200	20	610	13000	430	20000	2200	2600	11000	830	4000	53
S-14D	ND	5	ND	ND	3	3	12	23	4	25	ND	14	100	4	190	7	3	17	5	19	ND
S-6D	88	13	ND	22	3	4	14	22	3	16	ND	8	53	ND	270	ND	20	34	7	8	ND
S-1D	26	69	ND	6	28	14	18	78	22	32	ND	14	87	ND	98	ND	14	57	7	30	ND
S-9D	25	4	ND	70	2	ND	18	16	7	11	ND	2	40	ND	250	ND	25	220	42	33	2
S-4D	51	12	ND	46	11	6	21	44	3	13	ND	14	51	ND	260	ND	31	140	18	130	2
S2-D	120	16	ND	10	8	52	59	135	19	38	ND	88	200	ND	170	5	38	68	ND	15	ND
S-8D	56	11	ND	55	ND	ND	16	39	11	130	2	5	280	5	460	ND	25	580	12	10	2
S-5D	310	18	13	90	19	24	82	150	20	130	ND	60	270	ND	530	46	85	300	49	67	2
S-3D	80	110	14	86	86	11	17	46	5	67	ND	18	210	11	940	39	89	500	78	170	2
S-10D	370	110	ND	38	5	17	95	210	31	88	2	49	870	27	1000	86	97	120	20	41	1
Min	ND	4	ND	ND	ND	ND	12	16	3	11	ND	2	40	ND	98	ND	3	17	ND	8	ND
Med.	68	14	ND	42	7	9	18	45	9	35	ND	14	150	ND	270	3	28	130	15	31	1
Max	370	110	14	90	86	52	95	210	31	130	2	88	870	27	1000	86	97	580	78	170	2

3.4 Discussion

3.4.1 Identifying Likely Sources of AFFF

To gain insight into the fate of PFASs released at firefighter training sites, it is important to understand the composition of the AFFF formulations that were applied. Despite the absence of records on AFFF purchases and applications and an incomplete knowledge of the composition of AFFF manufactured prior to 1984, the archived AFFF samples and available information on the U.S. military's AFFF stockpiles provide an understanding of the likely composition of AFFF used onsite.

Most of the mass of PFASs in groundwater and solid samples resemble the PFASs in the archived 3M formulations produced between 1988 and 2001. The perfluorinated sulfonates, which were present at high concentrations in all 3M formulations (Figure 3.4a, Figure 3.5), accounted for 40% to 60% of the PFASs in groundwater and solid samples (Figure 3.5). The PFAA precursor PFHxSAm detected in archived 3M AFFF samples was also observed on soils and aquifer solids. FHxSA, which was detected frequently in groundwater and solids samples, was a probable transformation product of sulfonamide-based C_6 precursor compounds like PFHxSAm.

There is also evidence of the use of 3M formulations with a different PFAS composition than those of the archived samples. The C_8 sulfonamide-based precursor FOSA was detected at concentrations within an order of magnitude of PFOS in many soil and aquifer solids samples. This finding is inconsistent with the archived AFFF formulations manufactured by 3M between 1988 and 2001, which contained negligible concentrations of C_8 precursors (Figure 3.4b). A 1971 U.S. patent submitted by the 3M Company identified a C_8 sulfonamide-containing precursor in their AFFF products that may have been the source of FOSA [Francen 1971]. Prior to 1976, 3M's AFFF formulations reportedly contained high concentrations of perfluorinated carboxylates and their derivatives [Prevedouros *et al.* 2006, Tuve 1966, Chiesa 1974, Chiesa and Di Maio 1976]. The perfluorinated carboxylates detected in groundwater and solid samples could have originated from these early 3M formulations. They also could have been derived from transformation of fluorotelomer precursors in AFFF formulations from other manufacturers.

Although the 3M Company provided most of the military's AFFF prior to 2001 [Darwin 2004], AFFF manufactured by National Foam and Ansul were also approved for military use starting in 1976 [MIL-F-24385 2011]. AFFF formulations manufactured by one or both of these companies appear to have been used in the firefighter training area, as evidenced by the presence of transformation products of fluorotelomer compounds, such as 6:2 FtS and 8:2 FtS, in all contaminated media. These fluorotelomer compounds could not have been generated from 3M's precursors. However, fluorotelomer precursors that occur in the archived National Foam and Ansul AFFF (*e.g.* 6:2 FtTaOS or 6:2 FtSaB) were not measured in any contaminated samples. The absence of these AFFF-derived compounds implies that they were transformed after release. The abundance of 8:2 FtS on surficial soil samples indicates that the fluorotelomer-based AFFF applied in the firefighter training area contained a significant proportion of C_8 precursor compounds, similar to the archived Ansul AFFF formulations from 1984 and 1987 (Figure 3.3b).

3.4.2 AFFF Precursor Transformation

Despite the fact that they accounted for between 41% and 100% of the PFASs in AFFF formulations (Figure 3.5), the PFAA precursors observed in AFFF formulations were largely absent from groundwater, soil, and aquifer solid samples. PFHxSAm, the only precursor compound in AFFF that was detected onsite, was only detected sporadically. On average, the concentration of PFHxSAm was 5% of the molar concentration of PFHxS on soil samples. In the soil sample with the highest PFHxSAm concentration, the concentration of PFHxSAm was 25% of the concentration of PFHxS. In AFFF formulations from 1988 and 1989, the concentration of PFHxSAm was 60% of the concentration of PFHxS. In 3M formulations manufactured after 1989, PFHxSAm concentrations were five times higher than those of PFHxS. Because AFFF manufactured by 3M is the only source of these two compounds, these data suggest that most PFHxSAm released onsite was transformed.

The absence of AFFF precursors at this site is consistent with the two other studies where these compounds were measured at firefighter training areas [Backe *et al.* 2013, Moe *et al.* 2012]. In groundwater samples collected from facilities that operated contemporaneously with this site, AFFF precursor concentrations were below detection limits or were present at concentrations several orders of magnitude lower than the other PFASs [Backe *et al.* 2013]. A National Foam AFFF precursor (*i.e.,* 6:2 fluorotelomer sulfonamido betaine) was detected on soils outside a Norwegian airport, but concurrent detection of four of its putative transformation products suggests that it may not persist [Moe *et al.* 2012].

Several precursor compounds that were not present in any of the AFFF samples were detected at the site, including FOSA, FHxSA, 6:2 FtS and 8:2 FtS. It is likely that these compounds were formed from the biological or chemical transformation of precursors present in AFFF; various remedial activities that occurred onsite, including the installation of oxygen infusion wells [McGuire *et al.* 2013], may have promoted the biotransformation of these precursors. FOSA and FHxSA contain sulfonamide moieties adjacent to the perfluorinated chain, just like the precursors in AFFF formulations manufactured by 3M. FOSA is a known product of aerobic microbial transformation of sulfonamide-containing compounds such as *N*-ethyl perfluorooctane sulfonamidoethanol [Rhoads *et al.* 2008] that are oxidized through carboxylation and dealkylation reactions. It has also been proposed as a metabolite of numerous sulfonamide-containing precursors in humans [Lee and Mabury 2011] and biota [Houde *et al.* 2011]. FHxSA, a shorter-chain length analog of FOSA, is a potential product of biotransformation of C_6 sulfonamide compounds in AFFF manufactured by 3M. 6:2 FtS and 8:2 FtS could have been produced by one or more oxygen additions to the thio group followed by dealkylation of fluorotelomer thioamido sulfonates in Ansul AFFF or fluorotelomer sulfonamide betaines and amines in AFFF manufactured by National Foam. 6:2 FtS and 8:2 FtS have also been detected at relatively high concentrations at other sites where AFFF was used for firefighter training [Schultz *et al.* 2004, Backe *et al.* 2013].

Data from the precursor assay indicated that most samples contained PFAA precursors that were not identified by direct LC-MS/MS measurement of specific compounds (Figure 3.5). The distribution of perfluorinated carboxylates produced upon oxidation provides insight into the identity of the unknown precursors (Figure 3.6). The left side of Figure 3.6 consists of a series of bars that depicts the precursors measured in the sample next to the distribution of perfluorinated carboxylates expected after the sample is subjected to the oxidation assay. The right side of the figure compares the sum and distribution of the measured precursors and their expected perfluorinated carboxylate products with the sum and distribution of perfluorinated carboxylate products observed when the sample was oxidized. This comparison indicates that the measured precursors accounted for only 40% of the observed perfluorinated carboxylate products in the oxidized sample (Figure 3.6). The homolog profile of unattributed perfluorinated carboxylate production suggests that unidentified precursors mainly consisted of C_6 compounds: PFHxA measured after oxidation was 3.5 times higher than expected, while the concentration of PFOA produced upon oxidation was only ten percent higher. Because PFPeA and PFBA were also generated in greater concentrations than predicted, we estimate that up to 20% of the C_6 precursors had 6:2 fluorotelomer groups [Houtz and Sedlak 2012]. The remainder of the generated PFHxA in this sample appears to have been related to C_6 sulfonamide-containing molecules in 3M AFFF formulations.

Using the approach described for the single sample in Figure 3.6, the structures of the unidentified precursors in other samples were inferred by comparing the homolog pattern of the oxidation products with the precursors measured by LC-MS/MS (Figure 3.7). Of the perfluorinated carboxylate production that was not attributable to directly measured precursors in groundwater, PFBA and PFHxA accounted for an average of 85% (Figure 3.7). The absence of significant PFPeA production means that PFHxA was mainly generated from sulfonamide-containing precursor compounds [Houtz and Sedlak 2012]. Similarly, relatively high PFBA production compared to PFPeA production indicates the presence of C_4 precursors that were fluorotelomer-based or sulfonamide-based. Because hydrophilicity increases with decreasing perfluorinated chain length, it is unsurprising to observe a larger fraction of C_4 PFAA precursors in groundwater relative to solids.

The profile of perfluorinated carboxylates produced from unidentified precursor compounds in aquifer solids and soils was enriched with longer chain homologs (Figure 3.7). Like groundwater, PFHxA was the main product of unidentified precursors, but significant concentrations of PFHpA and PFOA were also generated from unidentified longer-chain precursors. The unattributed fraction of PFHxA on aquifer solids was mainly generated by sulfonamide-based C_6 precursors, as evidenced by the comparatively low production of PFPeA and PFBA. The composition of precursors on surficial soils is less certain because production of all homologs was significant.

There are two likely sources of the unidentified fraction of PFAA precursor compounds. First, the unidentified precursors could have been composed of precursors from AFFF formulations that were not present in the archived AFFF formulations and thus were not directly measured in our study. A second explanation is that the unidentified precursors were transformation products of AFFF-derived precursor compounds.

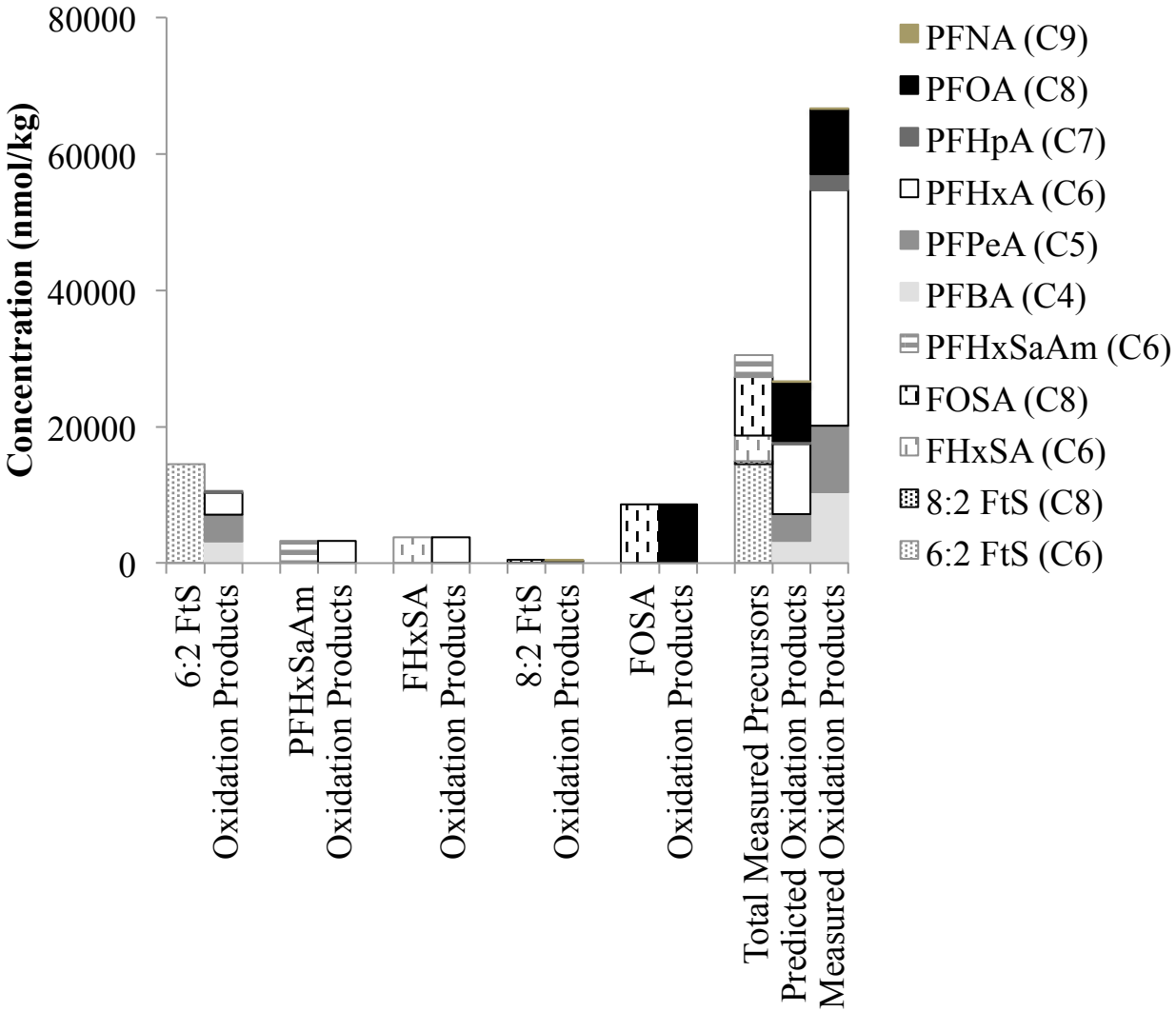


Figure 3.6: The measured PFAA precursor compounds in a contaminated soil sample extract (S5-S) compared with the expected and measured perfluorinated carboxylate products in the oxidized extract. The bars labeled "Total Measured Precursors" and "Predicted Oxidation Products" are summations of the respective bars to their left.

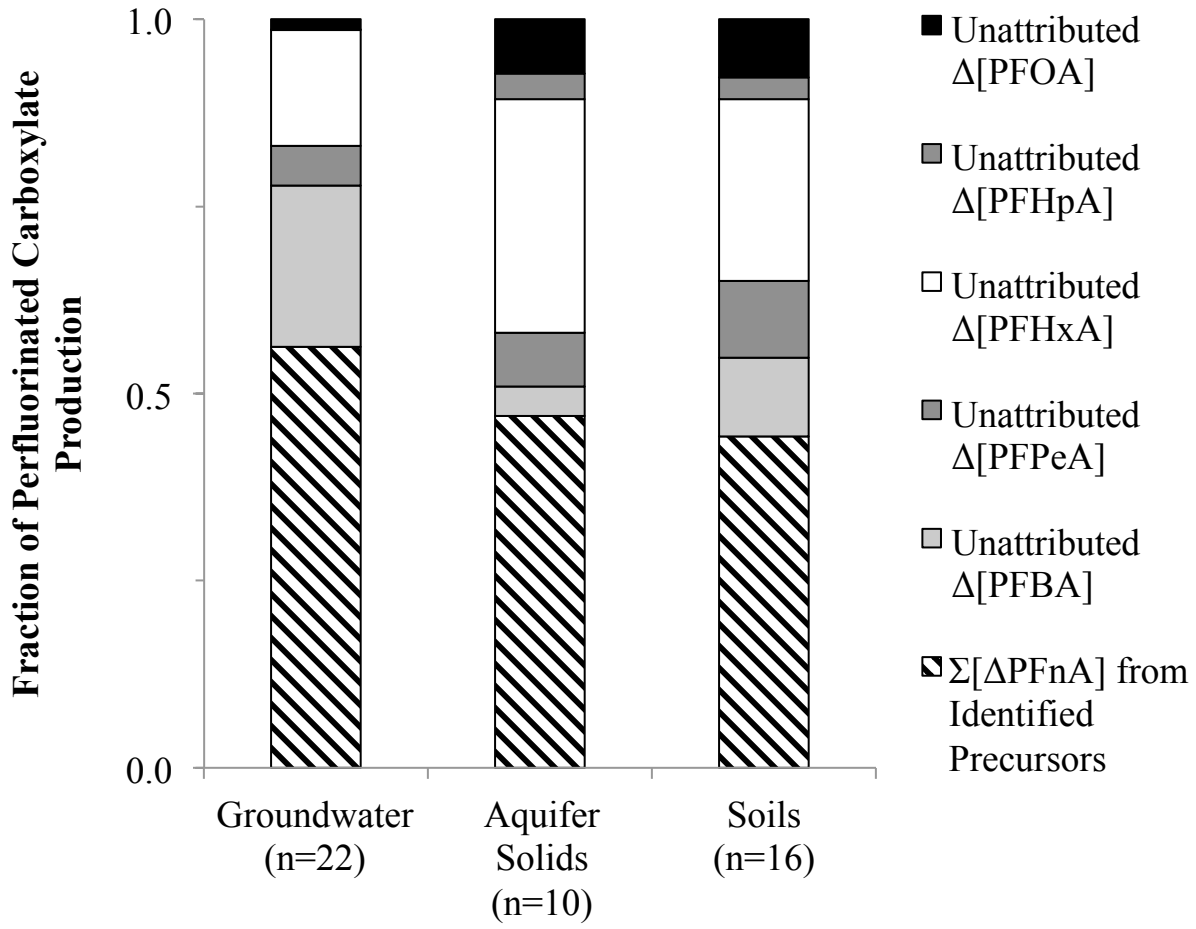


Figure 3.7: The average composition of perfluorinated carboxylates generated by precursor compounds in groundwater, aquifer solids, and soils from the firefighter training area.

Biotransformation of fluorotelomer precursor compounds with structures similar to those in AFFF has been observed previously [Wang *et al.* 2011, Wang *et al.* 2009]. In laboratory microcosms, fluorotelomer compounds, including 6:2 FtS, were transformed to perfluorinated carboxylates upon exposure to mixed cultures of aerobic bacteria [Wang *et al.* 2011, Wang *et al.* 2009]. For example, 6:2 FtS underwent desulfonation followed by oxidation and decarboxylation to be converted into PFHxA and PFPeA in activated sludge microcosms [Wang *et al.* 2011]. In most studies on biotransformation of n:2 fluorotelomer compounds, both the C_n and C_{n-1} perfluorinated carboxylates were produced at similar concentrations [Wang *et al.* 2011, Wang *et al.* 2009]. As a result, the occurrence of even C_n and odd C_{n-1} perfluorinated homologs in samples would support their origination from biotransformation of fluorotelomer compounds.

Despite the presence of relatively high concentrations of PFOA observed in many soil and groundwater samples, PFHpA was rarely detected. The absence of PFHpA suggests that biotransformation of 8:2 FtS was not the main source of PFOA, assuming that 8:2 FtS follows a similar transformation pathway to 6:2 FtS [Wang *et al.* 2011]. The ratio of PFHxA to PFPeA in most soil and groundwater samples was roughly consistent with the expected perfluorinated carboxylate product distribution from aerobic transformation of 6:2 FtS [Wang *et al.* 2011]. Much of the PFHxA and PFPeA detected onsite could have resulted from biotransformation of 6:2 fluorotelomer compounds, but it is impossible to test this hypothesis without specific knowledge of the composition of 3M AFFF formulations containing perfluorinated carboxylates manufactured in the early 1970's.

The isomer distribution of individual perfluorinated carboxylate homologs could be used in future research to identify their source [Benskin *et al.* 2010]. 3M historically used a manufacturing process that generates a mixture of roughly 30% branched and 70% linear isomers of perfluorinated compounds [Prevedouros *et al.* 2006], while the fluorotelomer manufacturing process only produces compounds with linear perfluorinated chains. Although the method used in this study did not quantify individual perfluorinated carboxylate isotopes, the ratio of linear to branched isomers of perfluorinated homologs could be used to distinguish the origins of perfluorinated carboxylates in an AFFF-contaminated area.

The transformation of precursors can serve as an important source of additional perfluorinated sulfonates. This is especially true for C_6 homologs. In the AFFF formulations from 3M, transformation of the precursors could have increased the concentration of PFHxS by a factor of four if all C_6 precursors were transformed to PFHxS (Figure 3.4a-b). Thus, an increase in the ratio of PFHxS to PFOS in contaminated samples relative to those observed in AFFF would imply that C_6 precursors had been transformed to terminal PFHxS. AFFF manufactured by 3M from 1988-1989 exhibited a PFHxS-to-PFOS mass ratio of 0.1. On surficial soils, PFHxS to PFOS ratios varied widely, but the ratio between the median concentrations of PFHxS and PFOS was 0.23. On the most contaminated sample analyzed, the PFHxS to PFOS ratio was 0.66. Similarly, the ratio between the median concentrations of PFHxS and PFOS was greater than 0.5 on aquifer solids and greater than 2 in groundwater. In all media, there was more than a two-fold increase in the PFHxS-to-PFOS mass ratio compared with the representative 3M formulations from 1988-1989, suggesting substantial transformation of 3M-derived C_6 precursors to PFHxS.

Transformation of AFFF-derived precursors to perfluorinated carboxylate and perfluorinated sulfonate products would also be indicated by a decrease in the percentage of precursor compounds relative to AFFF formulations. In all media, the average percentage of precursors as a share of total PFASs declined relative to Ansul and 3M AFFF formulations from the 1980's (Figure 3.5), which contained 100% and 41% PFAA precursors as a percentage of total PFAS concentrations, respectively. The declines in precursor content were more pronounced in groundwater (average = 23% precursors) than in soils and aquifer solids (average = 28% precursors). It is possible that older AFFF formulations containing smaller relative PFAA precursor concentrations than the archived AFFF accounted for some of these observations, but PFAA precursor compounds have been included in AFFF patents through the entire era of AFFF production and were likely present in older AFFF formulations [Prevedouros *et al.* 2006, Tuve 1966, Chiesa 1974, Francen 1971, Berger 1982, Fielding *et al.* 1977].

Overall, significant production of perfluorinated sulfonates and carboxylates from AFFF-derived PFAA precursors appears to have occurred in the firefighter training area. However, the presence of PFAA precursors more than twenty years after AFFF applications ceased indicates that the rate of transformation of precursors to the terminal PFAAs has been slow.

3.5 Environmental Implications

For at least three decades, AFFF formulations have contained polyfluorinated compounds that are potential precursors of perfluorinated carboxylates and perfluorinated sulfonates. The health effects of precursors from AFFF formulations currently are unknown, but perfluorinated carboxylates and perfluorinated sulfonates have been associated with adverse health effects in humans and biota [Nelson *et al.* 2009, Lau *et al.*. 2007, Fei *et al.* 2007, Sakr *et al.* 2007a, Costa *et al.* 2009, Sakr *et al.* 2007b]. The data collected in this study provide strong evidence that most of the PFAA precursors originally present in AFFF were transformed into other precursors and PFAAs over a period of several decades in the subsurface. The transformation products may be more mobile than the compounds originally present in AFFF thereby expanding the plume of PFAS contamination.

