AN ABSTRACT OF THE DISSERTATION OF

Melissa M. Schultz for the degree of Doctor of Philosophy in Chemistry

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Title: Determination of Fluorinated Alkyl Substances in Aqueous Systems.

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Douglas F. Barofsky

Fluorinated alkyl substances, which can be persistent, toxic, and bioaccumulative, have been quantitated in many densely populated and remote regions, including in air, surface waters, groundwater, and biota; however, little is known about their transport or behavior in the environment. Wastewater effluent is one of the principal routes for introducing environmental contaminants into aquatic environments. The partitioning behavior of fluorinated alkyl substances between aqueous and particulate phases is not well characterized; thus, sorption onto sludge can be a removal mechanism of fluorinated alkyl substances from the wastewater stream. This is another route into the environment if the biosolids are land-applied.

In an attempt to analyze for the fluorinated alkyl substances in wastewater, known aqueous-film-forming-foam (AFFF)-laden groundwater sampled from 3 military bases was used to develop an assay using liquid chromatography (LC), electrospray ionization (ESI) tandem mass

spectrometry (MS/MS). While working on the method development, fluorotelomer sulfonates were detected at Wurtsmith AFB, MI, and Tyndall AFB, FL, where total fluoroalkyl sulfonates ranged respectively from below quantitation (≤0.60 µg/L) to 182 µg/L and from 1100 µg/L to 14,600 µg/L.

The LC ESI-MS/MS method was modified to quantitate fluorinated alkyl sulfonates in wastewater by incorporating a high volume sample loop (500 µL), which lowered detection and quantitation limits by at least a factor of 50. This method was applied to 24 h composites of influents and effluents collected from treatment plants distributed nationwide. Fluorinated alkyl substances were observed at all 10 plants sampled, and each wastewater treatment plant was found to have a unique distribution of fluorinated alkyl substances, despite similar treatment processes. In 9 out of the 10 plants sampled, at least one class of fluorinated alkyl substance exhibited significant increases in the effluent as compared to the influent levels.

The high-volume-injection LC ESI-MS/MS method was also used to monitor the mass flows of perfluoroalkyl sulfonates and carboxylates through a municipal wastewater treatment plant for 10 d. The perfluoroalkyl carboxylates were overall removed by the wastewater treatment process (25-40% removal). Perfluoroalkyl sulfonates were found to increase significantly (~200%) in the final effluent, and the fluoroalkyl sulfonamide acetic acids were found to increase by approximately 500% throughout the sludge process. From this plant, significant quantities of fluorochemicals are discharged with treated wastewater and biosolids, indicating that wastewater treatment plants

are point sources of fluorinated alkyl substances and must be considered when determining origins and behavior of fluorinated alkyl substances in the environment.

Determination of Fluorinated Alkyl Substances in Aqueous Systems

by

Melissa M. Schultz

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APPROVED:

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Major Professor, representing Chemistry

Redacted for Privacy

Chair of the Department of Chemistry

Redacted for Privacy

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Melissa M. Schultz, Author

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"There is greatness all around you--use it. It is easy to be great when you get around great people." Quote from Bob Richards

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CONTRIBUTION OF AUTHORS

Drs. Douglas Barofsky and Jennifer Field provided guidance in all aspects of the dissertation. Christopher Higgins analyzed all sludge samples included in Chapter 5, and he, along with Dr. Richard Luthy, aided in interpreting results and edited Chapter 5.

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"It is good to have an end to

journey towards—but it is the journey

that matters, in the end."

-Ursula K. LeGuin

Dedicated to my parents, who provided support and encouragement for me to follow my ambitions and to choose what color to paint my parachute

DETERMINATION OF FLUORINATED ALKYL SUBSTANCES IN AQUEOUS SYSTEMS

1. INTRODUCTION

Research Objectives

Fluorinated alkyl substances, some of which are known to be persistent, toxic, and bioaccumulative, have been detected in many matrices; however, little is known about their transport or behavior in the environment. The specific goals of this dissertation were (1) to complete a thorough literature review of fluorinated alkyl substances to further understand the different fluorination chemistries, electrochemical fluorination and fluorotelomerization, to survey the available analytical methods and occurrence data, and to expose areas for further research; (2) to develop an analytical method based on liquid chromatography (LC) electrospray ionization (ESI) tandem mass spectrometry (MS/MS) and to use this technique to compare fluorotelomer sulfonate levels to the concentrations of electrochemically fluorinated surfactants found in contaminated groundwater systems; (3) to adapt this LC ESI-MS/MS method for the analysis of municipal wastewater influents and effluents collected from treatment plants distributed nationwide; and (4) to determine the mass-flows of fluorinated alkyl substances through a municipal wastewater treatment plant.