Firefighter training sites are often contaminated with halogenated solvents and hydrocarbons. As AFFF-impacted sites are remediated for these co-contaminants or for the perfluorinated carboxylates and sulfonates, the co-occurrence of AFFF-related precursors cannot be ignored. Some remedial activities targeted at co-contaminants may inadvertently accelerate the production of PFAAs. For example, *in situ* chemical oxidation often generates oxidants that convert precursors into perfluorinated carboxylates through the mechanism employed in the precursor assay. Additionally, the slow biotransformation of AFFF precursors contributes to the total concentration of PFAAs even if no action is taken at a site. As a result, the mass of precursors may need to be remediated along with the PFAAs for complete site closure if PFAAs are the target of the remediation effort.

CHAPTER 4. Biotransformation of AFFF-derived PFAA precursors in soil microcosms under aerobic and anaerobic conditions.

4.1 Introduction

Aqueous film-forming foam (AFFF) is a complex mixture of hydrocarbon and fluorocarbon surfactants that is used to extinguish hydrocarbon-based fuel fires at military bases, airports, oil refineries, and municipal firefighter training facilities [Moody and Field 2000]. Since its introduction in the 1960's, AFFF has been sold in a number of different formulations that have contained both the perfluoroalkyl acids (PFAAs) (*i.e.,* perfluorinated carboxylates and perfluorinated sulfonates) and a variety of polyfluorinated compounds that contain a perfluorinated moiety [Place and Field 2012, Houtz *et al.* 2013]. These polyfluorinated compounds, which are believed to be precursors to the PFAAs, have typically accounted for 40% to 100% of the poly- and perfluoroalkyl substances (PFASs) in AFFF from six of the most prominent U.S. manufacturers [Houtz *et al.* 2013]. Due to the abundance of PFAA precursors in AFFF formulations, their transformation has the potential to greatly increase the mass of perfluorinated carboxylates and perfluorinated sulfonates present in soil, sediments, and groundwater in places where AFFF has been deployed.

An understanding of the compounds produced when AFFF-derived precursors undergo transformation under field conditions is important to the management and remediation of AFFF-contaminated sites for several reasons. First, knowing the identities of intermediate products provides a basis for evaluating whether biotransformation has occurred. Second, knowing the conditions that yield different transformation products is important to human health risk assessment. For example, conditions that lead to the production of the perfluorinated sulfonates and the perfluorinated carboxylates are important because some of these compounds are associated with a number of adverse human health outcomes [Fei *et al.* 2007, Grandjean *et al.* 2012, Halldorsson *et al.* 2012, Vieira *et al.* 2013]. Finally, if bioremediation is going to be used as part of site treatment for PFASs, an understanding of the transformation potential of PFAA precursors in AFFF under different conditions is essential to a successful remediation strategy.

Measurements conducted on groundwater and soil samples collected from AFFF-impacted firefighter training areas indicate some likely transformation products of precursors found in AFFF. For example, 6:2 and 8:2 fluorotelomer sulfonate (FtS) [Schultz *et al.* 2004, Backe *et al*. 2012, Kärrman *et al.* 2012, Houtz *et al.* 2013] and 6:2 fluorotelomer sulfonamide alkyl betaine [Moe *et al.* 2012] detected at firefighter training sites were presumed transformation products of PFAA precursors originating in fluorotelomer-based AFFF formulations. Perfluorooctane sulfonamide and perfluorohexane sulfonamide detected in a firefighter training area were presumed transformation products of PFAA precursors found in the other main type of AFFF formulation, electrochemical fluorination-based AFFF manufactured by 3M [Houtz *et al.* 2013]. In addition, the concentrations and distributions of the perfluorinated carboxylates and perfluorinated sulfonates detected at one of these sites suggested that PFAA precursors had been transformed to terminal PFAA products [Houtz *et al.* 2013]. None of these field data indicated the conditions that led to the formation of these transformation products [Schultz *et al.* 2004, Backe *et al*. 2012, Kärrman *et al.* 2012, Houtz *et al.* 2013] nor did they include all polyfluorinated transformation products that may have been present in samples [Houtz *et al.* 2013].

Insight into potential biotransformation products of different types of PFAA precursors in AFFF under different redox conditions can be gained from previous laboratory studies using

similar compounds. Biotransformation of fluorotelomer precursors (Figure 1.5) in the presence of microbes from activated sludge reactors [Wang *et al.* 2005, Lee *et al.* 2010, Wang *et al.* 2011], aerobic soils [Wang *et al.* 2009, Liu *et al.* 2010, Dasu *et al.* 2012], and aerobic enrichments of ethanol-degrading bacteria [Dinglasan *et al.* 2004] has been observed. Two fluorotelomer precursors were also transformed under methanogenic conditions in anaerobic digester sludge [Zhang *et al.* 2013]. In each of these studies using fluorotelomer precursors, perfluorinated carboxylates were observed as minor products (*i.e.,* <10% of PFAA precursor transformed), in addition to intermediate fluorotelomer products, over the two to three month-long duration of these experiments. The microbial transformation of sulfonamide-containing precursors (Figure 1.5) with structures similar to the precursors in AFFF formulations manufactured by 3M was investigated under aerobic conditions [Rhoads *et al.* 2008]. In this study, perfluorooctane sulfonate (PFOS) accounted for the transformation of 5% to 40% of five different perfluorooctane sulfonamide-containing precursors that were incubated in activated sludge microcosms for ten days [Rhoads *et al.* 2008]. Overall, biotransformation of PFAA precursors under aerobic conditions often results in partial yields of PFAAs, however the aerobic biotransformation reactions of many types of precursors have not been studied. The potential biotransformation of most PFAA precursors under anaerobic conditions remains unknown.

The objective of this study was to characterize the microbial transformation of PFAA precursors in common AFFF formulations under controlled laboratory conditions that are relevant to AFFF-impacted sites. To accomplish this goal, microcosms were prepared using soil or sediment as inocula. The microcosms were established using five different terminal electron acceptors – oxygen, nitrate, ferric iron, sulfate, and carbon dioxide. PFAS transformation products were identified by mass spectrometry and the total concentration of PFAA precursors in microcosm slurries was measured by the precursor assay described in Chapter 3.

4.2 Materials and Methods

4.2.1 Materials

AFFF formulations were obtained from U.S. military bases as described previously [Place and Field 2012]. The composition of a typical AFFF sample consists of PFASs dissolved in a mixture of water and diethylene glycol butyl ether (DGBE) (Table 4.1). Concentrations of PFAA precursors in AFFF formulations (Figure 4.1A-C) used to construct microcosms are reported in Table 4.2.

All isotopically-labeled compounds, perfluorinated carboxylates, perfluorinated sulfonates, 6:2 fluorotelomer unsaturated carboxylate (6:2 FtUA), and fluorotelomer sulfonates (FtSs) (Figure 4.1D) used as analytical standards were obtained from Wellington Laboratories (Guelph, Ontario, CA). A commercial source material containing 6:2 fluorotelomer thioamido sulfonate (6:2 FtTAoS) (Figure 4.1C) was provided by the Fire Fighting Foam Coalition [Backe *et al.* 2013]. Diethylene glycol butyl ether (DGBE) and a mixture of 4:2, 6:2, 8:2, and 10:2 fluorotelomer thio carboxylates (FtTCA) (Figure 4.2E) sold as a product called Zonyl FSA were purchased from Sigma Aldrich. All other materials were purchased from Sigma Aldrich or Fisher Scientific at the highest purity possible.

Table 4.1: Typical composition of AFFF formulations [3M Company 2005, Wormald 2008, National Foam 2010, Chemguard 2006].

Component	Percentage by weight
Water	70 - 80%
Diethylene glycol butyl ether (DGBE)	5 - 20%
Hydrocarbon surfactants	1 - 10%
Fluorocarbon surfactants	1 - 6%
Other ingredients	1 - 8%

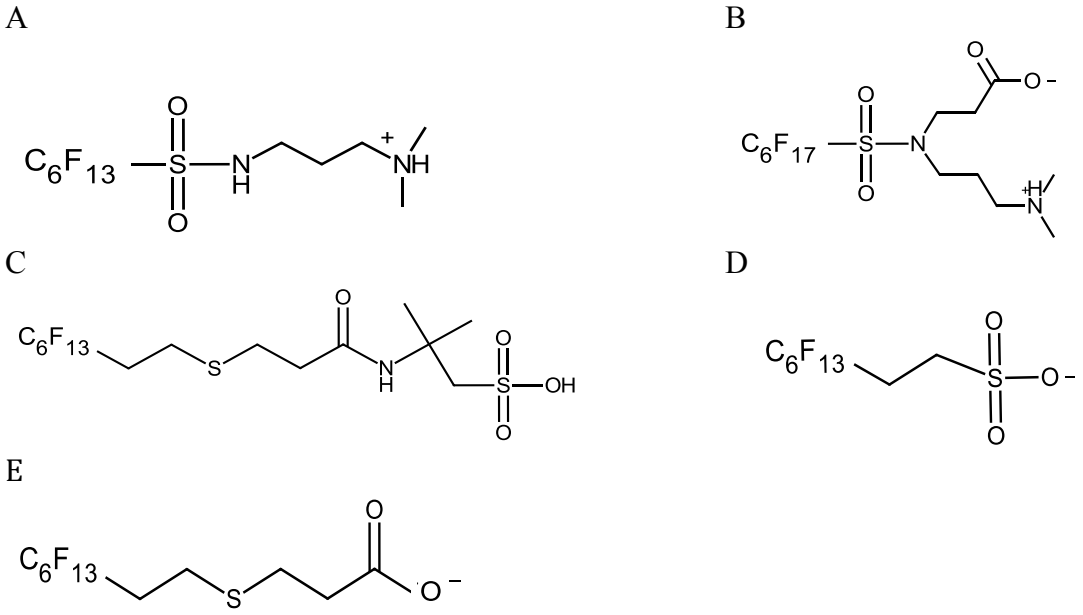


Figure 4.1: Structures of (A) PFHxSAm, (B) PFHxSAmA, (C) 6:2 FtTAoS, (D) 6:2 FtS, and (E) 6:2 FtTCA.

Table 4.2: Molar precursor concentrations in AFFF formulations added to microcosms.

AFFF Formulation	[PFAA Precursors], mmol/L						
	PFBSAm	PFPeSAm	PFHxSAm	PFBSAmA	PFPeSAmA	PFHxSAmA	Sum
3M 2001 (Aerobic/ Anaerobic)	1.6	1.1	8.7	3.2	1.4	6.6	23
	4:2 FtTAoS	6:2 FtTAoS	8:2 FtTAoS				Sum
Ansul 2010 (Aerobic)	<0.1	18	<0.1				18
Ansul 2009 (Anaerobic)	<0.1	30	<0.1				30

4.2.2 Inocula Sources

Aerobic microcosms were inoculated with soil collected from a firefighter training area at Ellsworth Air Force Base, South Dakota where AFFF had been released. Soil was stored in a 2-L plastic bottle at $4^{o}C$, except during transport, when it was maintained at ambient temperature.

Anaerobic microcosms were inoculated with sediment from the North Arm of Strawberry Creek on the campus of University of California, Berkeley using a PFAS-free metal shovel. Sediment was stored at room temperature in a 1-L jar in an anaerobic glove box filled with N_2 gas.

4.2.3 Microcosm Preparation and Maintenance

Aerobic live culture microcosms were prepared in triplicate in 250-mL bottles with 50 mL of modified ammonium mineral salts (AMS) medium [Parales *et al.* 1994] and 5 g of soil. The amount of ammonium sulfate was reduced from the standard AMS medium for a final microcosm concentration of 100 mg/L as N. A parallel set of triplicate abiotic ("autoclaved culture") controls were prepared identically to the live culture bottles and sterilized by three cycles of autoclaving and freezing for eight hours. Triplicate sterile medium controls that did not contain any soil were also included. All live culture, autoclaved culture, and medium control bottles were amended with 50 µL of pure AFFF. The headspace was pressurized to 1 bar with pure oxygen at the start of the experiment. The oxygen concentration in the headspace was measured by GC-TCD prior to the collection of every third sample to ensure that microcosms remained aerobic. The headspace was flushed with oxygen if the partial pressure of oxygen dropped below 0.15 bar. Live culture microcosms were periodically amended with an additional 50 µL of pure AFFF or 100 mg C/L autoclaved DGBE. Aerobic microcosms were incubated at room temperature on a shaker table rotating at 50 rpm.

Anaerobic microcosms were prepared in 120-mL bottles containing 50 mL of defined mineral salt medium [He *et al.* 2007]. Resazurin indicator and cysteine sulfide were omitted from the medium used in nitrate- and iron- reducing microcosms. Nitrate-reducing microcosms were amended with 50 mM $NaNO_3$, sulfate-reducing microcosms were amended with 50 mM K_2SO_4, and Fe-reducing microcosms were amended with 50 mM freshly prepared $FeOOH_{(s)}$. The FeOOH slurry was prepared according to Lovely *et al.* (1986). In a glove box maintained with N_2 atmosphere, 10 g of sediment was added to each microcosm and the bottles were resealed. Anaerobic microcosms were prepared in triplicate with similar medium and autoclaved culture controls as the aerobic incubations. All live culture, autoclaved culture, and medium control bottles were amended with 50 µL of pure AFFF. The headspace of each microcosm was pressurized to approximately 1 bar with a 20:80 CO_2:N_2 gas mix at the start of the experiment. Live culture microcosms were periodically amended with either an additional 50 µL AFFF (nitrate-reducing condition) or 100 mg C/L autoclaved DGBE (all anaerobic conditions), additional electron acceptor solutions, and CO_2/N_2 gas. Anaerobic microcosms were stored in the dark at room temperature.

4.2.4 Sampling and Preparation for Analysis

Well-mixed 1 to 2-mL slurry samples were collected and transferred to 2-mL centrifuge tubes from sealed aerobic and anaerobic microcosms on a periodic basis using sterile, disposable metal needles and plastic syringes. 200 µL of the sample was immediately removed and added to 200 µL of methanol in a 2-mL centrifuge tube, vortexed for 20 minutes, and centrifuged at 15,000 *g* for 10 minutes. A portion of this sample was diluted 1000 fold in a 50:50 methanol:water matrix, amended with 2 µg/L internal standard (IS) stock solution, and reserved for subsequent PFAS analysis by LC-MS/MS. Another 100 µL of the well-mixed sample was reserved for oxidation-based precursor analysis [Houtz and Sedlak 2012]. The remaining sample was centrifuged at 15,000 *g* for 5 minutes and the supernatant was removed for total organic carbon (TOC) analysis on a Shimadzu TOC-V. The concentrations of nitrate and sulfate in the supernatant were periodically analyzed with a Dionex DX-120 Ion Chromatograph.

For analysis that required a salt-free matrix (*i.e.,* high resolution MS), the supernatant of the sample stored in 50:50 MeOH was removed, diluted 20-fold in HPLC-grade water, and extracted on a Waters WAX SPE cartridge according to the method described in Chapter 2. The extract was eluted in two separate fractions, methanol and 0.1% NH_4OH in methanol, and reserved for subsequent analysis.

4.2.5 HPLC-MS/MS Analysis

Routine monitoring of PFAS analytes for which standards or reference materials were available was conducted as described in Chapter 3 using an Agilent 6410 LC-MS/MS.

The undiluted, centrifuged 50:50 methanol:sample collected from microcosms was used without addition of the internal standard stock to identify unknown intermediate products for which standards were not available. The Agilent LC-MS/MS was operated under the same chromatographic conditions in a molecular ion scanning mode (m/z 50 to 1000) using both positive and negative electrospray ionization to identify prominent but unknown molecular ions. Product ion scans were conducted on select molecular ions to aid in the identification of their structural characteristics. Once transformation product structures were tentatively identified, SPE-extracted samples were analyzed for their exact mass by direct infusion (50 μL) on a Thermo Scientific Finnigan LTQ FT high resolution mass spectrometer operating in negative electrospray ionization over a scan range of 150 to 850.

4.3 Results

4.3.1 3M AFFF-Amended Aerobic and Anaerobic Microcosms

No loss of PFAA precursors or production or loss of perfluorinated sulfonates was observed in either aerobic or anaerobic microcosms amended with 3M AFFF. These microcosms originally contained approximately 30 µM of perfluorinated sulfonates and approximately 23 µM of sulfonamide-containing precursors (Table 4.2).

Aerobic microcosms were incubated for 90 days. During this period, TOC decreased from approximately 150 mg/L to less than 10 mg/L after approximately twenty days. The

organic carbon was periodically replenished with 100 mg/L DGBE as C. The headspace was maintained above 0.15 bar O_2 by periodically flushing with pure O_2 gas.

Anaerobic microcosms were incubated for 65 days. TOC decreased to less than 10 mg/L in the live microcosms 44 days after incubation started and was not replenished before the experiment was terminated. Electron acceptors in the anaerobic microcosms with 3M AFFF were not measured; on the basis of similar incubations with Ansul AFFF, the length of the experiment was not long enough to deplete the electron acceptors.

4.3.2 Transformation of Ansul AFFF PFASs Under Aerobic Conditions

Incubation of Ansul AFFF formulations with live inocula from AFFF-acclimated soils under aerobic conditions led to complete transformation of the primary Ansul PFAA precursor, 6:2 FtTAoS (Figure 4.2A). Transformation of more than 90% of the initial 15 μM of 6:2 FtTAoS occurred within 20 days (Figure 4.2A). Ansul microcosms originally contained trace amounts (<0.1 μM) of 8:2 FtTAoS and 4:2 FtTAoS, both of which decreased to concentrations below detection limits within 15 days. After complete transformation of 6:2 FtTAoS from the first amendment of AFFF, two additional AFFF amendments were made to live culture bottles, with subsequent transformation of each 6:2 FtTAoS addition within approximately 20 days (Figure 4.2A). 6:2 FtTAoS concentrations in autoclaved culture microcosms were approximately constant until after day 60, when growth of microbes in the bottles appeared to occur and concentrations of 6:2 FtTAoS declined.

In live aerobic microcosms, production of 4:2, 6:2, and 8:2 FtS (Figure 4.2B) and the C_4 to C_8 perfluorinated carboxylates (Figure 4.2C) was observed as AFFF underwent transformation. 6:2 FtS accounted for approximately 6% of the 6:2 FtTAoS loss and 97% of the FtS compounds produced. Perfluorinated carboxylates accounted for approximately 2% of FtTAoS loss; PFHxA, PFPeA, and PFBA accounted for 44%, 45%, and 8% of the perfluorinated carboxylates produced, respectively. The small amounts of 4:2 FtS, 8:2 FtS, and C_7 and C_8 perfluorinated carboxylates were produced mainly from the transformation of 4:2 and 8:2 FtTAoS impurities in the AFFF formulation. While standards were unavailable to quantify 4:2 and 8:2 FtTAoS, their response relative to 6:2 FtTAoS was each less than 1% of the response observed at the start of incubation.

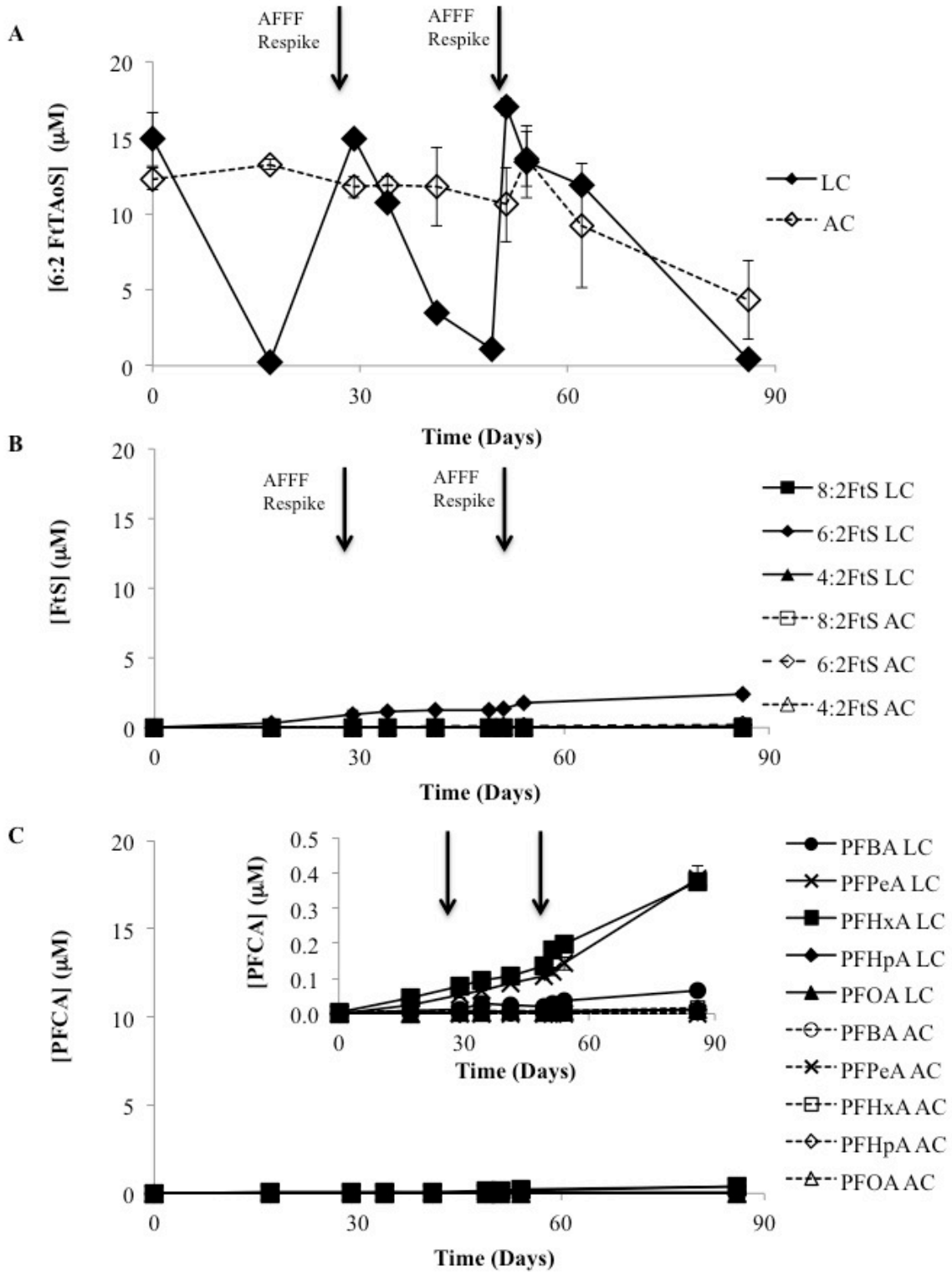


Figure 4.2: Time course of (A) 6:2 FtTAoS, (B) fluorotelomer sulfonates, and (C) perfluorinated carboxylates in live culture (LC) and autoclaved culture (AC) aerobic AFFF-acclimated soil microcosms.

Several other transformation products for which analytical standards were not available were detected in the live culture incubations after day 0. Initially, molecular ion scans were conducted on live and autoclaved samples taken from multiple time points to identify ions that increased in abundance primarily in the live microcosms. Two molecular ions, 602 and 618, were present in higher abundance in live aerobic microcosms after day 0. The molecular ion 602 was also detected in autoclaved culture and medium control microcosms after day 0, but its abundance increased at a faster rate in live culture microcosms (Figure 4.3B).

To calculate the exact atomic composition of these molecules, high-resolution MS exact mass measurements were performed on sample extracts (Table 4.3). The exact mass measurements confirmed within 5 ppm mass accuracy that these two molecules had the same atomic composition as 6:2 FtTAoS with one (602) or two (618) additional oxygen atoms (Table 4.3). Once the atomic compositions of the molecular ions were identified, sample extracts were analyzed on the LC-MS/MS operating in product ion mode: voltage was applied to the second quadrupole of the LC-MS/MS to generate a mass spectrum for each of the two molecular ions (Figure 4.5). Key structural features were proposed based on the mass spectrum observed for each molecular ion (Figure 4.5). The product ion scans of both 602 and 618 showed fragments corresponding to the entire non-fluorinated part of 6:2 FtTAoS adjacent to the thioether moiety (m/z 205) as well as the terminal betaine sulfonate moiety (m/z 135) contained within m/z 205 (Figure 4.5). Because these two fragments were also observed in the mass spectrum of 6:2 FtTAoS, they suggested that the oxygen additions occurred on the part of the molecule containing the fluorotelomer thioether moiety. Thus, the proposed structures for 602 and 618 contained oxygen on the thioether functional group of 6:2 FtTAoS. These compounds were named 6:2 FtSOAoS and 6:2 $FtSO_2AoS$.