History of Fluorochemicals

The carbon-fluorine (C-F) bond is the strongest single bond encountered in organic chemistry. Fluorination of organic chemicals dramatically changes their physical properties and chemical reactivities. In general, fluorochemicals have exceptional thermal and chemical stabilities relative to those of their hydrocarbon counterparts and, thus, find widespread applications (1,2). The key to the development of organofluorine chemistry, which was accomplished by Henri Moissan in 1886 when he first isolated fluorine (2), was overcoming the obstacle of preparing and handling the dangerous hydrogen fluoride. Despite this achievement, the field of organofluorine chemistry progressed slowly, and it was not until during World War II that organofluorine chemistry began to flourish commercially. Two distinct chemistries eventually emerged for the synthesis of organofluorine compounds: electrochemical fluorination and fluorotelomerization. The 3M Company acquired the commercial rights to electrochemical fluorination, which was developed by Joseph Simons at the Pennsylvania State University (1,3), whereas the DuPont Company developed the fluorotelomerization process (2). Both processes will be discussed in detail in Chapter 2. Many products have been manufactured from these organofluorine processes; arguably the two most prominent inventions are DuPont's Teflon® and 3M's Scotchgard™, which were first sold commercially in 1946 and 1953, respectively.

In 1968, Dr. Donald Taves, a dentist researching fluoride concentrations in the human body, unexpectedly detected organic fluorine in human serum by a

nonspecific analytical technique (4). The observed organic fluorine was unrelated to the fluoride being added to the public drinking water supply for the purpose of better dental hygiene; therefore, it was proposed that the source of organic fluorine in humans was exposure to industrial fluorochemicals (4,5). By using nuclear magnetic resonance (NMR), *Guy et al.* postulated that perfluorooctanoate (PFOA) or structurally-related compounds were the source of the observed organic fluorine in the human serum (6). This story continues into the 1990s.

Tandem Mass Spectrometry

Quadrupole Mass Spectrometry. Quadrupole mass spectrometers (MS) are powerful analytical tools that identify ions by measuring their mass-to-charge ratio (*m/z*). The quadrupole mass spectrometer is one kind of mass spectrometer that was invented by Wolfgang Paul in the mid-1950s (7). The quadrupole mass spectrometer, sometimes called a quadrupole mass filter, uses oscillating electric fields to separate ions according to their *m/z* values (8,9). The quadrupole consists of four parallel rods, which are electrically connected in pairs located opposite of each other (Figure 1.1). The pairs of rods have both fixed and alternating (RF) potentials applied to them. The polarity of the fixed voltages and the phases of the alternating voltages are adjusted oppositely on the two sets of rods so that the potentials produce a time-varying electric field that allows only ions of a particular *m/z* to pass along the axis of the quadrupole and reach the detector. All ions not having the

selected *m/z* will have a stable trajectory within the quadrupole and will eventually collide with one of the rods. Ions with different *m/z* values are detected by changing the magnitudes of the fixed and alternating voltages so that their ratio remains constant.