Production of 6:2 FtSOAoS was observed in all live, autoclaved, and medium control microcosms under aerobic conditions (Figure 4.3B). At the end of the second growth cycle, the observed response of 6:2 FtSOAoS in live cultures was 2.8 times the response in autoclaved microcosms, or 1.4 times relative to the concentration of AFFF that each set of microcosms received. Production of 6:2 $FtSO_2AoS$ was only observed in live culture aerobic microcosms (Figure 4.3C). Assuming equimolar instrumental responses for 6:2 FtTAoS, 6:2 FtSOAoS, and 6:2 $FtSO_2AoS$, 43% of the loss of 6:2 FtTAoS can be explained by production of 6:2 FtSOAoS and 6:2 $FtSO_2AoS$ in live microcosms on day 49 at the end of the second growth cycle.

Production of 6:2 fluorotelomer unsaturated carboxylate (6:2 FtUA) was confirmed in live culture microcosms using an authentic analytical standard. Due to low sensitivity of the MS for this compound, it could not be quantified. However, it was only observed in live culture microcosms after day 0 and was never detected in autoclaved or medium controls, suggesting it was a biological transformation product of 6:2 FtTAoS.

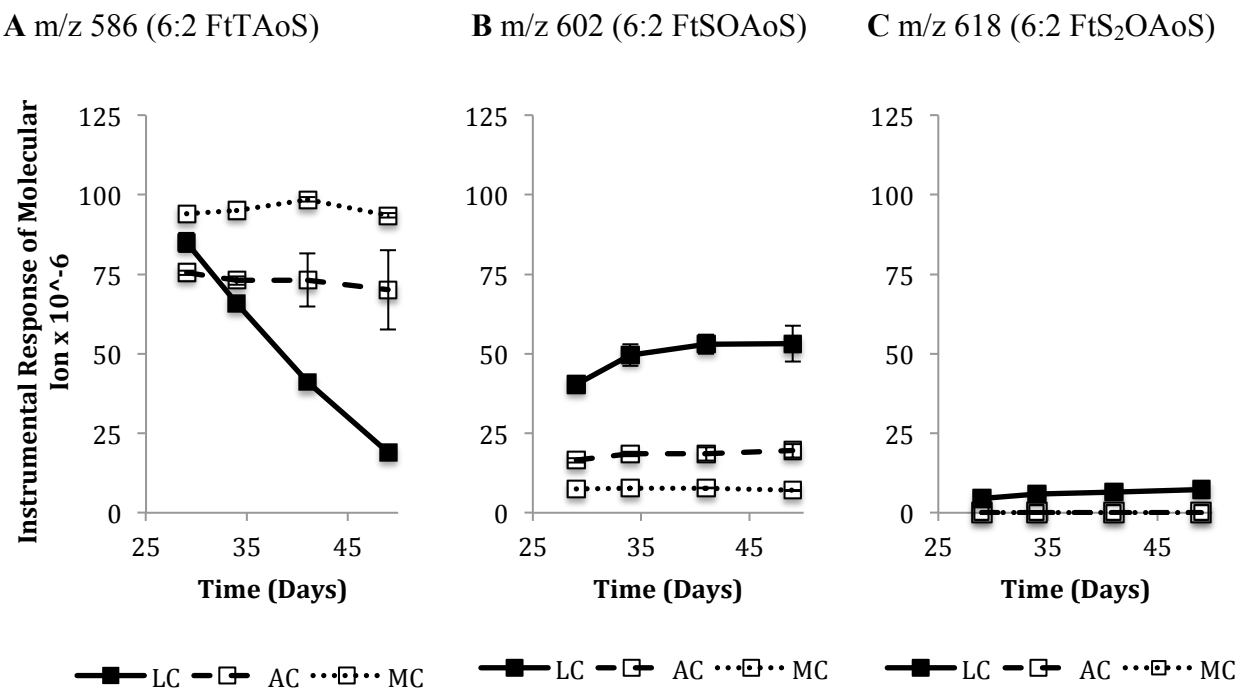


Figure 4.3: Time course of average molecular ion response of (A) m/z 586 (6:2 FtTAoS), (B) m/z 602 (6:2 FtSOAoS), and (C) m/z 618 (6:2 $FtSO_2AoS$) during the 2nd growth cycle in aerobic acclimated live culture (LC), autoclaved culture (AC), and medium control (MC) microcosms. Two or three replicates are averaged per time point for AC and LC, and one or two replicates are averaged for MC. On day 29, AC and MC microcosms had received one amendment of Ansul AFFF and LC microcosms had received two amendments of Ansul AFFF.

Figure 4.4: Chromatograph of 6:2 FtTAoS (m/z 586), 6:2 FtSOAoS (m/z 602), and 6:2 $FtSO_2AoS$ (m/z 618) molecular ions in an aerobic live microcosm on day 29 of incubation.

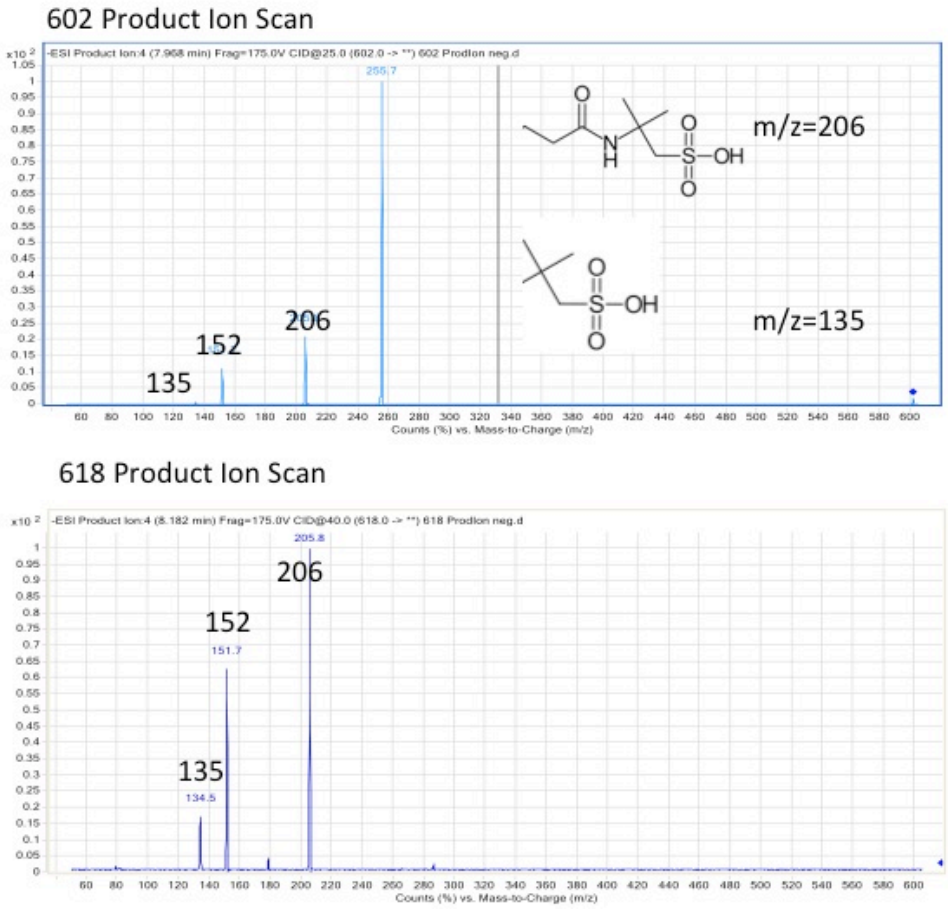


Figure 4.5: Product ion scans of m/z 602 (6:2 FtSOAoS) and 618 (6:2 $FtSO_2AoS$) and proposed structures of daughter ions.

Table 4.3: Measured mass, theoretical mass, and mass accuracy of intermediate products identified by high resolution mass spectrometry.

Compound	Conditions Observed	Retention Time, minutes	Composition	Measured Mass	Theoretical Mass	Mass Accuracy, ppm
6:2 $FtSO_2AoS$	Aerobic	8.1	$C_{15}H_{17}O_6NF_{13}S_2$	618.0276	618.0295	-3.11
6:2 FtSOAoS	Aerobic, Nitrate-reducing	8.0	$C_{15}H_{17}O_5NF_{13}S_2$	602.0329	602.0346	-2.84

To estimate the concentration of unidentified transformation products in live culture microcosms, the oxidation-based precursor assay developed in Chapter 3 was applied to the live culture microcosm slurries at the beginning and end of the third growth cycle (Figure 4.6). Although the concentration of 6:2 FtTAoS declined by 13 µM between days 54 and 85, the recovery of perfluorinated carboxylates from the oxidation of PFAA precursors in the slurry was constant between those two time points. While only 8% of 6:2 FtTAoS transformation was accounted for by FtS and perfluorinated carboxylate products, the consistency of the total precursor concentration suggests that unidentified intermediates generated over the third growth cycle were still present in the microcosm slurry.

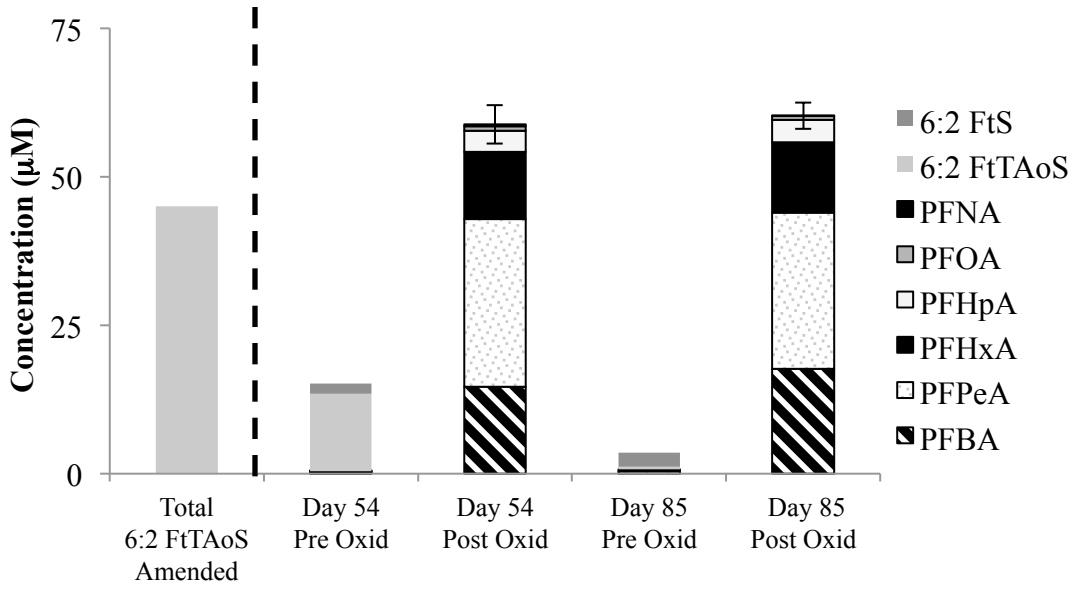


Figure 4.6: Average concentration of PFAA precursors and perfluorinated carboxylates measured in live culture microcosm slurries before and after oxidation at the beginning (day 54) and end (day 85) of the third growth cycle. Prior to the third growth cycle, LC microcosms had received three AFFF amendments containing approximately 15 µM 6:2 FtTAoS each.

4.3.3 Transformation of Ansul AFFF PFASs Under Anaerobic Conditions

6:2 FtTAoS was transformed under nitrate-reducing, sulfate-reducing, iron-reducing, and methanogenic conditions in live creek sediment microcosms (Figure 4.7A-D). Transformation of 6:2 FtTAoS was fastest under nitrate-reducing conditions (Figure 4.7A), with 90% loss of 6:2 FtTAoS 190 days after initiation of the experiment. After the second amendment of AFFF, 90% of the 6:2 FtTAoS was lost after 120 days. The accelerated transformation after the second AFFF amendment may have been related to more frequent DGBE additions to the microcosm during that period (Figure 4.7A). Under sulfate-reducing, iron-reducing, and methanogenic conditions, 71%, 66%, and 45% loss of 6:2 FtTAoS was observed, respectively, 317 days after initiation of incubation. No apparent loss of 6:2 FtTAoS was observed in any of the autoclaved or medium controls.

In the anaerobic microcosms, the only detected biotransformation product for which a reference material was available was 6:2 fluorotelomer thio carboxylate (6:2 FtTCA) (Figure 4.7A-D). No perfluorinated carboxylates were detected in any of the microcosms. Under nitrate-reducing and iron-reducing conditions, concentrations of 6:2 FtTCA initially increased then decreased after approximately half of the 6:2 FtTAoS had been transformed. Under sulfate-reducing and methanogenic conditions, the concentration of 6:2 FtTCA increased throughout the first 200 to 250 days of incubation and appeared to decrease slightly over the last 50 ,to 100 days

Concentrations of 6:2 FtTCA in the microcosms were estimated by making a series of assumptions about instrument response. A product sold as Zonyl FSA reportedly contained approximately 0.26 g/mL of five different FtTCA compounds in unknown proportions. Equimolar instrumental responses were assumed for each of the five FtTCA congeners in the standard. Using this information, the concentration of each congener was estimated by multiplying the total FtTCA concentration by its percent molar response relative to the response of the 6:2 FtS internal standard. Under all conditions except nitrate-reducing conditions, the maximum observed concentration of 6:2 FtTCA corresponded to approximately 50% of the amount of 6:2 FtTAoS lost. Under nitrate-reducing conditions, a maximum of 25% of FtTAoS was accounted for by 6:2 FtTCA.

6:2 FtSOAoS was detected after more than 100 days of incubation in nitrate-reducing live culture microcosms. The peak area of 6:2 FtSOAoS never exceeded 10% of the peak area corresponding to the initial concentration of 6:2 FtTAoS under nitrate reducing conditions, so its production was significantly less than in the live culture aerobic microcosms (Figure 4.3). Unlike the aerobic autoclaved culture and medium control microcosms, 6:2 FtSOAoS was not detected in any of the sterile microcosms under nitrate-reducing conditions. 6:2 FtSOAoS was also not detected in any live or sterile microcosms under the other anaerobic conditions.

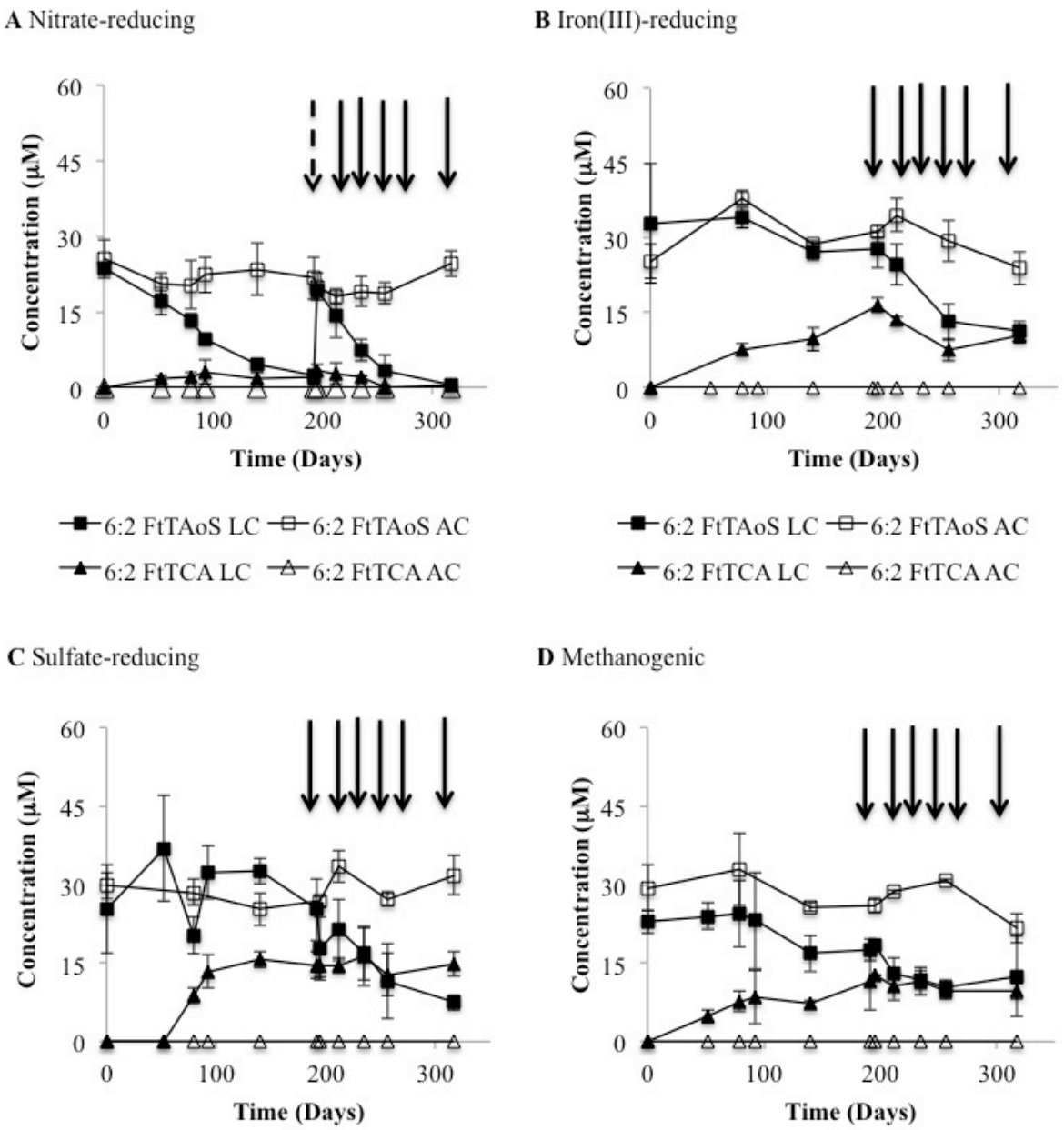


Figure 4.7: Time-course transformation of 6:2 FtTAoS and 6:2 FtTCA in UC Berkeley creek sediment live culture (LC) and autoclaved culture (AC) microcosms under (A) nitrate-reducing, (B) iron (III)-reducing, (C) sulfate-reducing, and (D) methanogenic conditions. Live culture microcosms were amended with 100 mg/L DGBE as C on days 195 (except nitrate-reducing), 215, 225, 249, 270, and 303 (denoted by solid arrows). Nitrate-reducing microcosms were amended with an additional 50 µL AFFF (denoted by dashed arrow) and nitrate on day 195. On day 270, all live culture nitrate-reducing, iron-reducing, and sulfate-reducing microcosms were amended with a second 50 mM dose of their respective electron acceptor. Methanogenic microcosm headspace was flushed with 80/20 N_2/CO_2 gas on day 270.

The total concentration of precursors in the anaerobic microcosm slurries was periodically measured using the oxidation assay. At the end of the incubation on day 317, the total concentration of PFAA precursors decreased by approximately 50% in the nitrate-reducing microcosms relative to autoclaved controls (Figure 4.8). Under the other anaerobic conditions, the total concentration of precursors in live culture microcosms decreased by 11% to 35% relative to autoclaved culture microcosms, although these differences were only statistically significant under iron-reducing and sulfate- reducing conditions (Figure 4.8).

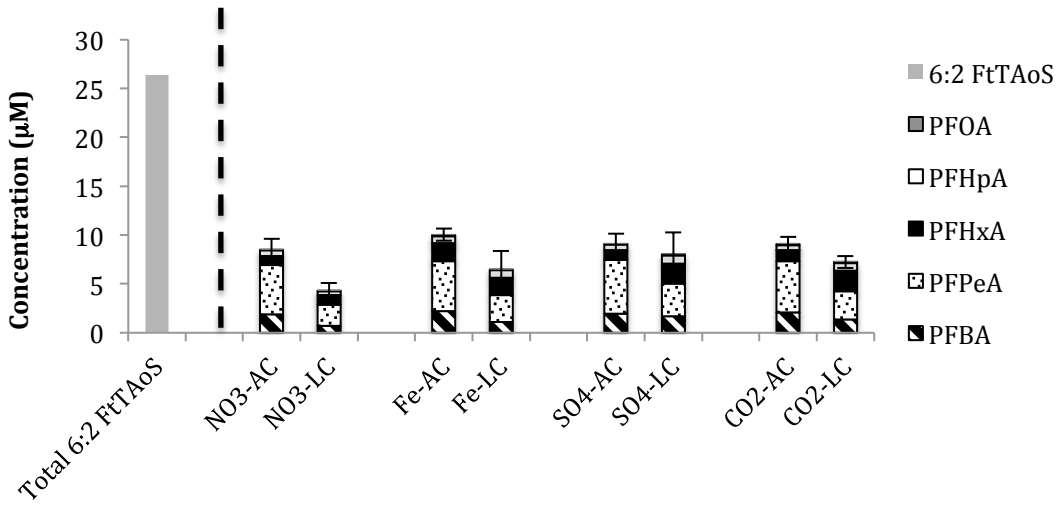


Figure 4.8: Average production of perfluorinated carboxylates measured from the oxidation of aqueous precursors in live culture (LC) and autoclaved culture (AC) anaerobic slurries on day 339. Concentrations in live culture nitrate-reducing bottles were divided by 2 to account for the two dosages of AFFF received relative to the one dosage all other bottles in the experiment received over this time period. Total 6:2 FtTAoS is the amount of 6:2 FtTAoS amended per AFFF dosage.

The live, nitrate-reducing microcosms were sub-cultured on day 360 to a new set of bottles containing 10% of slurry from the original microcosms and 90% fresh nitrate-reducing medium. These "low-solids subcultures" contained significantly less solids than the original microcosms. They were used to determine whether irreversible sorption to sediment particles was responsible for the loss of PFAA precursors in nitrate-reducing live culture microcosms relative to the nitrate-reducing autoclaved culture bottles (Figure 4.8). In the low-solids subculture, 71% of aqueous PFAA precursors as measured by the oxidation assay were lost over a 118-day incubation period in the live culture bottles (Figure 4.9). These data suggest that irreversible sorption of transformation products to solid particles is not responsible for the apparent decrease in the total concentration of precursors in the nitrate-reducing live culture microcosm slurries (Figures 4.8- 4.9).

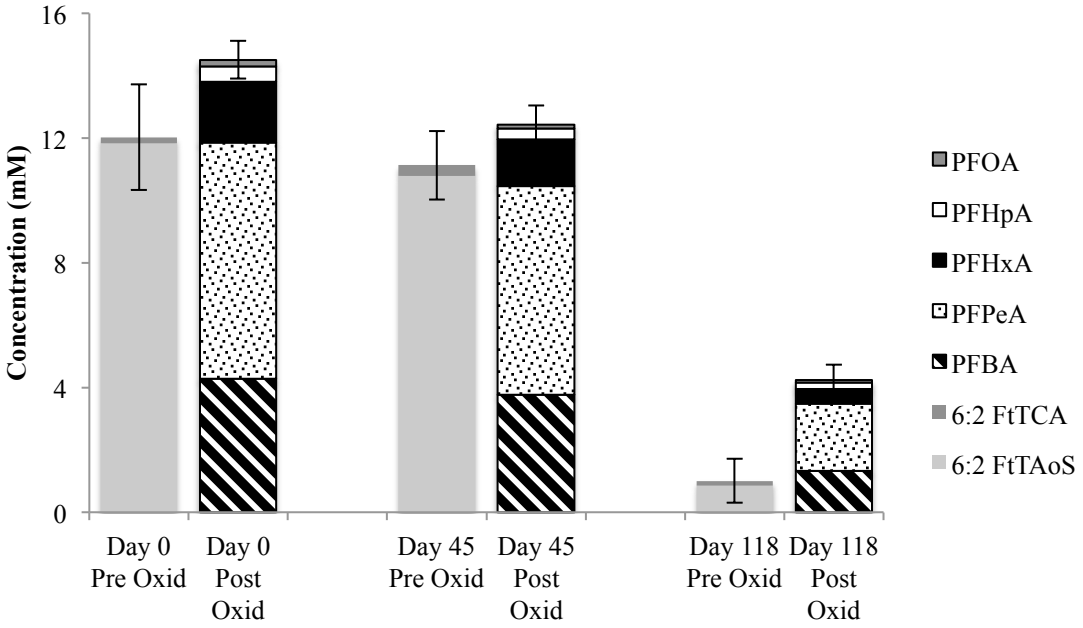


Figure 4.9: Average concentration of PFAA precursors and perfluorinated carboxylates measured before and after oxidation in triplicate live sub-cultured, low-solids, nitrate-reducing microcosms on days 0, 45, and 118 of a 118-day incubation.