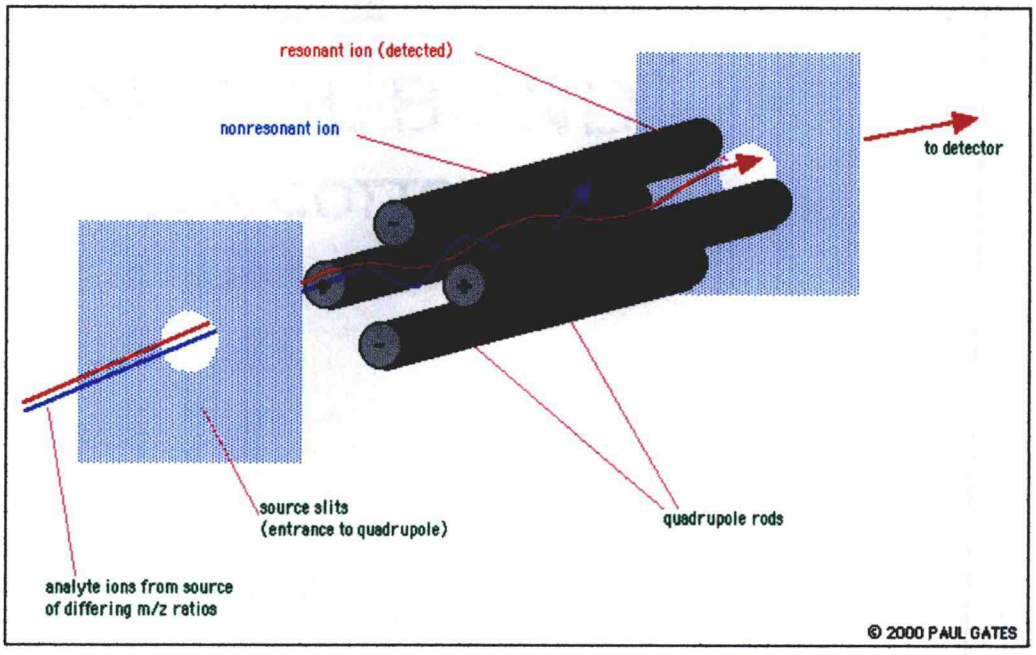

Figure 1.1. Schematic of a Quadrupole Mass Filter(10)

The selectivity of quadrupole mass filters was greatly increased with the advent of the triple quadrupole mass spectrometer, a tandem mass spectrometer invented by Richard A. Yost and Chris G. Enke (11-13). This mass spectrometer comprises a quadrupole mass filter, an "RF-only" collision-induced dissociation (CID) region, and a second quadrupole mass filter (Figure 1.2). Ion fragmentation occurs in the RF-only region where neutral gas atoms,

like argon atoms, are introduced to collide with and induce fragmentation of the precursor ions to form product ions. Single-Reaction-Monitoring (SRM) is the most sensitive and selective approach for acquiring quantitative data with a triple quadrupole mass spectrometer (14). In this mode of operation, a specific precursor/product ion transition is monitored (Figure 1.3). The information gathered from a SRM acquisition can elucidate organic molecules from complex mixtures, especially when coupled with a separation technique, such as liquid chromatography (LC). A triple quadrupole can be operated so that it performs several SRM acquisitions nearly simultaneously; this mode of operation is called Multiple-Reaction-Monitoring (MRM).

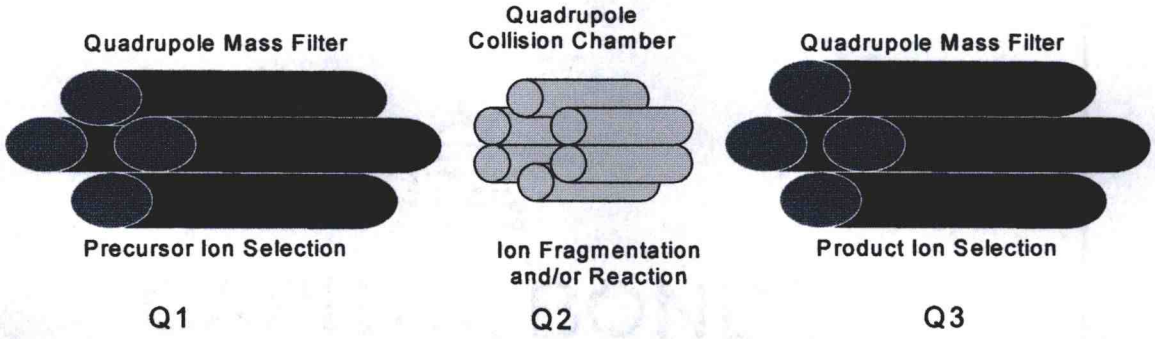

Figure 1.2. Diagram of the triple quadrupole mass spectrometer showing each component and its function.

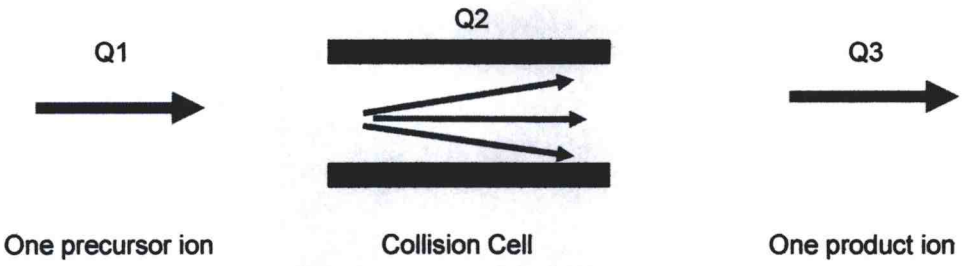

Figure 1.3. SRM mode where a specific mass transition is monitored.