4.4 Discussion

4.4.1 Mass Balance of PFASs in Ansul AFFF Aerobic Microcosms

In the aerobic live culture microcosms, approximately 8% of 6:2 FtTAoS loss was accounted for as transformation products, including perfluorinated carboxylates and fluorotelomer sulfonates, over the 90-day incubation period (Figure 4.2A-C). To determine if the apparent loss of 6:2 FtTAoS resulted in production of slurry-partitioned transformation products, the oxidation-based precursor assay was used to quantify the total concentration of PFAA precursors in live microcosms (Figure 4.6). Good agreement was observed between precursor concentrations in live microcosms at the beginning and end of the third, 31-day growth cycle (Figure 4.6), suggesting the biotransformation products were still present in the microcosm slurries that could not be accounted for by the measureable precursors.

The precursor assay also indicated a discrepancy between the concentration of 6:2 FtTAoS added to the aerobic microcosms by day 54 and the concentration of total precursors (Figure 4.6). Approximately 60 µM of precursors were measured by the oxidation assay in live culture microcosms on both days 54 and 85 whereas 45 µM of 6:2 FtTAoS had been added throughout the experiment. Additionally, the oxidation assay only captures 60% to 80% (Table 2.2) of the concentration of 6:2 fluorotelomer compounds as perfluorinated carboxylate products with a chain length of four or more carbons [Houtz and Sedlak 2012]. Thus, between 67% and 120% more perfluorinated carboxylate oxidation products occurred in microcosms than what was expected on the basis of the concentration of 6:2 FtTAoS amended to microcosms (Figure 4.2A, Figure 4.6).

There are several possible explanations for the phenomenon of over-recovery of PFAA precursors by the oxidation assay. First, because a reference material was used for 6:2 FtTAoS rather than a certified analytical standard, the concentration used in the calibration curve might not be accurate. Second, average 6:2 FtTAoS concentrations in medium controls were up to 20% higher than those measured in autoclaved controls (Figure 4.3A), suggesting that extraction of microcosm samples containing solids did not capture the entire concentration of 6:2 FtTAoS added to the microcosms. If 6:2 FtTAoS concentrations were underquantified for either of these reasons, then mass recovery of measureable transformation products (*i.e.,* FtS and perfluorinated carboxylates) would be lower than 8%. Finally, PFAA precursors other than 6:2 FtTAoS may have been present in the AFFF added to the microcosms. This explanation could also result in higher-than-expected concentrations of measureable transformation products attributable to 6:2 FtTAoS if some of those products were in reality attributable to other PFAA precursors.

Independent of measurement inconsistencies associated with comparison between the two techniques, consistent agreement was observed between the concentrations of PFAA precursors measured in the live microcosm slurries at the beginning and end of the final 31-day growth cycle (Figure 4.6). This consistency suggests that the biological transformation reactions of 6:2 FtTAoS altered the non-fluorinated part of the molecule and that the products were partitioned into the slurry. If cleavage of the perfluorinated group occurred during biological transformation, perfluorinated carboxylate production in live culture bottles would have resulted in a lower total precursor concentration over the course of the growth cycle. The consistent recovery of PFAA precursors over the growth cycle also indicates that biological transformation

products did not volatilize out of the microcosm slurry or adhere irreversibly to soil particles or the walls of the microcosms.

4.4.2 Aerobic Biotransformation Pathway of 6:2 FtTAoS

The proposed pathway for 6:2 FtTAoS biotransformation in aerobic microcosms may explain the production of a mixture of C_4, C_5, and C_6 perfluorinated carboxylates during incubation of an Ansul AFFF formulation (Figure 4.10). The first two steps in the transformation process appear to involve sequential addition of oxygen to the thioether group to produce 6:2 FtSOAoS and 6:2 $FtSO_2AoS$ (Figure 4.3). The existence of these two compounds was confirmed by high resolution mass spectrometry and daughter ion scans (Table 4.3, Figure 4.5). The next product in the proposed pathway, 6:2 FtS, was the result of a third oxygen addition to the thioether group and cleavage between the resulting sulfonate group and alkyl amido sulfonate group. The production of 6:2 FtS was confirmed by detection of low concentrations of the compound by LC-MS/MS. The formation pathway of a mixture of C_4 to C_6 perfluorinated carboxylates from 6:2 FtS has been previously reported under aerobic conditions in activated sludge [Wang *et al.* 2011].

The formation of 6:2 FtSOAoS from 6:2 FtTAoS also may proceed by an abiotic reaction in the presence of oxygen. The response of 6:2 FtSOAoS was approximately three times higher in autoclaved culture microcosms than in medium controls (Figure 4.3B). Thus, a constituent of the soil, such as a mineral surface, may catalyze the formation of 6:2 FtSOAoS under aerobic conditions.

The production of 6:2 FtUA and a suite of C_4 to C_6 perfluorinated carboxylates was consistent with previous research on fluorotelomer compounds in aerobic mixed culture microcosms [Lee *et al.*, 2010, Lieu *et al.* 2010, Wang *et al.* 2011, Dasu *et al.* 2012]. In this study and in earlier studies, the transformation of an n:2 fluorotelomer compound led to production of the C_n and C_{n-1} perfluorinated carboxylates in roughly equimolar quantities (Table 1.5) and production of the C_{n-2} perfluorinated carboxylate at concentrations 5 to 10 times lower than its C_n and C_{n-1} counterparts [Wang *et al.* 2011, Dasu *et al.* 2012]. Additional aerobic microbial transformation products that are formed between 6:2 FtS and 6:2 FtUA [Wang *et al.* 2011] and 6:2 FtUA [Wang *et al.* 2011, Liu *et al.* 2010] and the perfluorinated carboxylates have been observed elsewhere.

The rate of aerobic biotransformation of 6:2 FtTAoS appeared to be much faster than the rates of transformation of the intermediate biotransformation products. No decline in the concentration or response of any of the intermediate aerobic biotransformation products of 6:2 FtTAoS were observed, even though microcosms were incubated for approximately one week after 6:2 FtTAoS was fully depleted before being re-amended with AFFF.

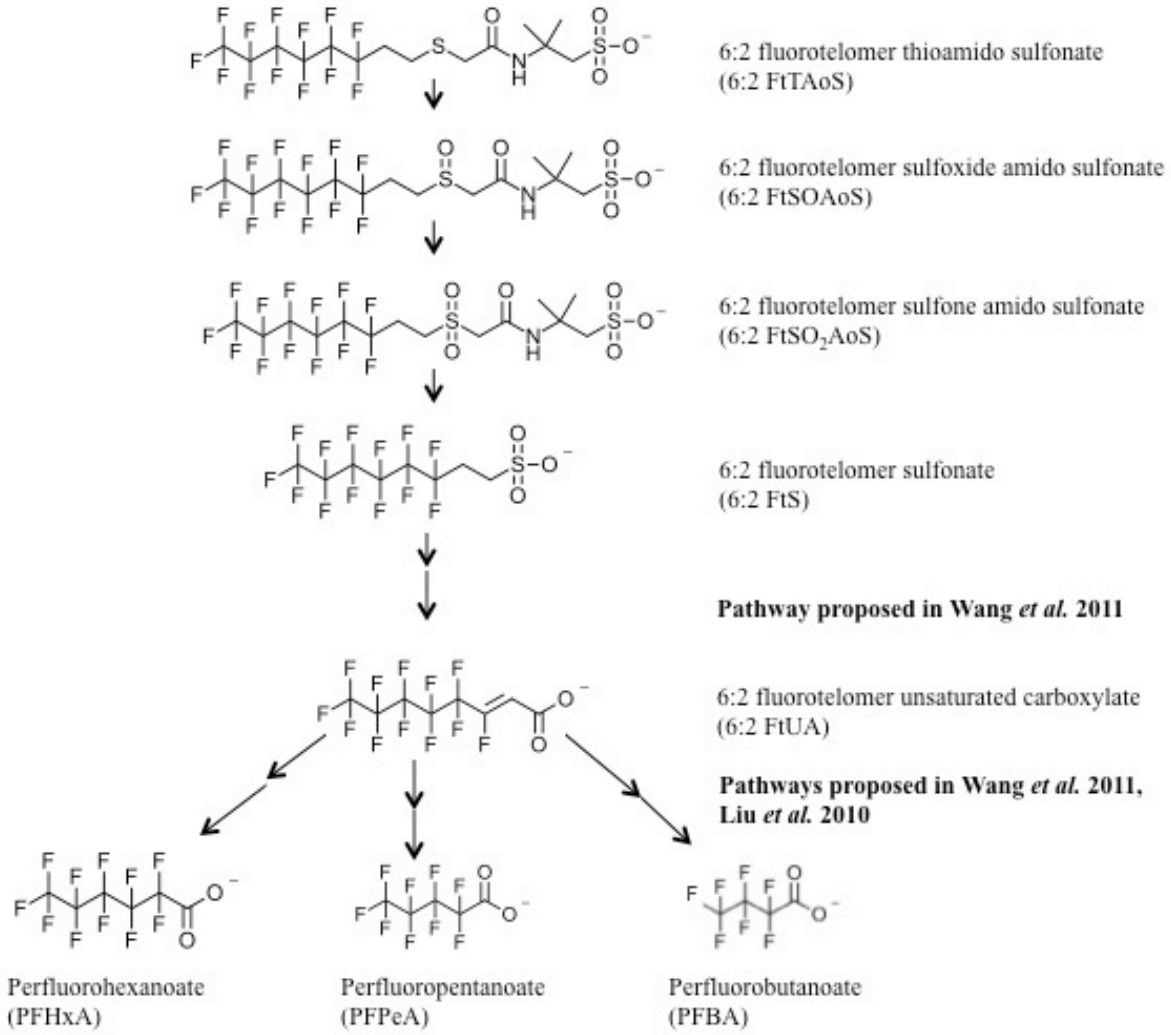


Figure 4.10: Proposed transformation pathway of 6:2 FtTAoS in the presence of aerobic soil microbes.

4.4.3 Mass Balance of PFASs in Ansul AFFF Anaerobic Microcosms

In Ansul AFFF anaerobic microcosms, the only measureable biotransformation product, 6:2 FtTCA, accounted for 25% to 50% of the observed loss of 6:2 FtTAoS under all conditions. Mass spectrometry scans of microcosm slurry extracts did not indicate any other transformation products.

To close the mass balance on transformation products for which standards were not available, the oxidation-based precursor assay was used to quantify the total concentration of PFAA precursors in live culture and autoclaved culture microcosms (Figures 4.8- 4.9). Apparent loss of PFAA precursors in live culture microcosms relative to autoclaved culture microcosms occurred under all conditions, but was most pronounced under nitrate-reducing conditions (50% difference). Nitrate-reducing, low sediment sub-cultures of the sediment-containing, live culture microcosms were prepared and substantial loss of PFAA precursors (*i.e.*, approximately 71%) measured by the oxidation assay was observed over the 118-day incubation (Figure 4.9).

In nitrate-reducing Ansul AFFF microcosms, it is possible that products were formed that partitioned to the gas phase; this phenomenon may also occur under other anaerobic conditions. First, the removal of solids did not result in increased detection of PFAA precursors in the nitrate-reducing live microcosm slurry. Irreversible partitioning to solids is, as a result, an unlikely explanation for the loss of PFAA precursors in the slurry observed in the original sediment-containing incubation. Second, a greater extent of removal of PFAA precursors was observed in the low-solids sub-culture slurries in roughly one third of the incubation time as the sediment-containing bottles. The removal of organic-rich solids appeared to increase the rate at which transformation products partitioned to the gas phase, which would be typical of possible volatile organofluorine transformation products.

It is also possible that transformation reactions occurred between a C-C or C-F bond on the perfluorinated chain, generating products that were not converted into perfluorinated carboxylates. However, this type of reaction is less thermodynamically favorable than reactions originating on the non-fluorinated part of the molecule.

Additional research is required to identify possible volatile transformation products in anaerobic microcosms and to fully account for the loss of 6:2 FtTAoS.

4.4.4 Co-metabolic Transformation

To provide an energy source to support co-metabolism of PFAA precursors, aerobic and anaerobic microcosms were periodically amended with DGBE or additional AFFF. Prior to additions of the carbon source, microcosms remained for periods of one to two weeks under depleted carbon conditions. In all Ansul AFFF-amended live culture microcosms, transformation of PFAA precursors required the presence of an additional source of carbon to stimulate growth: once dissolved organic carbon decreased below 10 mg/L, depletion of 6:2 FtTAoS (Figure 4.7 B-D) and formation of intermediate transformation products (Figure 4.2 B,C, Figure 4.7 A-D) slowed dramatically or halted. These results suggest that transformation of PFAA precursors in Ansul formulations and their partially fluorinated transformation products did not support

organisms capable of 6:2 FtTAoS transformation and that the transformation was driven by co-metabolic processes.

4.4.5 Implications for the Fate of AFFF in Contaminated Groundwater and Soil

It was unexpected that PFAA precursors in 3M AFFF formulations were not amenable to abiotic or biological transformation in any of the microcosms. In groundwater from numerous firefighter training sites, where it is likely that 3M AFFF has been present for decades, these precursors were absent or were found at extremely low concentrations relative to the perfluorinated sulfonates, which were also found exclusively in 3M AFFF [Backe *et al.* 2013]. The C_6 and C_8 perfluorinated sulfonamides, which are probable transformation products of PFAA precursors in 3M formulations, have been detected at concentrations that were 10% to 20% of the concentrations of the perfluorinated sulfonates in 3M AFFF-impacted groundwater and soil [Houtz *et al.* 2013]. The microcosm results indicate that 3M AFFF precursors might transform more slowly than Ansul AFFF precursors or require a better-acclimated inoculum.

The intermediates detected in the aerobic microcosms were consistent with data from previous studies of smaller PFAA precursor compounds and data from AFFF-impacted sites. For example, 6:2 FtS and 8:2 FtS accounted for up to 20% of PFAS analytes in AFFF-impacted groundwater and soil [Backe *et al.* 2012, Houtz *et al.* 2013], with concentrations as high as 14.6 mg/L 6:2 FtS [Schultz *et al.* 2004] in groundwater. Furthermore, the perfluorinated carboxylates have been detected at many AFFF sites, despite the fact that they are only present at trace concentrations in modern AFFF formulations [Backe *et al.* 2012, Schultz *et al.* 2004, Houtz *et al.* 2013].

6:2 FtTCA, the only aqueous transformation product of 6:2 FtTAoS observed under all four anaerobic conditions, has never been measured at AFFF-contaminated sites. Because no commonly-measured PFASs were observed as transformation products in the anaerobic microcosms, it is difficult to assess whether anaerobic processes have also played a role in the transformation of PFAA precursors at AFFF-impacted sites. The potential volatilization of anaerobic biotransformation products could partly explain why PFAA precursors account for a smaller percentage of PFASs in soil and groundwater than in representative AFFF formulations (Figure 3.5).

The new biotransformation products of PFAA precursors detected in the Ansul AFFF microcosms under aerobic conditions could explain part of the unidentified fraction of precursors measured in soil and groundwater in Chapter 3. Roughly half of PFAA precursors measured by the oxidation assay could not be identified in those AFFF-impacted samples. The slow transformation of 6:2 FtSOAoS and 6:2 $FtSO_2AoS$ relative to 6:2 FtTAoS indicate that these two compounds might be stable enough to persist in the subsurface over decades. The concentration of 6:2 FtTCA increased and then declined in concentration under all anaerobic conditions, so it is unlikely to persist over the twenty years or more residence time of AFFF in the subsurface at that site.

Transformation of PFAA precursors in Ansul AFFF occurred much faster in aerobic microcosms than anaerobic microcosms and resulted in the production of perfluorinated carboxylates, which were not observed in anaerobic microcosms. Additionally, the apparent loss of PFAA precursors under all anaerobic conditions (Figure 4.8, Figure 4.9) through volatilization or reactions occurring on the perfluorinated chain might result in a loss of PFAA precursor mass from groundwater and soil. These results suggest that an anaerobic *in situ* environment may result in less production of PFAAs, such as PFOA, from AFFF-derived precursor compounds and may also result in the removal of PFAA precursors.

CHAPTER 5. Conclusions

5.1 Summary

The research described in this dissertation investigated the occurrence and fate of precursors to a highly persistent class of anthropogenic contaminants, the perfluoroalkyl acids (PFAAs), in urban runoff and at firefighter training sites. New analytical techniques for indirectly measuring PFAA precursors were developed for stormwater, groundwater, and soil matrices along with new methods for quantifying compounds in aqueous film forming foam (AFFF)-contaminated soil and groundwater. The runoff study concluded that modest quantities of precursors that are predominately short-chained are present in San Francisco Bay Area runoff. Runoff storage in aquifers or reservoirs may result in production of additional PFAAs upon abiotic or biological transformation of precursors. At a firefighter training site where large quantities of AFFF were deployed, high concentrations of PFAA precursors persisted twenty or more years in soil and groundwater. Both field measurements and laboratory soil incubations in microcosms amended with AFFF suggested that PFAA precursors in AFFF formulations are transformed slowly to the PFAAs. The rates and mechanisms of these reactions are affected by the type of terminal electron acceptor present. These findings suggest a need to address both PFAAs and their precursors at contaminated firefighter training sites.

5.2 Fluorochemicals in Urban Runoff

Early investigations of PFAAs in urban runoff suggested the concurrent presence of readily transformable PFAA precursors at concentrations similar to PFAAs [Kim and Kannan 2007, Murakami *et al.* 2008, Murakami *et al.* 2009a]. However, measured concentrations of specific PFAA precursors were often below detection limits or too low to substantially alter the concentration of PFAAs upon their transformation [Kim and Kannan 2007, Meyer *et al.* 2011, Nguyen *et al.* 2011]. Collectively, these early findings indicated that PFAA precursors might be important in runoff but that existing measurement techniques were incapable of detecting the precursors that were present.

In Chapter 2, a new method was developed to quantify concentrations of difficult-to-measure and unidentified precursors of PFAAs in urban runoff. To measure PFAA precursors, water samples were exposed to hydroxyl radicals generated by thermolysis of persulfate under basic pH conditions. PFAA precursors were transformed to perfluorinated carboxylates of related perfluorinated chain length. By comparing perfluorinated carboxylate concentrations before and after oxidation, the total concentration of PFAA precursors was inferred.

Thirty-three urban runoff samples collected from locations around the San Francisco Bay, CA were analyzed for concentrations of PFAAs, individual PFAA precursors, and total precursors. PFOS (2.6 to 26 ng/L), PFOA (2.1 to 16 ng/L), and PFHxA (0.9 to 9.7 ng/L) were the predominant poly- and perfluoroalkyl substances (PFASs) detected prior to oxidation. After oxidation, the concentration of perfluorinated carboxylates increased in samples by 2.8 to 56 ng/L. The total concentration of directly measured precursors (i.e., <0.2 and 2.9 ng/L) could explain only a small fraction of the precursors measured by oxidation. For example, direct measurements of several common precursors to PFOS and PFOA (*e.g.* perfluorooctanesulfonamide and 8:2 fluorotelomer sulfonate) in the runoff samples accounted for less than 25% of the observed increases in PFOA generated upon oxidation. While the specific structures of additional precursors measured by the oxidation assay could not be identified, analysis of the homologs of perfluorinated carboxylates produced upon oxidation suggested that

C_6 and shorter precursors were more prevalent in runoff samples than C_8 precursors, despite lower concentrations of their corresponding PFAAs prior to oxidation. The homolog analysis also indicated that C_6 (6:2) fluorotelomer precursors were the main type of C_6 precursors present. This precursor characterization was consistent with the reported manufacturing changes that have occurred in the fluorochemical industry.

The results presented in Chapter 2 suggest that storage of urban runoff may lead to an increase in the concentration of PFAAs through the transformation of PFAA precursors. The precursor assay provides a fast and convenient method of measuring the concentration of PFAA precursors in runoff or surface waters where PFAS contamination is of concern. These runoff samples did not contain PFAAs or PFAA precursors at concentrations that would lead to exceedance of drinking water guidelines, but runoff in more polluted environments or in areas where a manufacturing source is present is likely to contain higher concentrations. Toxicological studies conducted on animals suggest that total concentrations of C_8 PFAAs and C_8 PFAA precursors in drinking water in excess of a few hundred ng/L may result in negative human health impacts such as thyroid disruption [Lau *et al.* 2006, Seacat *et al.* 2002]; however, positive correlations in non-occupationally exposed humans have been observed between PFOA and PFOS serum concentrations and health outcomes such as obesity [Halldorsson *et al.* 2012] and total cholesterol [Nelson *et al.* 2009]. If the runoff investigated in this study was used to augment a drinking water supply, it would contribute to the potentially deleterious background exposure of these compounds to humans.

5.3 The Fate of Fluorochemicals at AFFF-Impacted Firefighter Training Areas

Firefighter training with AFFF has led to the occurrence of high concentrations of PFAAs in nearby groundwater and soils [Moody *et al.* 2003, Weiss *et al.* 2012, Schultz *et al.* 2004, Backe *et al.* 2013]. In 2012, PFAAs and twenty-two previously uninvestigated PFAA precursors were identified in AFFF formulations [Place and Field 2012]. Because the PFAA precursors had never been measured at AFFF-impacted sites, their behavior in the environment was unknown. It was important to elucidate the fate and transport of the AFFF-derived PFAA precursors in the subsurface because the successful remediation of PFAA-contaminated sites requires consideration of all PFASs.

In Chapter 3, the PFAS contamination in the subsurface underlying a historical firefighter training area was investigated. Groundwater, soil, and aquifer solids were obtained in 2011 from an unlined firefighter training area at a U.S. Air Force Base where AFFF was used between 1970 and 1990. To quantify the total concentration of PFAA precursors in archived AFFF formulations and AFFF-impacted environmental samples, the oxidation-based assay developed in Chapter 2 was used. The assay was adapted for analysis of soil and aquifer solids. This assay was employed along with direct measurement of the twenty-two PFAA precursors in AFFF formulations and a suite of other PFASs that could have been formed upon transformation of those precursors.

The results collected from the firefighter training area in Chapter 3 demonstrated that much of the mass of PFAA precursors in AFFF formulations used onsite had been transformed to smaller precursors and terminal PFAAs. On a molar basis, precursors accounted for 41% to 100% of the total concentration of PFASs in archived AFFF formulations; in the training area, precursors measured by the oxidation assay accounted for an average of 23% and 28% of total

PFASs (*i.e.,* precursors and perfluorinated carboxylates and sulfonates) in groundwater and solids samples, respectively. One precursor in AFFF, perfluorohexane sulfonamide amine, was detected on several highly contaminated soil and aquifer solids samples at low concentrations. No other precursors also measured in AFFF formulations were detected in any samples at the field site. Suspected intermediate transformation products of precursors in AFFF that were directly measured accounted for approximately half of the total precursor concentration in samples from the training site. The fraction of PFASs consisting of PFAAs was greater in groundwater and solid samples than in any of the archived AFFF formulations, suggesting that much of the mass of precursors released at the site was converted to perfluorinated carboxylates and sulfonates.

To characterize AFFF-derived PFAA precursor transformation mechanisms, a series of microcosms were constructed with soil and sediment inocula (Chapter 4). The microcosms were incubated under different redox conditions with two different types of AFFF. Live culture, autoclaved culture, and medium control microcosms were monitored over time for PFAS, dissolved organic carbon, and electron acceptor concentrations. Additional PFAS products not detected by the existing LC-MS/MS method (Chapter 3) were identified through additional mass spectrometry techniques.

Live culture microcosms amended with AFFF manufactured by 3M demonstrated an ability to utilize the carbon in AFFF under all redox conditions, however no changes in PFAS concentrations were observed in any live culture or sterile microcosms over 60 to 90 day incubation periods. The lack of PFAA precursor transformation in soil and sediment microcosms was surprising in light of the PFAA precursor occurrence data measured in Chapter 3. PFAA precursors in 3M AFFF were detected only at relatively low concentrations on a few soil samples, whereas suspected transformation products made up 3% to 5% of total PFASs detected in groundwater and solid samples.

Changes in PFAS concentrations were observed over time in live culture soil and sediment microcosms amended with AFFF manufactured by Ansul. Under aerobic conditions, complete transformation of 15 µM of the main PFAA precursor, 6:2 fluorotelomer thioamido sulfonate (6:2 FtTAoS), was observed within 20 days in AFFF-acclimated soil microcosms. After 60 days of incubation and three AFFF amendments, 6% of 6:2 FtTAoS loss was measured as 6:2 fluorotelomer sulfonate and 2% was measured as perfluorinated carboxylates (0.9% PFHxA, 0.9% PFPeA, and 0.2% PFBA). Two additional products proposed to contain one and two oxygens associated with the thioether moiety of 6:2 FtTAoS were also identified but were not quantified. When the oxidation-based precursor assay (Chapter 3) was applied to aerobic microcosm slurries, the concentration of PFAA precursors in live culture microcosms remained steady throughout the duration of a growth cycle. This result indicated that aerobic transformation products remained in the microcosm slurry. In live culture anaerobic microcosms inoculated with creek sediments and amended with Ansul AFFF, complete loss of 30 µM of 6:2 FtTAoS was observed under nitrate-reducing conditions after 200 days and 45% to 71% loss was observed under sulfate-reducing, iron-reducing, and methanogenic conditions after 319 days. One common transformation product was identified among all live culture anaerobic microcosms, a carboxylate hydrolysis product of the 6:2 FtTAoS amide group. Relative to autoclaved culture microcosms, the total concentration of PFAA precursors in the microcosm

slurry decreased throughout incubation in all anaerobic live culture microcosms. This result indicated that anaerobic 6:2 FtTAoS biotransformation products may have volatilized.

Transformation products observed in Ansul AFFF microcosms provided insight into the PFASs measured in the subsurface of firefighter training areas. The aerobic transformation products of soil inocula exposed to Ansul AFFF, 6:2 FtS and perfluorinated carboxylates were observed in soil, aquifer solids, and groundwater at the site investigated in Chapter 3 and at other firefighter training areas [Schultz *et al*. 2004, Backe *et al*. 2012]. The other transformation products of 6:2 FtTAoS identified in the aerobic microcosms might be partially responsible for precursors measured by the oxidation assay that could not be attributed to any of the measured precursors. The apparent loss of PFAA precursors from the slurries of live culture anaerobic microcosms indicates that some loss of PFAA precursors at firefighter training sites might be converted to volatile transformation products in the absence of oxygen.

The research conducted in Chapters 3 and 4 confirmed that PFAA precursors in AFFF slowly form intermediate PFAA precursors as well as PFAA terminal products under laboratory and field conditions. Remediation activities targeted at PFAAs or other contaminants may accelerate transformation of the PFAA precursors. To fully address PFAA contamination at AFFF-impacted sites, the fate of PFAA precursors must also be taken into consideration.

AFFF-impacted sites, along with areas surrounding fluorochemical manufacturing facilities, are among the largest sources of PFAS contamination. The concentrations of PFOS and PFOA in AFFF-impacted groundwater often exceed their U.S. EPA drinking water Provisional Health Advisories by three orders of magnitude, and their precursors are also present at relatively high concentrations in soil and groundwater. The shorter-chain PFAAs and precursors, which are generally thought to require greater exposure than the C_8 compounds before resulting in adverse health effects, also cannot be ignored at AFFF sites because they occur at similar or higher concentrations than their analogous C_8 forms. Thus, drinking water procurement, food production, and biosolids harvesting must be isolated from AFFF firefighter-training sites and accidental large scale releases of AFFF for the protection of human health.

5.4 Future Research and Recommendations

While the precursor assay was applied to aqueous and solid samples in Chapters 2-4, many PFAA precursors such as fluorotelomer alcohols are volatile and are partitioned to the gas phase. The precursor assay could be adapted for the measurement of PFAA precursors in air using samples captured on carbon filters. This technique might be useful, for instance, in quantifying volatile biological transformation products in microcosm experiments or quantifying PFAA precursor emissions outside fluorochemical facilities creating point source contamination.

The precursor assay might also have utility in quantifying PFAA precursors in biological tissues such as serum or breast milk. *In vivo* transport, partitioning, and metabolism are relatively well understood for PFAAs in humans, but not for PFAA precursors. In addition to the challenges associated with analysis of specific PFAA precursors, their potential to metabolize into multiple products makes individual PFAA precursors difficult to monitor. The precursor analysis would enable the quantification of all PFAA precursors, including any partially fluorinated metabolites. The measurement of precursors is important because they might induce toxicity through different mechanisms than the PFAAs. For example, PFAA precursors tend to

have more hydrophobic structures than PFAAs and might partition more strongly to fats, resulting in different toxicological effects.

The application of the precursor assay in runoff and in AFFF-contaminated soil and groundwater indicates that there are a number of PFAA precursors not included in existing HPLC-MS/MS protocols. The large number of PFAA precursors used in commerce makes it challenging to capture all of these compounds using a single analytical technique. The fluorochemical research community, ideally in partnership with the major fluorochemical manufacturers, should adopt a set of indicator PFAA precursor compounds that reflects the range of years, products, and major production technologies used in fluorochemical manufacturing. Companies such as Wellington Laboratories that manufacture analytical standards could be enlisted to generate a mixed suite of the indicator compounds. By analyzing for a comprehensive but streamlined set of standard indicator compounds, more researchers would be able to include PFAA precursors in their analytical protocols and address these important sources of indirect PFAA contamination.

There is almost no understanding of the transformation potential of fluoropolymers. The few studies that have been conducted are disputed [Russell *et al.* 2010b, Washington *et al.* 2010a], and they only investigated the biotransformation potential of fluoropolymers in soils [Russell *et al.* 2009, Russell *et al.* 2010a, Washington *et al.* 2009]. The majority of the volume of fluorochemicals has been sold in the form of polymerized products [Prevedouros et al. 2006, Paul et al. 2009, Ahrens 2011, Martin et al. 2010], so their fate is very important to the ultimate burden of PFAAs in the environment. The biotransformation of fluoropolymers has thus far been observed to occur extremely slowly (*e.g.*, half-lives greater than ten years) in the presence of soil microbes, so researchers may be discouraged from studying fluoropolymer transformation potential. However, recycling and disposal processes that involve exposing fluoropolymer treated products to high temperatures or other extreme conditions might offer relevant abiotic transformation mechanisms to investigate on more favorable timescales. The precursor assay could also be tested on polymerized products to rapidly determine their susceptibility to hydroxyl radical deterioration. If polymerized products yield PFAAs upon exposure to hydroxyl radical, their long-term ability to serve as a source of PFAAs would seem more likely. Additionally, experiments with other radical species, such as sulfate radical, might indicate other abiotic conditions under which fluoropolymers would transform.

The toxicity of PFAA precursors and the non-C_8 PFAA compounds have not been investigated in detail. There are a number of known PFAA precursors that are relatively stable, such as the 5:3 fluorotelomer unsaturated carboxylate [Wang *et al.* 2011], that warrant toxicological examination. Based on their persistence over multi-decadal residence times at historical AFFF-impacted sites, 6:2 FtS, FOSA, and their related homologs are also relatively stable [Schultz *et al.* 2004, Backe *et al.* 2012, Houtz *et al.* 2013]. As the fluorochemical manufacturing industry has shifted production to C_4 and C_6-based products, it has become more important to understand the effects of these shorter-chain compounds on humans and biota, even if they are not as bioaccumulative as the C_8 PFAAs.

As with many environmental contaminants, developing treatment processes that can transform PFASs to benign products requires a tremendous amount of resources. It is still an open question whether C_8-replacement PFASs will eventually garner the same concern as PFOS and PFOA. That question will need to be answered before determining whether or not sites

contaminated with shorter-chain PFASs warrant treatment or remediation efforts. It is generally easier to defluorinate PFAA precursors, especially fluorotelomer precursors, than it is to defluorinate PFAAs. It is arguable that treatment technologies should target PFAA precursors rather than PFAAs in areas where they occur at comparable concentrations to one another, such as firefighter training areas.

The perfluoroalkyl group is one of the most thermodynamically stable chemical moieties. This attribute of recalcitrance is doubly challenging because the PFAAs last far longer than other environmental contaminants and they are also far more challenging and expensive to treat. Because of their extreme recalcitrance and their negative effects on human and animal health, the use of PFASs could be restricted to essential uses as a means of lowering their environmental burden and avoiding the high costs associated with their cleanup. Alternatively, attributes such as stain and water repellence could be achieved through the development of more benign chemical alternatives that avoid the use of the perfluoroalkyl group.

REFERENCES

3M Company. Voluntary Use and Exposure Information Profile Perfluorooctanesulfonyl fluoride (POSF). **1998**. AR226–0576.

3M Company. Material Safety Data Sheet for FC-203CF Light Water AFFF (3%). January **2005**.

Ahrens, L. Polyfluoroalkyl compounds in the aquatic environment: a review of their occurrence and fate. *J. Environ. Monit.* **2011**, *13*, 20-31.

Ahrens, L. Barber, J.L.; Xie, Z.; Ebinghaus, R. Longitudinal and latitudinal distribution of perfluoroalkyl compounds in the surface water of the Atlantic Ocean. *Environ. Sci. Technol.* **2009a**, *43*, 3122-3127.

Ahrens, L.; Felizeter, S.; Sturm, R.; Xie, Z.; Ebinghaus, R. Polyfluorinated compounds in waste water treatment plant effluents and surface waters along the River Elbe, Germany. *Mar. Pollut. Bull.* **2009b**, *58*, 1326-1333.

Armitage, J.M.; MacLeod, M.; Cousins, I.T. Comparative assessment of the global fate and transport pathways of long-chain perfluorocarboxylic acids (PFCAs) and perfluorcarboxylates (PFCs) emitted from direct sources. *Environ. Sci. Technol.* **2009**, *43*, 5830-5836.

Armitage, J.M.; Schenkler, U.; Scheringer, M.; Martin, J.W.; MacLeod, M.; Cousins, I.T. Modeling the global fate and transport of perfluorooctane sulfonate (PFOS) and precursor compounds in relation to temporal trends in wildlife exposure. *Environ. Sci. Technol.* **2009**, *43*, 9247-9280.

Awad, E.; Zhang, X.; Bhavsar, S.P.; Petro, S.; Crozier, P.W.; Reiner, E.J.; Fletcher, R.; Tittlemier, S.A.; Braekevelt, E. Long-term environmental fate of perfluorinated compounds after accidental release at Toronto airport. *Environ. Sci. Technol.* **2011**, *45*, 8081-8089.

Backe, W.J.; Christensen, K.E.; Field, J.A. Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from U.S. Military bases by non-aqueous large-volume injection HPLC-MS/MS. *Environ. Sci. Technol.* **2013**, *47*, 5226-5234 .

Begley, T.H.; Hsu, W.; Noonan, G.; Diachenko, G. Migration of fluorochemical-paper additives from food-contact paper into foods and food simulants. *Food Additives & Contaminants: Part A* **2008**, *25*, 384-390.

Begley, T.H.; White, K.; Honigfort, P.; Twaroski. M.L.; Neches, R.; Walker, R.A. Perfluorochemicals: potential sources of and migration from food packaging. *Food Additives & Contaminants: Part A* **2005**, *22*, 1023-1031.

Benskin, J.P.; De Silva, A.O.; Martin, J.W. Isomer profiling of perfluorinated substances as a

tool for source tracking: a review of early findings and future applications. *Rev. of Environ. Contam. and Toxicol.* **2010**, *208*, 111-160.

Benskin, J.P.; Muir, D.C.G.; Scott, B.F.; Spencer, C.; De Silva, A.O.; Kylin, H.; Martin, J.W.; Morris, A.; Lohmann, R.; Tomy, G.; Rosenberg, B.; Taniyasu S.; Yamashita, N. Perfluoroalkyl acids in the Atlantic and Canadian Arctic Oceans. *Environ. Sci. Technol.* **2012**, *46*, 5815-5823.

Berger, T.W. Aqueous Film-Forming Foam Fire Extinguisher. U.S. Patent 4,359,096. Nov. 16, **1982**.

Bischel, H.N.; MacManus-Spencer, L.A.; Luthy, R.G. Noncovalent interactions of long-chain perfluoroalkyl acids with serum albumin. *Environ. Sci. Technol*. **2010**, *44*, 5263-5269.

Björklund, J.A.; Thuresson, K.; de Wit, C.A. Perfluoroalkyl compounds (PFCs) in indoor dust: concentrations, human exposure estimates, and sources. *Environ. Sci. Technol.* **2009**, *43*, 2276-2281.

Boulanger, B.; Vargo, J.D.; Schnoor, J.L.; Hornbuckle, K.C. Evaluation of perfluorooctane surfactants in a wastewater treatment system and in a commercial surface protection product. *Environ. Sci. Technol.* **2005**, *39*, 5524-5530.

Butt, C.M.; Muir, D.C.G.; Mabury, S.A. Elucidating the pathways of poly- and perfluorinated acid formation in rainbow trout. *Environ. Sci. Technol.* **2010**, *44*, 4973-4980.

Chemguard. Material Safety Data Sheet for Chemguard 3% AFFF C-301MS. January **2006**.

Chiesa, P.J. Film-forming fire fighting composition. U.S. Patent 3,849,315. Nov. 19, **1974**.

Chiesa, P.J.; Di Maio, L.R. Fire fighting. U.S. Patent, 3,957,658. May 18, **1976**.

Colosi, L.M.; Pinto, R.A.; Huang, Q.; Weber, W.J. Peroxidase-mediated degradation of perfluorooctanoic acid. *Environ. Toxicol. Chem.*, **2009**, *28*, 264-271.

Commission Regulation No 552/2009. *Office Journal of the European Union*. **2009**, *164*, 7-31.

Conder, J.M.; Hoke, R.A.; De Wolf, W.; Russell, M.H.; Buck, R.C. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. *Environ. Sci. Technol.* **2008,** *42*, 995-1003.

Costa, G.; Sartori, S.; Consonni, D. Thirty years of medical surveillance in perfluorooctanoic acid production workers. *J. Occup. Environ. Med.* **2009**, *51*, 364-372.

Darwin, R. L. Estimated Quantities of Aqueous Film Forming Foam (AFFF) in the United States; Baltimore, MD, August, **2004**.

Dasu, K.; Liu, J.; Lee, L.S. Aerobic soil biodegradation of 8:2 fluorotelomer stearate monoester. *Environ. Sci. Technol.* **2012**, *46*, 3831-3836.

D'Eon, J.; Crozier, P.; Furdui, V.; Reiner, E. Perfluorinated phosphonic acids in Canadian surface waters and wastewater treatment plant effluent: Discovery of a new class of perfluorinated acids. *Environ. Toxicol. Chem.* **2009**, *28*, 2101-2107.

D'Eon, J.; Hurley, M.D.; Wallington, T.J.; Mabury, S.A. Atmospheric chemistry of N-methyl perfluorobutane sulfonamidoethanol: Kinetics and mechanism of reactions with OH. *Environ. Sci. Technol.* **2006**, *40*, 1862-1868.

D'Eon, J.; Mabury, S.A. Production of perfluorinated carboxylic acids (PFCAs) from biotransformation of polyfluoroalkyl phosphate surfactants (PAPs): Exploring routes of human contamination. *Environ. Sci. Technol.* **2007**, *41*, 4799-4805.

D'Eon, J.; Mabury, S.A. Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? *Environ. Sci. Technol.* **2011**, *45*, 7974-7984.

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.

DuPont Global PFOA Strategy. Presentation to the U.S.EPA, January 31, 2005. Public Docket AR226-1914; Washington, DC, January 31, **2005**.

Eaton, A.D.; Clesceri, L.S.; Greenberg, A.E. editors. Standard methods for the examination of water and wastewater, 20th ed. Washington DC: American Public Health Association, American Water Works Association, Water Environment Federation Publishers, **1998**.

Ellis, D.A.; Martin, J.W.; DeSilva, A.O.; Mabury, S.A.; Hurley, M.D.; Andersen, M.P.S.; Wallington, T.J. Degradation of fluorotelomer alcohols: a likely atmospheric source of perfluorinated carboxylic acids. *Environ. Sci. Technol.* **2004**, *38*, 3316-3321.

Emmett, E.A.; Shofer, F.S.; Zhang, H.; Freeman, D.; Desai, C.; Shaw, L.M. Community exposure to perfluorooctanoate: relationships between serum concentrations and exposure sources. *J. Occup. Environ. Med.* **2006**, *48*, 759-770.

Eschauzier, C.; Beerendonk, E.; Scholte-Veenendaal, P.; de Voogt, P. Impact of treatment processes on the removal of perfluoroalkyl acids from the drinking water production chain. *Environ. Sci. Technol.* **2012**, *46*, 1708-1715.

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.

Fielding, H.C.; Hutchinson, J.; Webster, S.J. Fire-fighting Foam Compositions. U.S. Patent 4,020,903. May 3, **1977**.

Francen, V.L. Fire extinguishing composition comprising a fluoroaliphatic surfactant and a fluorine-free surfactant. U.S. Patent 3,562,156. Feb. 9, **1971**.

Fromme, H.; Schlummer, M.; Möller, A.; Gruber, L.; Wolz, G.; Ungewiss, J.; Böhmer, S.; Dekant, W.; Mayer, R.; Liebl, B.; Twardella, D. Exposure of an adult population to perfluorinated substances using duplicate diet portions and biomonitoring data. *Environ. Sci. Technol.* **2007**, *41*, 7928-7933.

Gallo, V.; Leonardi, G.; Genser, B.; Lopez-Espinosa, M.; Frisbee, S.J.; Karlsson, L.; Ducatman, A.M.; Fletcher, T. Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure. *Environ. Health Perspect.*, **2012**, *120*, 655-660.

Gauthier, S.A.; Mabury, S.A. Aqueous photolysis of 8:2 fluorotelomer alcohol. *Environ. Toxicol. Chem.* **2005**, *24*, 1837-1846.

Giesy, J.P.; Kannan, K. Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **2001**, *35*, 1339-1342.

Glynn, A.; Berger, U.; Bignert, A.; Ullah, S.; Aune, M.; Lignell, S.; Darnerud, P. O. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996−2010. *Environ. Sci. Technol.* **2012**, *46*, 9071−9079.

Grandjean, P.; Wreford Andersen, E.; Budtz-Jorgensen, E.; Nielsen, F.; Molbak, K.; Weihe, P.; Heilmann, C. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *J. American Med. Assoc.* **2012***, 307*, 391-397.

Guo, R.; Sim, W.J.; Lee, E.S.; Lee, J.H.; Oh J.E. Evaluation of the fate of perfluoroalkyl compounds in wastewater treatment plants. *Water Res*, **2010**, *44*, 3476-3486.

Halldorsson, T.I.; Rytter, D.; Smastuen, H.; Hammer Bech, B.; Danielsen, G.B.; Henriksen, T.B.; Olsen, S.F. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort srtudy. *Environ. Health Perspect.* **2012**, *120*, 668-673.

Harada, K.; Saito, N.; Inoue, K.; Yoshinaga, T.; Watanabe, T.; Sasaki, S.; Kamiyama, S.; Koizumi, A. The influence of time, sex and geographic factors on levels of perfluorooctane sulfonate and perfluorooctanoate in human serum over the last 25 years. *J. Occup. Health* **2004**, *46*, 141−147.

Haug, L.S.; Huber, S.; Becher, G.; Thomsen, C. Characterization of human exposure pathways to perfluorinated compounds – comparing exposure estimates with biomarkers of exposure. *Env. Int.* **2011**, *37*, 687-693.

Haug, L.S.; Thomsen, C.; Bechert, G. Time trends and influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. *Environ. Sci. Technol.* **2009**, *43*, 2131-2136.

He, J.; Holmes, V.F.; Lee, P.K.H.; Alvarez-Cohen, L. Influence of vitamin B12 and cocultures on the growth of Dehalococcoides isolates in defined medium. *Appl. Environ. Microbiol.* **2007**, *73*, 2847–2853.

Higgins, C.P.; Field, J.A.; Criddle, C.S.; Luthy, R.G. Quantitative determination of perfluorochemicals in sediments and domestic sludge. *Environ. Sci. Technol.* **2005**, *39*, 3946-3956.

Higgins, C.P.; Luthy, R.G. Sorption of perfluorinated surfactants on sediments. *Environ. Sci. Technol.* **2006**, *40*, 7251-7256.

Higgins, C.P.; Luthy, R.G. Modeling sorption of anionic surfactants onto sediment materials: an a priori approach for perfluoroalkyl surfactants and linear alkylbenzene sulfonates. *Environ. Sci. Technol.* **2007**, *41*, 3254-3261.

Hori, H.; Hayakawa, E.; Einaga, H.; Kutsuna, S.; Koike, K.; Ibusuki, T.; Kiatagawa, H.; Arakawa, R. Decomposition of environmentally persistent perfluorooctanoic acid in water by photochemical approaches. *Environ. Sci. Technol.* **2004**, *38*, 6118-6124.

Hori, H.; Yamamoto, A.; Hayakawa, E.; Taniyasu, S.; Yamashita, N.; Kutsuna, S.; Kiatagawa, H.; Arakawa, R. Efficient decomposition of environmentally persistent perfluorocarboxylic acids by use of persulfate as a photochemical oxidant. *Environ. Sci. Technol.* **2005**, *39*, 2383-2388.

Houde, M.; Martin, J.W.; Letcher, R.J.; Solomon, K.R.; Muir, D.C.G. Biological monitoring of polyfluoroalkyl substances: a review. *Environ. Sci. Technol.* **2006**, *40*, 3463-3473.

Houde, M.; De Silva, A.O.; Muir, D.C.G.; Letcher, R.J. Monitoring of perfluorinated compounds in aquatic biota: an updated review. *Environ. Sci. Technol.* **2011**, *45*, 7962-7973.

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.

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.

Huset, C.A.; Barlaz, M.A.; Barofski, D.F.; Field, J.A. Quantitative determination of fluorochemicals in municipal landfill leachates. *Chemosphere* **2011**, *82*, 1380-1386.

Huset, C.A.; Chiaia, A.C.; Barofsky, D.F.; Jonkers, N.; Kohler, H.E.; Ort, C.; Giger, W.; Field, J.A. Occurrence and mass flows of fluorochemicals in the Glatt Valley Watershed, Switzerland. *Environ. Sci. Technol.* **2008**, *42*, 6369-6377.

Jackson, D.A.; Wallington, T.J.; Mabury, S.A. Atmospheric oxidation of polyfluorinated amides: historical source of perfluorinated carboxylic acids to the environment. *Environ. Sci. Technol.* **2013**, *47*, 4317-4234.

Jin, Y.; Saito, N.; Harada, K. H.; Inoue, K.; Koizumi, A. Historical trends in human serum levels of perfluorooctanoate and perfluorooctane sulfonate in Shenyang, China. *Tohoku J. Exp. Med.* **2007**, *212*, 63−70.

Johnson, R.L.; Tratnyek, P.G.; Johnson, R.O. Persulfate persistence under thermal activation conditions. *Environ. Sci. Technol.* **2008**, *42*, 9350-9356.

Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K.S.; Loganathan, B.G.; Mohd, M.A.; Olivero, J.; Van Wouwe, N.; Yang, J.H.; Aldous, K.M. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* **2004**, *38*, 4489-4495.

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

Kato, K.; Wong, L.-Y.; Jia, L.T.; Kuklenyik, Z.; Calafat, A.M. Trends in exposure to polyfluoroalkyl chemicals in the U.S. population: 1999-2008. *Environ. Sci. Technol.* **2011**, *45*, 8037-8045.

Kim, S.; Kannan, K. Perfluorinated acids in air, rain, snow, surface runoff, and lakes: relative importance of pathways to contamination of urban lakes. *Environ. Sci. Technol.* **2007**, *41*, 8328-8334.

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

Lau C.; Thibodeaux JR, Hanson R.G.; Narotsky, M.G.; Rogers, J.M.; Lindstrom, A.B.; Strynar, M.J. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicol. Sci.* **2006**, *90*, 510-518.

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.

Lee, H.; Mabury, S.A. A pilot survey of legacy and current commercial fluorinated chemicals in human sera from United States donors in 2009. *Environ. Sci. Technol.* **2011**, *45*, 8067-8074.

Liu, C.; Gin, K.Y.H.; Chang, V.W.C.; Goh, B.P.L.; Reinhard, M. Novel perspectives on the bioaccumulation of PFCs – the concentration dependency. *Environ. Sci. Technol.* **2011**, *45*, 9758-9764.

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. *Chemosphere* **2010**, *80*, 716-723.

Lopez-Espinosa, M.; Mondal, D.; Armstrong, B.; Bloom, M.S.; Fletcher, T. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. *Environ. Health Perspect.* **2012**, *120*, 1036-1041.

Lovely, D.R.; Phillips, E.J. Organic matter mineralization with reduction of ferric irion in anaerobic sediments. *Appl. Environ. Microbiol.* **1986**, *51*, 683-689.

Martin, J.W.; Asher, B.J.; Beesoon, S.; Benskin, J.P.; Ross, M.S. PFOS or PreFOS? Are perfluorooctane sulfonate precursors (PreFOS) important determinants of human and environmental perfluorooctane sulfonate (PFOS) exposure? *J. Environ. Monitor.* **2010**, *12*, 1979-2004.

Martin, J.W.; Ellis, D.A.; Mabury, S.A. Atmospheric chemistry of perfluoroalkanesulfonamides: kinetic and product studies of OH radical and Cl atom initiated oxidation of N-Ethyl perfluorobutanesulfonamide. *Environ. Sci. Technol.* **2006**, *40*, 864-872.

Martin, J.W.; Mabury, S.A.; O'Brien, P.J. Metabolic products and pathways of fluorotelomer alcohols in isolated rat hepatocytes. *Chem-Biol Interact*. **2005**, *155*, 165-180.

Martin, J.W.; Smithwick, M.M.; Braune, B.M.; Hoekstra, P.F.; Muir, D.C.G.; Mabury, S.A. Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environ. Sci. Technol.* **2004**, *38*, 373-380.

McGuire, M.E.; Schaefer, C.; Richards, T.; Backe, W.; Field, J.A.; Houtz, E.F.; Sedlak, D.L.; Guelfo, J.L.; Higgins, C.P. An in-depth site characterization of poly and perfluoroalkyl substances (PFASs) at an abandoned fire training area. *In Preparation.*

Meyer, T.; De Silva, A.O.; Spencer, C.; Wania, F. Fate of perfluorinated carboxylates and sulfonates during snowmelt within an urban watershed. *Environ. Sci. Technol.* **2011,** *45*, 8113-8119.

MIL-F-24385 QPL/QPD History for Type 6 AFFF. US Naval Sea Systems Command. July 1, **2011**.

Mitch, W.A.; Gerecke, A.C.; Sedlak, D.L. A *N*-Nitrosodimethylamine (NDMA) precursor analysis for chlorination of water and wastewater. *Water Res.* **2003**, *37,* 3733-3741.

Miyake, Y.; Yamashita, N.; Rostkowski, P.; So, M.K.; Taniyasu, S.; Lam, P.K.S.; Kannan, K. Determination of trace levels of total fluorine in water using combustion ion chromatography for fluorine: a mass balance approach to determine individual perfluorinated chemicals in water. *J. Chromatogr. A* **2007a**, *1143*, 98-104.

Miyake, Y.; Yamashita, N.; So, M.K.; Rostkowski, P.; Taniyasu, S.; Lam, P.K.S.; Kannan, K. Trace analysis of total fluorine in human blood using combustion ion chromatography for fluorine: a mass balance approach for the determination of known and unknown organofluorine compounds. *J. Chromatogr. A* **2007b**, *1154*, 214-221.

Moe, M.K.; Huber, S.; Svenson, J.; Hagenaars, A.; Pabon, M.; Trümper, M.; Berger, U.; Knapen, D.; Herzke, D. The structure of the fire fighting foam surfactant Forafac® 1157 and its biological and photolytic transformation products. *Chemosphere* **2012**, *89*, 869-875.

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.

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.

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.

Moriwaki, H.; Takagi, Y.; Tanaka, M.; Tsuruho, K.; Okitsu, K.; Maeda, Y. Sonochemical decomposition of perfluorooctane sulfonate and perfluorooctanoic acid. *Environ. Sci. Technol.* **2005**, *39*, 3388-3392.

Müller, C.E.; Spiess, N.; Gerecke, A.C.; Scheringer, M.; Hungerbühler, K. Quantifying diffuse and point inputs of perfluoroalkyl acids in a nonindustrial river catchment. *Environ. Sci. Technol.* **2011**, *45*, 9901-9909.

Murakami, M.; Kuroda, K.; Sato, N.; Fukushi, T.; Takizawa, S.; Takada, H. Groundwater pollution by perfluorinated surfactants in Tokyo. *Environ. Sci. Technol.* **2009a,** *43*, 3480-3486.

Murakami, M.; Sato, N.; Anegawa, A.; Nakada, N.; Harada, A.; Komatsu, T.; Takada, H.; Tanaka, H.; Ono, Y.; Furumai, H. Multiple evaluations of the removal of pollutants in road runoff by soil infiltration. *Water Res.* **2008,** *42*, 2745-2755.

Murakami, M.; Shinohara, H.; Takada, H. Evaluation of wastewater and street runoff as sources of perfluorinated surfactants (PFSs). *Chemosphere* **2009b**, *74*, 487-493.

National Foam. Material Safety Data Sheet for Aer-O-Water 3EM 3% AFFF Liquid Concentrate. November **2010**.

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.

Nguyen, V.T.; Reinhard, M.; Karina, G.Y.-H. Occurrence and source characterization of perfluorochemicals in an urban watershed. *Chemosphere* **2011**, *82*, 1277-1285.

Niu, J.; Lin, H.; Xu, J.; Wu, H.; Li, Y. Electrochemical mineralization of perfluorocarboxylic acids (PFCAs) by Ce-doped modified porous nanocrystalline PbO_2 Film Electrode. *Environ. Sci. Technol.* **2012**, *46*, 10191-10198.

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. *Aquatic Toxicol.* **2010**, *98*, 120-129.

Ochoa-Herrera, V.; Sierra-Alvarez, R.; Somogyi, A.; Jacobsen, N.E.; Wysocki, V.H.; Field, J.A. Reductive defluorination of perfluorooctane sulfonate. *Environ. Sci. Technol.* **2008**, *42*, 3260-3264.

Olsen, G.W.; Burris, J.M.; Ehresman, D.J.; Froehlich, J.W.; Seacat, A.M.; Butenhoff, J.L.; Zobel, L.R. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ. Health Perspect.* **2007**, *115*, 1298-1305.

Olsen, G.W.; Huang, H.Y.; Helzlsouer, K.J.; Hansen, K.J.; Butenhoff, J.L.; Mandel, J.H. Historical comparison of perfluorooctanesulfonate, perfuorooctanoate and other fluorochemicals in human blood. *Environ. Health Perspect.* **2005**. *113*, 539-545.

Olsen, G. W.; Lange, C. C.; Ellefson, M. E.; Mair, D. C.; Church, T. R.; Goldberg, C. L.; Herron, R. M.; Medhdizadehkashi, Z.; Nobiletti, J. B.; Rios, J. A.; Reagen, W. K.; Zobel, L. R. Temporal trends of perfluoroalkyl concentrations in American Red Cross adult blood donors, 2000−2010. *Environ. Sci. Technol*. **2012**, *46*, 6330−6338.

Parales, R. E.; Adamus, J. E.; White, N.; May, H. D. Degradation of 1,4-dioxane by an actinomycete in pure culture. *Appl. Environ. Microbiol.* **1994**, *60*, 4527–4530.

Park, H.; Vecitis, C.D.; Cheng, J.; Mader, B.T.; Hoffmann, M.R. Reductive defluorination of aqueous perfluorinated alkyl surfactants: effects of ionic headgroup and chain length. *J. Phys. Chem. A* **2009**, *113*, 690-696.

Parsons, J.R.; Saez, M.; Dolfing, J. de Voogt, P. Biodegradation of perfluorinated compounds. *Rev. Environ. Contam. and Toxicol.* **2008**, *196*, 53-71.

Paul, A.G.; Jones, K.C.; Sweetman, A.J. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. *Environ. Sci. Technol.* **2009**, *43*, 386-392.

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.

Plumlee, M.H.; Larabee, J.; Reinhard, M. Perfluorochemicals in water reuse. *Chemosphere* **2008**, *72*, 1541-1547.

Plumlee, M.H.; McNeill, K.; Reinhard, M. Indirect photolysis of perfluorochemicals: hydroxyl radical-initiated oxidation of N-ethyl perfluorooctane sulfonamido acetate (*N*-EtFOSAA) and other perfluoroalkanesulfonamides. *Environ. Sci. Technol.* **2009**, *43*, 3662-3668.

Prevedouros, K.; Cousins, I.T.; Buck, R.C.; Korzeniowski, S.H. Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol.* **2006**, *40*, 32-44.

Qu, Y.; Zhang, C.; Li, F.; Qi, Z. Photo-reductive defluorination of perfluorooctanoic acid in water. *Water Res.* **2010**, *44,* 2939-2947.

Rhoads, K.R.; Janssen, E.M.-L.; Luthy, R.G.; Criddle, C.S. Aerobic biotransformation and fate of N-ethyl perfluorooctane sulfonamidoethanol (*N*-EtFOSE) in activated sludge. *Environ. Sci. Technol.* **2008**, *42*, 2873-2878.

Russell, M.H.; Berti, W.R.; Szostek, B.; Buck, R.C. Investigation of the biodegradation potential of a fluoroacrylate polymer product in aerobic soils. *Environ. Sci. Technol.* **2008**, *42*, 800-807.

Russell, M.H.; Berti, W.R.; Szostek, B.; Wang, N.; Buck, R.C. Evaluation of PFO formation from the biodegradation of a fluorotelomer-based urethane polymer product in aerobic soils. *Polymer Degradation and Stability* **2010a**, *95*, 79-85.

Russell, M.H.; Wang, N.; Berti, W.R.; Szostek, S.; Buck, R.C. Comment on "Degradability of an acrylate-linked, fluorotelomer polymer in soil". *Environ. Sci. Technol.* **2010b**, *44*, 848-848.

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.* **2007a**, *49*, 1086-1096.

Sakr, C.J.; Leonard, R.C.; Kreckmann, K.H.; Slade, M.D.; Cullen, M.R.; Longitudinal study of serum lipids and liver enzymes in workers with occupational exposure to ammonium perfluorooctanoate. *J. Occup. Environ. Med.* **2007b**, *49*, 872-879.

Schröder, H.F.; Meesters, R.J.W. Stability of fluorinated surfactants in advanced oxidation processes – A follow up of degradation products using flow injection mass spectrometry, liquid chromatography-mass spectrometry and liquid chromatography-multiple stage mass spectrometry. *J. Chromatogr. A* **2005**, *1082*, 110-119.

Schultz, M. M.; Day, T.C.; Field, J. A. Quantitative determination of fluorotelomer sulfonates in groundwater by LC MS/MS. *Environ. Sci. Technol*. **2004**, *38*, 1828−1835.

Schultz, M.M.; Higgins, C.P.; Huset, C.A.; Luthy, R.G.; Barofsky, D.F.; Field, J.A. Fluorochemical mass flows in a municipal wastewater treatment facility. *Environ. Sci. Technol.* **2006**, *40*, 7350-7357.

Seacat, A.M.; Thomford, P.J.; Hansen, K.J.; Olsen, G.W.; Case, M.T.; Butenhoff, J.L. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in Cynomolgus monkeys. *Toxicol. Sci.* **2002**, *68*, 249-264.

Sepulvado, J.G.; Blaine, A.C.; Hundal, L.S.; Higgins, C.P. Occurrence and fate of perfluorochemicals in soil following the land application of municipal biosolids. *Environ. Sci. Technol.* **2011**, *45,* 8106-8112.

Stevens, A.A.; Symons, J.M. Measurement of trihalomethane and precursor concentration changes. *J. AWWA* **1977**, *October*, 546-54.

Taniyasu, S.; Kannan, K.; So, M.K.; Gulkowska, A.; Sinclair, E.; Okazawa, T.; Yamashita, N. Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. *J. Chromatogr. A* **2005**, *1093*, 89-97.

Telomer Research Program Update. Presentation to the U.S. EPA, November 25, 2002. Public Docket AR226-1141; Washington, DC, November 25, **2002**.

Trudel, D.; Horowitz, L.; Wormuth, M.; Scheringer, M.; Cousins, I.T.; Hungerbühler. Estimating consumer exposure to PFOS and PFOA. *Risk Anal.* **2008**, *28*, 251-269.

Tsao, M.; Wilmarth, W.K. The aqueous chemistry of inorganic free radicals. I. The mechanism of the photolytic decomposition of aqueous persulfate ion and evidence regarding the sulfate-hydroxyl radical interconversion equilibrium. *J. Phys. Chem.* **1959**, *63*, 346-353.

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

U.S. EPA. Office of Pollution Prevention and Toxics. 2010/2015 PFOA Stewardship Program. October **2006**.

U.S. EPA. Provisional Health Advisories for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS). **2009.**

U.S. EPA. Revisions to the Unregulated Contaminant Monitoring Regulations (UCMR 3) for Public Water Systems. April **2012.**

Vecitis, C.D.; Park, H.; Cheng, J.; Mader, B.T. Treatment technologies for aqueous perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA). *Front. Environ. Sci. Eng. China* **2009**, *3*, 129-151.

Vecitis, C.D.; Park, H.; Cheng, J.; Mader, B.T.; Hoffmann, M.R. Kinetics and mechanism of the sonolytic conversion of the aqueous perfluorinated surfactants, perfluroocotanoate (PFOA), and perfluorooctane sulfonate (PFOS) into inorganic products. *J. Phys. Chem. A* **2008**, *112*, 4261-4270.

Vestergren, R.; Berger, U.; Glynn, A.; Cousins, I.T.; Dietary exposure to perfluoroalkyl acids for the Swedish population in 1999, 2005, and 2010. *Environ. Int.* **2012**, *49*, 120-127.

Vestergren, R.; Cousins, I.T. Tracking the pathways of human exposure to perfluorocarboxylates. *Environ. Sci. Technol.* **2009**, *43,* 5565-5575.

Vestergren, R.; Cousins, I.T. Trudel, D.; Wormuth, M.; Scheringer, M. Estimating the contribution of precursor compounds in consumer exposure to PFOS and PFOA. *Chemosphere* **2008**, *73*, 1617-1624.

Vieira, V.M.; Hoffman, K.; Shin, H.M.; Weinberg, J.M.; Webster, T.F.; Fletcher, T. Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: A geographic analysis. *Environ. Health Perspect.* **2013**, *121*, 318-323.

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.

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.

Wang, N.; Szostek, B.; Buck, R.C.; Folsom, P.W.; Sulecki, L.M.; Gannon, J.T. 8:2 fluorotelomer alcohol aerobic soil biodegradation: pathways, metabolites, and metabolite yields. *Chemosphere* **2009**, *75*, 1089-1096.

Washington, J.W.; Ellington, J.J.; Jenkins, T.M.; Evans, J.J.; Yoo, H.; Hafner, S.C. Degradability of an acrylate-linked, fluorotelomer polymer in soil. *Environ. Sci. Technol.* **2009**, *43*, 6617-6623.

Washington, J.W.; Ellington, J.J.; Jenkins, T.M.; Evans, J.J.; Yoo, H. Response to Comments on "Degradability of an Acrylate-Linked, Fluorotelomer Polymer in Soil". *Environ. Sci. Technol.* **2010a**, *44*, 849-850.

Washington, J.W.; Yoo, H.Y.; Jackson Ellington, J.; Jenkins, T.M.; Libelo, E.L. Concentrations, distribution, and persistence of perfluoroalkylates in sludge-applied soils near Decatur, Alabama, USA. *Environ. Sci. Technol.* **2010b**, *44*, 8390-8396.

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.

Wormald. Material Safety Data Sheet for Ansulite 3 % AFFF AFC-5-A. April **2008**.

Yamada, T.; Taylor, P.H.; Buck, R.C.; Kaiser, M.A.; Giraud, R.J. Thermal degradation of fluorotelomer treated articles and related materials. *Chemosphere* **2005**, *61*, 974-984.

Yeung, L.W.Y; Miyake, Y.; Taniyasu, S.; Wang, Y.; Yu, H.; So, M.K.; Jiang, G.; Wu, Y.; Li, J. Giesy, J.P.; Yamashita, N.; Lam, P.K.S. Perfluorinated compounds and total extractable organic fluorine in human blood samples from China. *Environ. Sci. Technol.* **2008**, *42*, 8140-8145.

Yeung, L.W.-Y.; Robinson, S.; Koschorreck, J.; Mabury, S.A. Part I. A temporal study of PFCAs and their precursors in human plasma from two German cities 1982-2009. *Environ. Sci. Technol.* **2013a,** *47*, 3865-3874.

Yeung, L.W.-Y.; Robinson, S.; Koschorreck, J.; Mabury, S.A. Part II. A temporal study of PFOS and its precursors in human plasma from two German cities 1982-2009. *Environ. Sci. Technol.* **2013b**, *47*, 3875-3882.

Yoo, H.Y.; Washington, J.W.; Jackson Ellington, J.; Jenkins, T.M.; Neill, M.P. Concentrations, distribution, and persistence of fluorotelomer alcohols in sludge-applied soils near Decatur, Alabama, USA. *Environ. Sci. Technol.* **2010**, *44*, 8397-8402.

Zhuo, Q.; Deng, S.; Yang, B.; Huang, J.; Yu, G. Efficient electrochemical oxidation of perfluorooctanoate using a Ti/SNO_2-Sb-Bi anode. *Environ. Sci. Technol.* **2011**, *45*, 2973-2979.

Zushi, Y.; Masunaga, S. First-flush loads of perfluorinated compounds in stormwater runoff from Hayabuchi River basin, Japan served by separated sewerage system. *Chemosphere* **2009a**, *76*, 833-834.

Zushi, Y.; Masunaga, S. Identifying the nonpoint source of perfluorinated compounds using a geographic information system based approach. *Environ. Toxicol. Chem.* **2009b**, *28*, 691-700.

Zushi, Y.; Takeda, T.; Masunaga, S. Existence of nonpoint source of perfluorinated compounds and their loads in the Tsurumi River basin, Japan. *Chemosphere* **2008**, *71*, 1566-1573.

Appendix: Chapter 2

Table A2.1: Perfluoroalkyl and polyfluorinated substances (PFASs) and stable isotope surrogate standards.

Analyte Abbreviation	Analyte Name	Surrogate Standard
PFCAs		
PFBA[1]	Perfluorobutanoate	[$^{13}C_4$] PFBA
PFPeA	Perfluropentanoate	[$^{13}C_2$] PFHxA
PFHxA	Perfluorohexanoate	[$^{13}C_2$] PFHxA
PFHpA	Perfluoroheptanoate	[$^{13}C_2$] PFHxA
PFOA	Perfluorooctanoate	[$^{13}C_4$] PFOA
PFNA	Perfluorononanoate	[$^{13}C_5$] PFNA
PFDA	Perfluorodecanoate	[$^{13}C_2$] PFDA
PFUnA	Perfluroundecanoate	[$^{13}C_2$] PFUnA
PFDoA	Perfluorododecanoate	[$^{13}C_2$] PFDoA
PFTrA	Perfluorotridecanoate	[$^{13}C_2$] PFDoA
PFTeA	Perfluorotetradecanoate	[$^{13}C_2$] PFDoA
PFSAs		
PFBS	Perfluorobutanesulfonate	[$^{18}O_2$] PFHxS
PFHxS	Perfluorohexanesulfonate	[$^{18}O_2$] PFHxS
PFOS	Perfluorooctanesulfonate	[$^{13}C_4$] PFOS
PFDS	Perfluorodecanesulfonate	[$^{13}C_4$] PFOS
PFAA Precursors		
6:2 FtS	6:2 fluorotelomer sulfonate	[$^{13}C_4$] PFOA
8:2 FtS	8:2 fluorotelomer sulfonate	[$^{13}C_2$] PFDA
FOSA	Perfluorooctanesulfonamide	None[2]
N-EtFOSE	2-(N-Ethyl-perfluoro-1-octanesulfonamido) ethanol	None[2]
N-MeFOSAA	N-Methyl-perfluoro-1-octanesulfonamideoacetic acid	[$^{2}D_5$] *N*-EtFOSAA
N-EtFOSAA	N-Ethyl-perfluoro-1-octanesulfonamidoacetic acid	[$^{2}D_5$] *N*-EtFOSAA
6:2 diPAP[1]	6:2 polyfluoroalkyl phosphate diester	None[2]
8:2 diPAP[1]	8:2 polyfluoroalkyl phosphate diester	None[2]

[1] Analytes not reported for stormwater samples.

[2] Analytes were quantified without surrogate recovery standards, which were not available at the time of research.

Table A2.2: Method and instrument detection limits and limits of quantification (LOQ) for each PFAS analyte. Instrument detection limit and LOQ are the concentrations at which the signal to noise ratio of the analyte is equal to 3 and 9, respectively, over triplicate injections. The method detection limit and limit of quantification account for enhanced sensitivity achieved by WAX SPE extraction and subsequent concentration.

	Method Detection Limit (ng/L)	Method LOQ (ng/L)	Instrument Detection Limit (ng/L)	Instrument LOQ (ng/L)
PFBA	N/A	N/A	200	500
PFPeA	0.3	0.9	75	225
PFHpA	0.2	0.6	50	150
PFHxA	0.3	0.9	75	225
PFOA	0.2	0.6	50	150
PFNA	0.3	0.9	75	225
PFDA	0.2	0.6	50	150
PFUnA	0.3	0.9	75	225
PFDoA	0.2	0.6	50	150
PFTrA	0.3	0.9	75	225
PFTeA	0.3	0.9	75	225
FOSA	0.2	0.5	40	120
PFBS	0.5	1.4	115	345
PFHxS	0.2	0.5	40	120
PFOS	0.1	0.3	25	75
PFDS	0.2	0.5	40	120
N-EtFOSE	0.2	0.7	40	180
N-EtFOSAA	0.2	0.5	40	120
N-MeFOSAA	0.2	0.5	40	120
6:2 FtS	0.2	0.5	40	120
8:2 FtS	0.2	0.5	40	120
6:2 diPAP	N/A	N/A	2000	5000
8:2 diPAP	N/A	N/A	2000	5000

Table A2.3: Recovery of perfluorinated carboxylates, perfluorinated sulfonates, and fluorotelomer sulfonates after WAX-SPE extraction when spiked (n=4 per condition) at 10 ng/L and 50 ng/L into HPLC water and a representative stormwater sample. Inconsistent and poor recovery of PFBA prevented the reporting of PFBA for samples. Recovery is reported after correction for its surrogate standard recovery.

Analyte	10 ng/L in HPLC-grade water	50 ng/L in HPLC-grade water	10 ng/L in stormwater[3]	50 ng/L in stormwater[3]
PFPeA	0.90± 0.19	0.75± 0.03	0.82± 0.11	0.72± 0.04
PFHxA	0.98± 0.03	0.98± 0.03	0.96± 0.03	1.02± 0.09
PFHpA	0.92± 0.04	1.00± 0.05	0.92± 0.03	1.02± 0.02
PFOA	1.03± 0.04	1.03± 0.03	0.91± 0.02	1.02± 0.07
PFNA	0.96± 0.04	0.99± 0.03	0.90± 0.03	1.01± 0.08
PFDA	0.95± 0.06	1.01± 0.03	0.87± 0.07	1.08± 0.06
PFUnA	0.95± 0.03	0.92± 0.04	0.85± 0.07	0.97± 0.07
PFDoA	0.82± 0.11	0.70± 0.21	0.65± 0.06	0.77± 0.08
PFTrA	1.00± 0.07	0.83± 0.07	1.07± 0.06	0.94± 0.18
PFTeA	0.79± 0.09	0.76± 0.20	0.65± 0.05	0.48± 0.17
PFBS	0.61± 0.05	0.66± 0.05	0.63± 0.22	0.69± 0.06
PFHxS	0.93± 0.11	1.00± 0.01	0.89± 0.10	1.02± 0.06
PFOS	0.98± 0.08	0.96± 0.03	0.93± 0.04	0.98± 0.07
PFDS	0.85± 0.15	0.71± 0.18	0.60± 0.01	0.67± 0.10
6:2 FtS	2.65± 0.25	2.00± 0.08	2.55± 0.20	2.98± 0.23
8:2 FtS	1.44± 0.14	1.33± 0.014	1.46± 0.09	1.42± 0.14

[3] Recovery = $[\text{Analyte}]_{\text{spiked stormwater}} / ([\text{Analyte}]_{\text{unspiked stormwater}} + \text{Spike Concentration})$

Table A2.4: Recovery of perfluorooctane sulfonamide-containing precursors after WAX-SPE extraction when spiked (n=4 per condition) at 2 ng/L and 10 ng/L into HPLC water and a representative stormwater sample. Recovery is reported after correction for its surrogate standard recovery, except for the recoveries of *N*-EtFOSE and FOSA, which did not have surrogate standards.

Analyte	2 ng/L in HPLC-grade water	10 ng/L in HPLC-grade water	2 ng/L in stormwater[3]	10 ng/L in stormwater[3]
N-EtFOSE	0.88± 0.06	0.82± 0.26	0.78± 0.21	0.60± 0.14
N-EtFOSAA	0.97± 0.20	0.95± 0.07	0.86± 0.04	0.99± 0.10
N-MeFOSAA	1.00± 0.17	1.10± 0.15	0.90± 0.12	1.06± 0.07
FOSA	0.60± 0.04	0.77± 0.18	0.73± 0.07	0.74± 0.01

Table A2.5: Monitored Ion Transitions and MS Conditions

Analyte	Molecular Ion	Fragmenter Voltage (V)	Quantifier Product Ion	Collision Energy (V)	Qualifier Product Ion	Collision Energy (V)	Polarity
PFBA	213	50	169	2			Negative
PFPeA	263	60	219	2			Negative
PFHpA	363	80	319	2	169	2	Negative
PFHxA	313	80	269	2	119	15	Negative
PFOA	413	80	369	3	169	14	Negative
PFNA	463	80	419	2	219	15	Negative
PFDA	513	80	469	5	169	10	Negative
PFUnA	563	80	519	10	269	15	Negative
PFDoA	613	100	569	5	169	25	Negative
PFTrA	663	100	619	10			Negative
PFTeA	713	100	669	10			Negative
FOSA	498	180	77.8	40			Negative
PFBS	299	120	80	30	99	30	Negative
PFHxS	399	160	80	80	99	50	Negative
PFOS	499	180	80	80	99	50	Negative
PFDS	599	150	80	50	99	50	Negative
N-EtFOSE	630	90	59	10			Negative
N-EtFOSAA	584	110	419	15	526	20	Negative
N-MeFOSAA	570	120	419	20	512	20	Negative
6:2 FtS	427	140	407	25	80	35	Negative
8:2 FtS	527	140	507	30	80	40	Negative
6:2 diPAP	789	160	442	20	97	40	Negative
8:2 diPAP	989	180	543	20	97	45	Negative
[$^{13}C_4$] PFBA	217	50	172	5			Negative
[$^{13}C_2$] PFHxA	315	60	270	5			Negative
[$^{13}C_4$] PFOA	417	70	372	2			Negative
[$^{13}C_5$] PFNA	468	70	423	5			Negative
[$^{13}C_2$] PFDA	515	70	470	10			Negative
[$^{13}C_2$] PFUnA	565	80	520	10			Negative
[$^{13}C_2$] PFDoA	615	80	570	10			Negative
[$^{18}O_2$] PFHxS	403	150	103	40			Negative
[$^{13}C_4$] PFOS	503	190	80	60			Negative
[$^{2}D_5$] *N*-EtFOSAA	589	120	419	15			Negative

Discussion of how to predict [C8 PFAA precursors] from oxidative increases of PFCAs.

As demonstrated in the control experiments with pure compounds, sulfonamide-containing C8 precursor compounds are quantitatively converted into PFOA while 8:2 fluorotelomer-containing precursor compounds react to form a mixture of PFCAs, where PFOA is 19% to 21% (average = 20%) of the product yield for each perfluorinated group in the molecule (Table 2.2). Δ[PFOA] evolved in a sample upon oxidation with the precursor test can be predicted by the following expression:

$$\Delta[\text{PFOA}] = \sum [\text{C8 SNP}]_i + 0.20 \cdot \sum p_j\,[\text{C8 FtP}]_j + \sum x_n \cdot p_k\,[\text{n:2 (n>8) FtP}]_k \qquad \text{(Equation A2.1)}$$

where SNP is a sulfonamide-containing precursor, FtP is a fluorotelomer precursor, p is the number of perfluorinated chains in the fluorotelomer molecule, and x_n is the fraction of n:2 fluorotelomer precursors that form PFOA.

The oxidation of 8:2 FtS and 8:2 diPAP resulted in relative PFHpA to PFOA product yields of 1.1 to 1.3 (average = 1.2) (Table 2.2). If all PFHpA production resulted from the oxidation of 8:2 fluorotelomer compounds, then the maximum concentration of PFOA evolved from 8:2 fluorotelomer compounds can be modeled by the following expression:

$$\Delta[\text{PFOA}]_{\text{8:2 FtP, max}} = 0.20 \sum p_j\,[\text{C8 FtP}]_{j,\,\text{max}} = \Delta[\text{PFHpA}]/1.2 \qquad \text{if } \Delta[\text{PFHpA}]/1.2 \le \Delta[\text{PFOA}]$$
$$\Delta[\text{PFOA}]_{\text{8:2 FtP, max}} = 0.20 \sum p_j\,[\text{C8 FtP}]_{j,\,\text{max}} = \Delta[\text{PFOA}] \qquad \text{if } \Delta[\text{PFHpA}]/1.2 > \Delta[\text{PFOA}$$

(Equation A2.2)

In these samples, the first condition of Equation A2.2 was always applicable.

No C9 or greater fluorotelomer precursors were tested in control experiments, so their behavior under oxidation will be extrapolated from control experiments of shorter chain compounds. For C6 and C8 FtS and diPAP, Δ[PF(n-1)A]/Δ[PFnA] ranged between 1.15 and 1.45 (average = 1.3), *e.g.* Δ[PFPeA]/Δ[PFHxA] = 1.23 for 6:2 FtS (Table 2.2). If Δ[PFNA] is entirely produced by 9:2 and higher order fluorotelomer precursors, then Δ[PFOA] resulting from the oxidation of 9:2 and higher precursors is expected to be less than or equal to 1.3; this ratio would be less if some of the precursors were of greater chain length, as PF(n-1)A is the product found in greatest yield from the oxidation of an n:2 fluorotelomer precursor (Table 2.2). The maximum value of the final term in Equation A2.1 can be approximated by the following expression:

$$\Delta[\text{PFOA}]_{\text{n>8:2 FtP, max}} = \sum x_n \cdot (p_k \cdot [\text{C(n>8) FtP}]_{k,})_{,\,\text{max}} = \Delta[\text{PFNA}] \cdot 1.3 \quad \text{if } \Delta[\text{PFNA}] \cdot 1.3 \le \Delta[\text{PFOA}]$$
$$\Delta[\text{PFOA}]_{\text{n>8:2 FtP, max}} = \sum x_n \cdot (p_k \cdot [\text{C(n>8) FtP}]_{k,})_{,\,\text{max}} = \Delta[\text{PFOA}] \quad \text{if } \Delta[\text{PFNA}] \cdot 1.3 > \Delta[\text{PFOA}]$$

(Equation A2.3)

In these samples, the first condition of Equation A2.3 was always applicable.

Equations A2.2 and A2.3 can be used to predict the minimum and maximum concentration of C8 PFAA precursors based on results from the precursor oxidation method. The minimum estimate of [C8 precursors] assumes that PFOA is not generated from 8:2 fluorotelomer compounds and that all PFNA production is from oxidation of 9:2 and higher chain-length fluorotelomer compounds that also contribute to PFOA production.

$$[\text{C8 precursors}]_{min} = \Delta[\text{PFOA}] - \Delta[\text{PFOA}]_{n>8:2\ \text{FtP, max}} \qquad \text{(Equation A2.4)}$$

The maximum concentration estimate of C8 PFAA precursors assumes all of Δ[PFHpA] is attributable to the oxidation of 8:2 fluorotelomer compounds and that Δ[PFNA] is attributable to oxidation of non-fluorotelomer C9 PFAA precursors that do not contribute to PFOA production.

$$[\text{C8 precursors}]_{max} = \Delta[\text{PFOA}] + \sum[\text{C8 FtP}]_{j,\ max} * (1-0.2) \qquad \text{(Equation A2.5)}$$

The following example calculation demonstrates the range of [C8 precursors] possible from the observed oxidative increases of PFCAs in a stormwater sample.

Example:

A stormwater sample has the following characteristics:

- Δ[PFOA] = 6.4 pM
- Δ[PFHpA] = 1.6 pM
- Δ[PFNA] = 0.9 pM
- $\sum[\text{Precursors}]_{\text{LC-MS/MS}}$ = 0.9 pM or 0.4 ng/L as PFOA
- According to Equation A2.2,

- $\Delta[\text{PFOA}]_{\text{8:2 FtP, max}} = \Delta[\text{PFHpA}]/1.2 = 1.6\ \text{pM}/1.2 = 1.3\ \text{pM}$
- $\sum[\text{C8 FtP}]_{\text{j, max}} = \Delta[\text{PFOA}]_{\text{8:2 FtP, max}}/0.2 = 1.3/0.2 = 6.5\ \text{pM}$

According to Equation A2.3,

- $\Delta[\text{PFOA}]_{\text{n>8:2 FtP, max}} \approx \Delta[\text{PFNA}] \cdot 1.3 = 0.9 \cdot 1.3 = 1.2\ \text{pM}$

Thus, according to Equations A2.4 and A2.5,

- $[\text{C8 precursors}]_{\text{min}} = \Delta[\text{PFOA}] - \Delta[\text{PFOA}]_{\text{n>8:2 FtP, max}}$
  $[\text{C8 precursors}]_{\text{min}} = 6.4 - 1.2\ \text{pM} = 5.2\ \text{pM}$ or 2.1 ng/L as PFOA

- $[\text{C8 precursors}]_{\text{max}} = \Delta[\text{PFOA}] + \sum[\text{C8 FtP}]_{\text{j, max}} * (1\text{-}0.2)$
  $[\text{C8 precursors}]_{\text{min}} = 6.4 + 6.5 \cdot (1\text{-}0.2) = 11.6\ \text{pM}$ or 4.8 ng/L as PFOA

The range of [C8 PFAA precursors] is 81 to 181% of Δ[PFOA]. The [C8 PFAA precursors] measured by LC/MS/MS is less than 25% of the minimum concentration estimated by oxidation of the sample.

Table A2.6: Application of oxidation results to predict the minimum and maximum concentrations of C8 PFAA precursors (ng/L as PFOA) and their proportion to Δ[PFOA] generated upon oxidation in stormwater samples (n=33).

	$[C8\text{-precursors}]_{min}$[4]	$[C8\text{-precursors}]_{max}$[5]	$[C8\text{-precursors}]_{min}$/ Δ[PFOA]	$[C8\text{-precursors}]_{max}$/ Δ[PFOA]
Minimum	<0.1	<0.1	N/A	N/A
10th Percentile	0.5	1.1	0.50	1.0
Median	1.8	4.5	0.87	1.11
90th Percentile	4.8	10.6	1.0	3.03
Maximum	7.0	16.0	1.00	4.38

[4] Equation A2.4
[5] Equation A2.5

Discussion of how to predict [C6 PFAA precursors] from oxidative increases of PFCAs.

Analytical standards of C6 PFAA precursors are not as widely available their C8 counterparts, so control experiments with sulfonamide-containing C6 precursors were unable to be conducted. However, it is reasonable to assume that sulfonamide-containing C6 precursors would behave the same as their C8 analogs under the oxidation method, as the oxidation reaction affects the non-fluorinated part of the molecule. Thus, C6 sulfonamide-containing precursors would produce PFHxA in a one to one molar ratio upon oxidation with hydroxyl radical. The PFCA product profile of C6 fluorotelomer compounds exposed to hydroxyl radical can be predicted from the control experiments with representative compounds (Table 2.2). Δ[PFHxA] evolved in a sample upon oxidation with the precursor test can be predicted by the following expression analogous to Equation A2.1 for D[PFOA]:

$$\Delta[\text{PFHxA}] = \sum[\text{C6 SNP}]_i + 0.19 \cdot \sum p_j\,[\text{C6 FtP}]_j + \sum x_n \cdot p_k\,[\text{n:2 (n>6) FtP}]_k \qquad \text{(Equation A2.6)}$$

Prediction of the range of possible [C6 precursors] in a sample follows closely from the discussion of [C8 precursors]. The maximum possible [C6 precursors] assumes that PFHxA is produced only from the oxidation of C6 fluorotelomer compounds and C6 sulfonamide-containing precursors. Using the results in Table 2.2 and following the discussion outlined for $\Delta[\text{PFOA}]_{8:2\ \text{FtP, max}}$, the maximum possible contribution of Δ[PFHxA] from 6:2 fluorotelomer precursors can be estimated by the following expression:

$$\Delta[\text{PFHxA}]_{6:2\ \text{FtP, max}} = 0.19 \cdot \sum p_j\,[\text{C6 FtP}]_{j,\ \text{max}} = \Delta[\text{PFPeA}]/1.3 \qquad \text{if } \Delta[\text{PFPeA}]/1.3 \leq \Delta[\text{PFHxA}]$$

$$\Delta[\text{PFHxA}]_{6:2\ \text{FtP, max}} = 0.19 \cdot \sum p_j\,[\text{C6 FtP}]_{j,\ \text{max}} = \Delta[\text{PFHxA}] \qquad \text{if } \Delta[\text{PFPeA}]/1.3 > \Delta[\text{PFHxA}]$$

(Equation A2.7)

In many of the samples measured in this study, Δ[PFPeA]/1.3 ≥ Δ[PFHxA], so a conditional equation is needed to calculate $\Delta[\text{PFHxA}]_{6:2\ \text{FtP, max}}$ in these samples.

The minimum possible [C6 precursors] assumes PFHxA production arises from (n>6):2 fluorotelomer precursors and C6 sulfonamide-containing precursors. Using Table 2.2 and following the discussion outlined for $\Delta[\text{PFOA}]_{n>8:2\ \text{FtP, max}}$, the following expression can be used to estimate $\Delta[\text{PFHxA}]_{n>6:2\ \text{FtP, max}}$:

$$\Delta[\text{PFHxA}]_{n>6:2\ \text{FtP, max}} = \sum x_n \cdot p_k\,([\text{C(n>6) FtP}]_{k,})_{,\ \text{max}} = \Delta[\text{PFHpA}] \cdot 1.3 \qquad \text{if } \Delta[\text{PFHpA}] \cdot 1.3 \leq \Delta[\text{PFHxA}]$$

$$\Delta[\text{PFHxA}]_{n>6:2\ \text{FtP, max}} = \sum x_n \cdot p_k\,([\text{C(n>6) FtP}]_{k,})_{,\ \text{max}} = \Delta[\text{PFHxA}] \qquad \text{if } \Delta[\text{PFHpA}] \cdot 1.3 > \Delta[\text{PFHxA}]$$

(Equation A2.8)

In a few of the samples measured in this study, Δ[PFHpA] · 1.3 > Δ[PFHxA], so a conditional equation is needed to calculate $\Delta[\text{PFHxA}]_{n>6:2\ \text{FtP, max}}$ in these samples.

Equations A2.7 and A2.8 can be used to predict the minimum and maximum concentration of C6 precursors based on results from the precursor oxidation method. The minimum estimate of [C6 precursors] assumes that PFHxA is not generated from 6:2 fluorotelomer compounds and that all PFHpA production is from oxidation of 7:2 and higher chain-length fluorotelomer compounds that also contribute to PFHxA production.

$$[\text{C6 precursors}]_{min} = \Delta[\text{PFHxA}] - \Delta[\text{PFHxA}]_{n>6:2\ \text{FtP, max}} \qquad \text{(Equation A2.9)}$$

The maximum concentration estimate of C6 PFAA precursors assumes all of Δ[PFPeA] is attributable to the oxidation of 6:2 fluorotelomer compounds and that Δ[PFHpA] is attributable to oxidation of non-fluorotelomer C7 PFAA precursors that do not contribute to PFHxA production.

$$[\text{C6 Precursors}]_{max} = \Delta[\text{PFHxA}] + \Delta[\text{PFHxA}]_{6:2\ \text{FtP, max}} * (1-0.19) \qquad \text{(Equation A2.10)}$$

Table A2.7: Application of oxidation results to predict the minimum and maximum concentrations of C6 PFAA precursors (ng/L as PFHxA) and their proportion to Δ[PFHxA] generated upon oxidation in stormwater samples (n=33).

	$[C6\text{-precursors}]_{min}$[6]	$[C6\text{-precursors}]_{max}$[7]	$[C6\text{-precursors}]_{min}$/ Δ[PFHxA]	$[C6\text{-precursors}]_{max}$/ Δ[PFHxA]
Minimum	0.0	0.7	0.0	1.0
10th Percentile	0.0	3.8	0.0	3.0
Median	2.3	14.3	1.0	5.3
90th Percentile	7.6	30.4	1.0	5.3
Maximum	28.6	87.3	1.0	5.3

[6] Equation A2.9
[7] Equation A2.10

Appendix: Chapter 3

Table A3.1: Monitored ion transitions, MS Conditions, and internal standard used for quantification of each analyte.

Electrochemical Fluorination- Based Compounds								
Compound	Internal Standard	Molecular Ion	Fragmentor Voltage (V)	Quantifier Ion (m/z)	Collision Energy (V)	Qualifier Ion (m/z)	Collision Energy (V)	Polarity
Perfluoroalkyl sulfonamide amino carboxylates (PFSAmA)								
PFBSAmA	[$^{18}O_2$] PFHxS	457	135	85	30	70	60	Positive
PFPeSAmA	[$^{18}O_2$] PFHxS	507	135	85	30	70	60	Positive
PFHxSAmA	[$^{18}O_2$] PFHxS	557	135	85	30	70	60	Positive
Perfluoroalkyl sulfonamido amines (PFSAm)								
PFBSAm	[$^{18}O_2$] PFHxS	385	135	85	30	58	60	Positive
PFPeSAm	[$^{18}O_2$] PFHxS	435	135	85	30	58	60	Positive
PFHxSAm	[$^{18}O_2$] PFHxS	485	135	85	30	58	60	Positive
Perfluorinated sulfonates								
PFBS	[$^{18}O_2$] PFHxS	299	120	80	70	99	30	Negative
PFHxS	[$^{18}O_2$] PFHxS	399	160	80	80	99	50	Negative
PFHpS	[$^{13}C_4$] PFOS	449	160	80	80	99	50	Negative
PFOS	[$^{13}C_4$] PFOS	499	180	80	80	99	50	Negative
PFDS	[$^{13}C_4$] PFOS	599	150	80	50	99	50	Negative
Perfluoroalkyl sulfonamides								
FHxSA	[$^{13}C_8$] FOSA	398	180	78	40			Negative
FOSA	[$^{13}C_8$] FOSA	498	180	78	40			Negative
Fluorotelomer - Based Compounds								
Compound		Molecular Ion	Fragmentor Voltage (V)	Quantifier Ion (m/z)	Collision Energy (V)	Qualifier Ion (m/z)	Collision Energy (V)	Polarity
Fluorotelomer betaines (FtB)								
5:1:2 FtB	[$^{13}C_2$] 6:2 FtS	432	180	58	50			Positive
5:3 FtB	[$^{13}C_2$] 6:2 FtS	414	180	58	60			Positive
7:1:2 FtB	[$^{13}C_2$] 6:2 FtS	532	180	58	60			Positive
7:3 FtB	[$^{13}C_2$] 6:2 FtS	514	180	58	60			Positive
9:1:2 FtB	[$^{13}C_2$] 6:2 FtS	632	180	58	60			Positive
9:3 FtB	[$^{13}C_2$] 6:2 FtS	614	180	58	60			Positive
Fluorotelomer thio hydroxy ammonium (FtTHN+)								
6:2 FtTHN+	[$^{13}C_2$] 6:2 FtS	496	180	393	40	437	30	Positive
Fluorotelomer thioamido sulfonates (FtTAoS)								
4:2 FtTAoS	[$^{13}C_2$] 6:2 FtS	486	180	206	45	135	45	Negative
6:2 FtTAoS	[$^{13}C_2$] 6:2 FtS	586	190	135	45	206	40	Negative
8:2 FtTAoS	[$^{13}C_2$] 6:2 FtS	686	180	206	50	135	50	Negative
Fluorotelomer sulfonates (FtS)								
6:2 FtS	[$^{13}C_2$] 6:2 FtS	427	140	407	25	80	35	Negative
8:2 FtS	[$^{13}C_2$] 6:2 FtS	527	140	507	30	80	40	Negative
Fluorotelomer sulfonamido betaines (FtSaB)								

6:2 FtSaB	[$^{13}C_2$] 6:2 FtS	571	180	58	40	104	40	Positive
8:2 FtSaB	[$^{13}C_2$] 6:2 FtS	671	180	104	40	58	60	Positive
10:2 FtSaB	[$^{13}C_2$] 6:2 FtS	771	180	104	40	58	60	Positive
12:2 FtSaB	[$^{13}C_2$] 6:2 FtS	871	180	104	40	58	60	Positive
Fluorotelomer sulfonamido amines (FtSaAm)								
6:2 FtSaAm	[$^{13}C_2$] 6:2 FtS	513	180	58	60	86	60	Positive
8:2 FtSaAm	[$^{13}C_2$] 6:2 FtS	613	180	86	60	58	80	Positive
Perfluorinated carboxylates - Electrochemical or Fluorotelomer-Based								
PFBA	[$^{13}C_4$] PFBA	213	50	169	2			Negative
PFPeA	[$^{13}C_4$] PFBA	263	60	219	2			Negative
PFHxA	[$^{13}C_2$] PFHxA	313	80	269	2	119	15	Negative
PFHpA	[$^{13}C_2$] PFHxA	363	80	319	2	169	2	Negative
PFOA	[$^{13}C_4$] PFOA	413	80	369	3	169	14	Negative
PFNA	[$^{13}C_5$] PFNA	463	80	419	2	219	15	Negative
PFDA	[$^{13}C_2$] PFDA	513	80	469	5	169	10	Negative
PFUnA	[$^{13}C_2$] PFUnA	563	80	519	10	269	15	Negative
PFDoA	[$^{13}C_2$] PFDoA	613	100	569	5	169	25	Negative
PFTrdA	[$^{13}C_2$] PFDoA	663	100	619	10			Negative
PFTeDA	[$^{13}C_2$] PFDoA	713	100	669	10			Negative
Internal Standards								
[$^{13}C_8$] FOSA		506	150	77.8	50			Negative
[$^{18}O_2$] PFHxS		403	150	103	40			Negative
[$^{13}C_4$] PFOS		503	190	80	60			Negative
[$^{13}C_4$] PFBA		217	50	172	5			Negative
[$^{13}C_2$] PFHxA		315	60	270	5			Negative
[$^{13}C_4$] PFOA		417	70	372	2			Negative
[$^{13}C_5$] PFNA		468	70	423	5			Negative
[$^{13}C_2$] PFDA		515	70	470	10			Negative
[$^{13}C_2$] PFUnA		565	80	520	10			Negative
[$^{13}C_2$] PFDoA		615	80	570	10			Negative
[$^{13}C_2$] 6:2 FtS		429	140	409	25			Negative

Description of Spike Recovery Experiments in Groundwater

A groundwater sample that did not contain any background signal for PFASs was used for spike recovery of PFASs and determination of method precision.

The perfluorinated carboxylates, perfluorinated sulfonates, and 6:2 and 8:2 fluorotelomer precursor compounds were amended from a 60 µg/L mixed methanolic stock solution to a 6-mL aliquot of the uncontaminated groundwater sample for final concentrations of 2 µg/L per analyte. The FtB compounds were amended to the same sample from a methanolic stock for individual final concentrations ranging from 0.5 to 2 µg/L per analyte. Three replicates were prepared. After equilibrating overnight, the spiked samples were analyzed as described in the section on groundwater analysis in the Materials and Methods. Recovery was calculated as the average concentration measured in the spiked groundwater sample divided by the expected concentration (Table A3.2). Precision was calculated by taking the percent relative standard deviation of the concentrations measured in the three replicate spiked samples (Table A3.2). The instrumental limit of detection (LOD) of an analyte was defined as the minimum concentration that produced a signal to noise ratio of 3. Because matrix effects were minimal in groundwater, the whole method LOD (Table A3.2) was defined as the instrumental limit of detection multiplied by the groundwater analysis dilution factor, 2.5.

Because no quantitative standards were available for the 3M AFFF-derived PFAA precursors, spike recovery of those compounds in groundwater was conducted with an AFFF solution. A 3M AFFF formulation manufactured in 1998 was diluted 10^3 times in methanol and added to 6-mL aliquots of an uncontaminated groundwater sample for a final dilution of 10^5, or approximately 1 to 50 µg/L per analyte. Three replicates were prepared. Preparation for LC-MS/MS analysis was identical to that described above for non-3M PFAA precursor analytes. Recovery of a 3M AFFF-derived PFAA precursor was calculated as the average instrumental response measured in the spiked groundwater sample divided by the instrumental response of the analyte in the parent AFFF formulation measured at the same total dilution factor (Table A3.2). Precision was calculated by taking the percent relative standard deviation of the instrumental response of the three replicate spiked samples (Table A3.2). The instrumental LOD and whole method LOD (Table A3.2) were calculated as above using the conversion from instrumental response to concentration described below and in Table A3.4.

Table A3.2: Recovery, precision, and whole method LOD of PFASs in groundwater.

	Percentage Recovery	Precision	Whole Method LOD, µg/L
PFBA	94%	3.8%	0.5
PFPeA	130%	0.9%	0.2
PFHxA	107%	4.9%	0.1
PFHpA	116%	2.8%	0.2
PFOA	115%	5.1%	0.1
PFNA	86%	1.2%	0.2
PFDA	57%	3.8%	0.1
PFBS	90%	9.8%	0.3
PFHxS	95%	3.5%	0.1
PFHpS	102%	3.3%	0.1
PFOS	84%	4.9%	0.1
FOSA	94%	6.5%	0.1
6:2 FtS	107%	11%	0.1
8:2 FtS	90%	9.4%	0.1
6:2 FtTAoS	96%	9.2%	0.1
6:2 FtSaAm	60%	4.4%	0.3
5:1:2 FtB	114%	2.9%	0.3
5:3 FtB	109%	1.4%	0.3
7:1:2 FtB	105%	5.0%	0.3
7:3 FtB	100%	8.4%	0.3
9:1:2 FtB	56%	2.6%	0.4
PFBSAm	133%	1.3%	0.3
PFPeSAm	97%	0.9%	0.3
PFHxSAm	131%	0.0%	0.1
PFBSAmA	98%	1.6%	0.3
PFPeSAmA	160%	0.5%	0.3
PFHxSAmA	85%	0.3%	0.1

Description of Spike Recovery Experiments in Soils

A soil sample that did not contain any background signal for PFASs was used for spike recovery of PFASs and determination of method precision.

A methanol stock containing 10 ng of perfluorinated carboxylates and perfluorinated sulfonates and 50 ng of 6:2 FtS, 8:2 FtS, and FOSA was amended to 250 mg of uncontaminated soil in triplicate. Soil samples were vortexed in this methanol slurry for twenty seconds, dried under N_2 gas, and aged at room temperature for 72 hours before extraction. The spiked samples were extracted and analyzed using the methodology described in the section on soil analysis in the Materials and Methods. Recovery was calculated as the average concentration measured in the spiked groundwater sample divided by the expected concentration (Table A3.3). Precision was calculated by taking the percent relative standard deviation of the concentrations measured in the three replicate spiked samples (Table A3.3). The use of ENVI-carb minimized matrix effects, so the whole method LOD (Table A3.3) was defined as the instrumental LOD multiplied by the soil extract concentration factor, 15 L/kg.

To measure recoveries of Ansul and 3M PFAA precursor compounds, 10^3 diluted Ansul and 3M AFFF formulations in methanol were separately added to three replicates each of uncontaminated soil samples at approximately 1 to 10 ng per compound. Soil samples were treated as in the above recovery experiment. Recovery, precision, and whole method LOD were calculated for Ansul-derived PFAA precursors (*i.e.,* 6:2 FtTAoS and 8:2 FtTAoS) as described for other PFASs above. Recovery of the 3M AFFF-derived PFAA precursor compounds was calculated as the average instrumental response measured in the spiked soil sample divided by the instrumental response of the analyte in the parent AFFF formulation measured at the same total dilution factor (Table A3.3). Precision was calculated by taking the percent relative standard deviation of the instrumental response of the three replicate spiked samples (Table A3.3). The instrumental LOD and whole method LOD (Table A3.3) were calculated as above using the conversion from instrumental response to concentration described below and in Table A3.4.

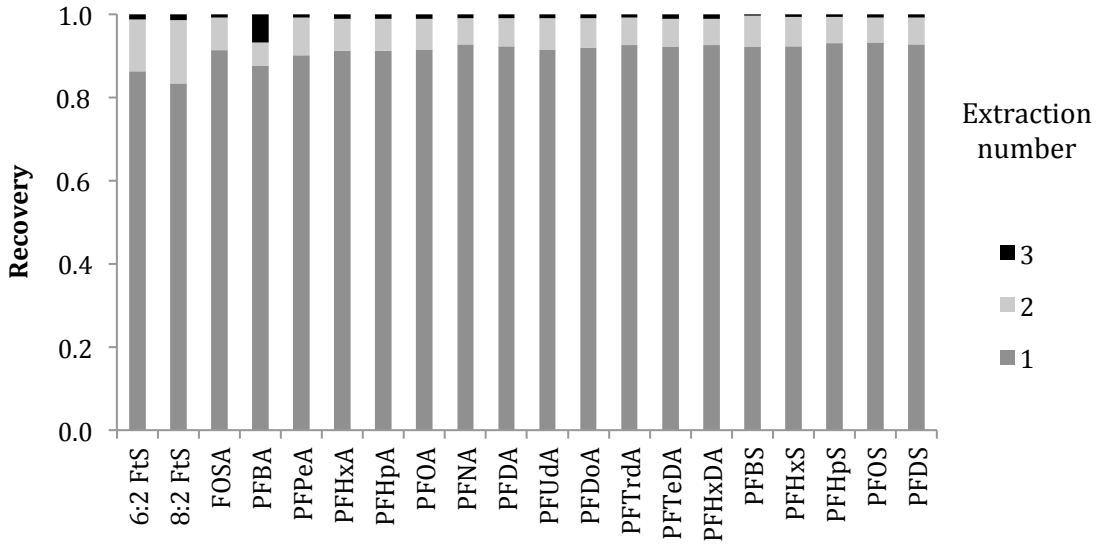


Figure A3.1: Fractional recovery of PFASs added to an uncontaminated soil sample. The 500 mg soil sample was extracted sequentially three times with 2.5 mL 0.1% NH_4OH in methanol per extraction. The data depict the recovery of compounds in each fraction; around 1% was recovered in the third fraction for most compounds.

Table A3.3: Recovery, precision, and whole method LODs of PFASs in soil and aquifer solids samples.

Analyte	Percentage Recovery	Precision	Whole Method LOD, µg/kg
6:2 FtS	110%	5%	0.4
8:2 FtS	121%	3%	0.6
FOSA	110%	6%	0.6
PFBA	95%	9%	3.0
PFPeA	105%	13%	1.1
PFHxA	110%	7%	0.8
PFHpA	113%	10%	1.1
PFOA	106%	4%	0.8
PFNA	109%	6%	1.1
PFBS	112%	8%	1.7
PFHxS	116%	13%	0.6
PFOS	104%	3%	0.4
PFDS	106%	4%	0.6
PFBSAm	100%	1%	1.5
PFBSAmA	56%	1%	1.5
PFPeSAmA	131%	1%	1.5
PFHxSAm	85%	2%	0.8
PFHxSAmA	70%	1%	0.8
6:2 FtTAoS	91%	16%	0.8
8:2 FtTAoS	98%	14%	0.8

Sample Preparation for Precursor Assay

Duplicate 140 µL aliquots of each AFFF formulation diluted 1000x in methanol were added to 7-mL HDPE vials and evaporated under nitrogen. The evaporated samples were reconstituted in 6 mL of 60 mM potassium persulfate and 125 mM sodium hydroxide and reacted for 6 hours at 85°C. Samples were brought to room temperature, neutralized with concentrated HCl to a pH value between 4 and 10, and amended with 1 mL methanol to enhance dissolution of PFASs. The reacted AFFF samples were analyzed at 10^6 total dilution.

Duplicate 3 mL aliquots of each groundwater sample were diluted 1:1 in a persulfate and NaOH stock solution in 7-mL HDPE tubes to achieve the same reaction conditions as in the oxidation of AFFF samples. Following reaction, samples were treated identically to AFFF and analyzed at an appropriate dilution factor previously determined in the analysis of unreacted groundwater samples.

The basic methanolic soil extracts were evaporated to dryness and subsequently reacted to measure the perfluorinated carboxylate products of precursor compounds. Complete removal of measureable precursor compounds in the methanolic extract was verified through spike recovery experiments (Table A3.3). Analysis of oxidized soil sample extracts was less time consuming and produced more precise data than treatment of suspended soils with persulfate followed by solid-phase extraction. To determine if the extraction method yielded lower concentrations than oxidation of suspended particulates, one sample was measured with both approaches. Reacting a whole soil sample at 2 to 4 g soil/L yielded similar concentrations of perfluorinated carboxylate products as reacting the evaporated soil extract and produced less variability (Figure A3.2). Using an ENVI-CARB- exposed extract did not remove any precursor compounds, as demonstrated by consistent perfluorinated carboxylate production upon reaction with and without ENVI-CARB exposure (Figure A3.3).

A 1 mL aliquot of each ENVI-CARB exposed soil extract was added to a 7-mL HDPE tube and evaporated for use in the oxidation assay. The evaporated extract was reconstituted in the same persulfate and NaOH mixture used in AFFF formulation analysis. Reacted soil extracts were analyzed at appropriate dilutions previously determined from the analysis of unreacted soils.

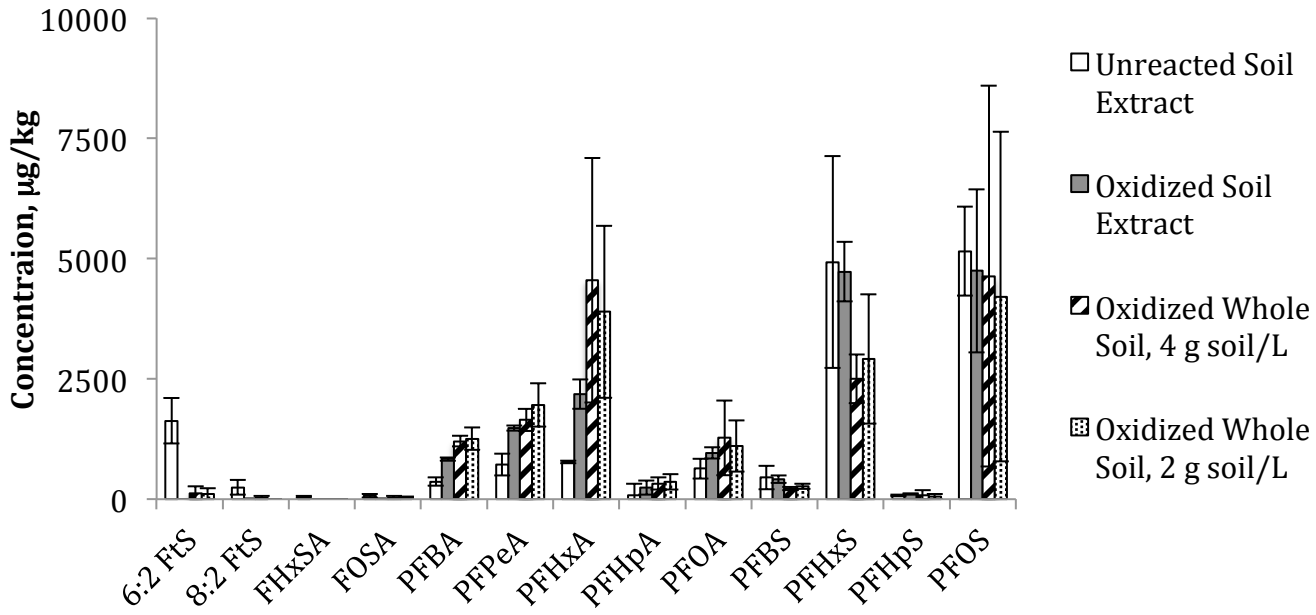


Figure A3.2: Comparison of whole soil sample oxidation and soil extract oxidation approach for a single sample. The unreacted soil extract is shown for comparison. Soil extracts were evaporated under N_2 prior to oxidation. All samples were reacted in triplicate in 60 mM potassium persulfate and 125 mM NaOH. Error bars represent standard deviation of three measurements.

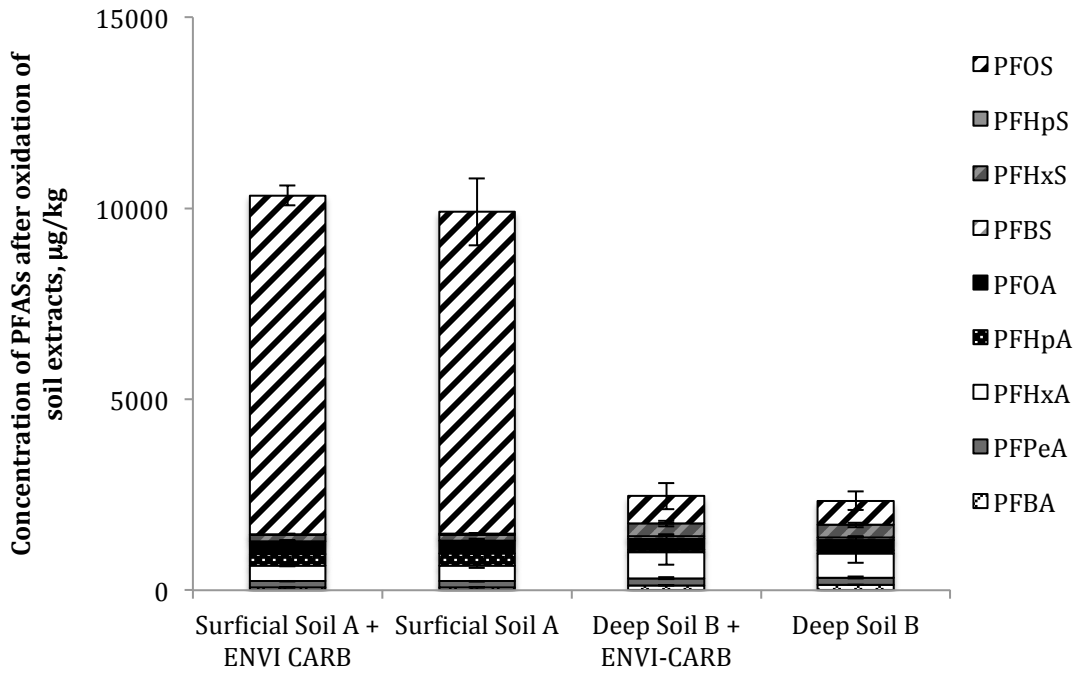


Figure A3.3: Effect of ENVI-CARB on precursor recovery in soil extracts. Concentrations of PFAA analytes are shown after reaction of two AFFF-contaminated soil sample extracts (one sandy surficial soil sample and one clay-rich aquifer solids sample) with and without an ENVI-CARB addition before blow down of basic methanolic extract.

Quantification of analytes for which authentic standards were not available:

Fluorotelomer compounds where a standard was available for one homolog in a class of compounds:

For all 6:2 fluorotelomer compounds in AFFF, a commercial material was used for quantification. No commercial material was available for the longer chain versions of these compounds. All of these compounds were quantified relative to the 6:2 FtS internal standard using the calibration of the 6:2 compound of related structure. The calibration was adjusted slightly, for differences in response of compounds due to different perfluorinated chain length. The chain length adjustment factors are as follows:

8:2 FtTAoS: $\frac{8{:}2\ FtS\ slope}{6{:}2\ FtS\ slope}$ - both slopes calculated relative to 6:2 FtS IS response.

10:2 FtSaB: $\frac{PFDA\ slope}{PFHxA\ slope}$ - both slopes calculated relative to PFHxA IS response.

8:2 FtSaB: $\frac{8{:}2\ FtS\ slope}{6{:}2\ FtS\ slope}$ - both slopes calculated relative to the 6:2 FtS IS response.

FHxSA

No reference material or standard was available for FHxSA. To quantify FHxSA, an MRM method was designed using the same ion transition as FOSA adjusted for the mass difference due to the shorter perfluorinated chain length. The retention time of FHxSA was approximated relative to FOSA using typical spacing between other C8 and C6 compounds (*i.e.* about 1 minute). FHxSA was quantified using the calibration curve for FOSA with an adjustment for chain length in the following manner:

$[FHxSA] = \frac{FHxSA\ response}{FOSA\ IS\ response} \times FOSA\ slope \times Chain\ length\ adjustment\ factor)$, where the FOSA slope is calculated relative to the FOSA IS response.

$Chain\ length\ adjustment\ factor\ = \frac{PFHxS\ slope}{PFOS\ slope}$, where both slopes were calculated relative to the PFOS IS response.

3M PFAA Precursor compounds:

No quantitative standards were available for the 3M-derived C_4 to C_6 perfluorinated sulfonamide amines (PFnSAm) and C_4 to C_6 perfluorinated sulfonamide amino carboxylates (PFnSAmA). The internal standard-relative responses of these compounds in various 3M formulations were compared to the concentration of perfluorinated carboxylates generated upon oxidation of each of these formulations. As these precursors contain a sulfonamide-linkage, one mole of a precursor molecule with a perfluorinated chain length of n will generate one mole of the C_n perfluorinated carboxylate under the oxidation conditions used [Houtz and Sedlak 2012]. Thus, the molar concentration of PFHxA generated should be equal to the combined molar

concentration of PFHxSAm and PFHxSAmA, assuming that these compounds are the only C_6 precursors present in the formulation. For environmental samples, the following procedure was used to estimate the concentration of each C_6 precursor:

3M AFFF Sample A, diluted 1 x 10^6 prior to analysis

$$\frac{PFHxSAm\ response}{PFHxS\ IS\ response} = 9.2$$

$$\frac{PFHxSAmA\ response}{PFHxS\ IS\ response} = 5.3$$

$$Combined\ PFHxS\ IS\ relative\ response\ =\ 14.5$$

3M AFFF Sample A, reacted at 10^4 dilution in the presence of 60 mM $S_2O_8^{2-}$, 125 mM NaOH at 85C for 6 hours; analysis performed at 10^6 dilution.

$$\Delta\ [PFHxA]\ =\ 10.1\ nM$$

The relative responses of PFHxSAm and PFHxSAmA were assumed to be equal on a molar basis. The production of PFHxA was distributed accordingly.

$$[PFHxSAm] = \frac{9.2}{14.5} \times 10.1\ nM\ =\ 6.4\ nM \times \frac{485\ \mu g}{1000\ nM}\ =\ 3.1\ \mu g/l$$

$$[PFHxSAmA] = \frac{5.3}{14.5} \times 10.1\ nM\ =\ 3.7\ nM\ *\ \frac{557\ \mu g}{1000\ nM}\ =\ 2.0\ \mu g/l$$

$$\frac{[PFHxSAm]}{\frac{PFHxSAm\ response}{PFHxS\ IS\ response}} = \frac{3.1\frac{\mu g}{l}}{9.2} = 0.34\frac{\mu g}{l}\ PFHxSAm\ per\ relative\ response$$

$$\frac{[PFHxSAmA]}{\frac{PFHxSAmA\ response}{PFHxS\ IS\ response}} = \frac{2.0\frac{\mu g}{l}}{5.3} = 0.38\frac{\mu g}{l}\ PFHxSAmA\ per\ relative\ response$$

This procedure was also used to estimate the concentrations of the C4 and C5 precursor compounds manufactured by 3M. A total of five 3M formulations were analyzed to determine an average concentration coefficient for each of the 3M fluorochemicals. Quantitative estimates of each compound in AFFF samples, groundwater, and soils were made using the coefficients determined in Table A3.4.

The accuracy of this approach depends in large part on the validity of assuming that each C_n homolog produces the same instrumental response per mole. Based on the quantification of other sulfonamide-containing precursors with authentic analytical standards, this assumption seems reasonable. For example, *N*-ethyl perfluorooctane sulfonamide (*N*-EtFOSA) and *N*-ethyl perfluorooctane sulfonamide acetic acid (*N*-EtFOSAA) differ by a 58 mass unit amidic- methyl carboxylate group and produce less than 15% difference in instrument signal per mole, with the smaller compound ionizing slightly more efficiently. Similarly, PFHxSAm and PFHxSAmA

differ by a 72 mass unit amidic-ethyl carboxylate group. It follows that the difference in instrumental response per mole would be comparable to the difference between *N*-EtFOSA and *N*-EtFOSAA. Thus, the PFHxSAm concentration might be underestimated by approximately 15% to 25% in samples. PFHxSAm is the only 3M precursor compound detected in environmental samples, and it is also less than 10% of the total molar precursor concentration in any sample in which it is detected. On the whole, a small error in quantification of PFHxSAm would not introduce any significant changes to the quantitative or qualitative interpretation of the data presented in this study.

Table A3.4: Coefficients used to compute concentrations of precursors in 3M formulations without quantitative standards. The relative response refers to the response of the analyte versus the response of the PFHxS internal standard.

µg/L per Relative Response					
PFBSAm	PFPeSAm	PFHxSAm	PFBSAmA	PFPeSAmA	PFHxSAmA
0.62	**0.40**	**0.35**	**0.74**	**0.47**	**0.40**