Electrospray Ionization. Given that approximately half of all inorganic chemistry, organic chemistry, and biochemistry involves ions in solution (15), the potential utility for on-line coupling of LC to mass spectrometry (MS) was identified very early. However, despite the fact that LC is an older technique than gas chromatography (GC), GC-MS was an established technique by the time the first successful LC MS/MS experiments were in progress in the 1970s (16). Many technical obstacles had to be overcome before LC could be successfully coupled to MS, including the introduction of high liquid flows into a high vacuum system and the elimination of solvent. Early LC-MS ionization techniques included particle beam and thermospray (17). Although these interfaces were routinely used in environmental analyses, they had numerous shortcomings, the most serious of which were lack of sensitivity and selectivity.

The development of electrospray ionization (ESI) forever changed the field of LC-MS. ESI was independently reported by Fenn *et al.* and Aleksandrov *et al.* in the mid-1980s (18-22); it refers to the dispersion of a liquid into small droplets by the application of an electric field. As depicted in Figure 1.4, the injected analyte (in solution) enters a sharp hypodermic needle. At the tip of the needle, a relatively high voltage is applied, the resulting electric field works to overcome the surface tension of the liquid emerging from the end of the needle and, thus, to disperse the sample into a fine spray of droplets. The droplets are driven by the potential gradient (i.e. the electric field) and migrate toward the capillary inlet that eventually leads to the vacuum environment of the quadrupole analyzer. The multiply charged droplets rapidly shrink as they

approach the capillary inlet. The decrease in size of the droplets increases their surface charge density to a point called the Rayleigh limit. At this limit, electrostatic repulsion overcomes the droplet's surface tension, and a Coulombic explosion breaks the droplet into smaller droplets. This process repeats itself until solvated gas phase ions appear by an, as yet, unexplained process. These ions are guided by the capillary (and desolvated in the process) into the mass filters.

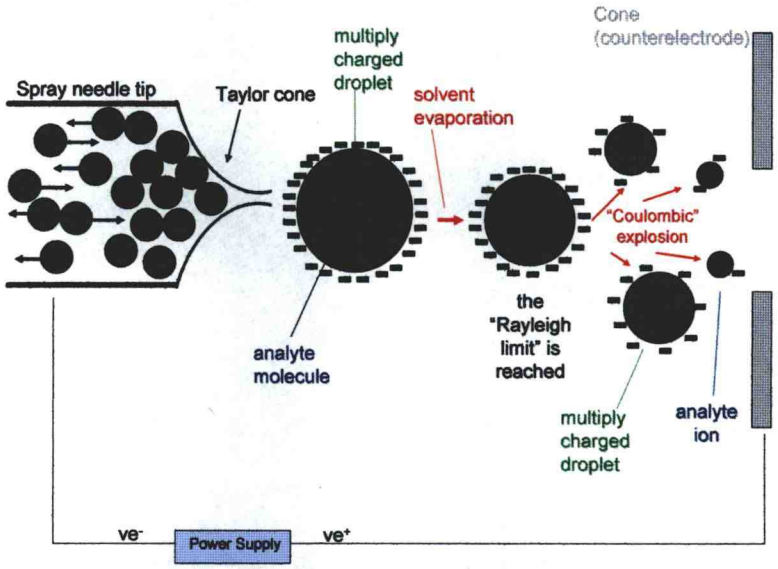

Figure 1.4. Schematic showing negative ion electrospray ionization. For positive ion electrospray ionization, the needle would be held at positive potential.

LC MS/MS applications boomed as a result of the invention of ESI. Its growing importance is depicted in the following graph showing the increase in ESI publications with time that culminated in over 1500 publications by 2001 (Figure 1.5) (23). These numbers are conservative estimates since pharmaceutical companies and other industrial companies, major consumers of LC ESI-MS/MS technology, typically do not publish their work. As a result of

the ionization technique's impact, John Fenn was awarded the 2002 Nobel Prize in chemistry.

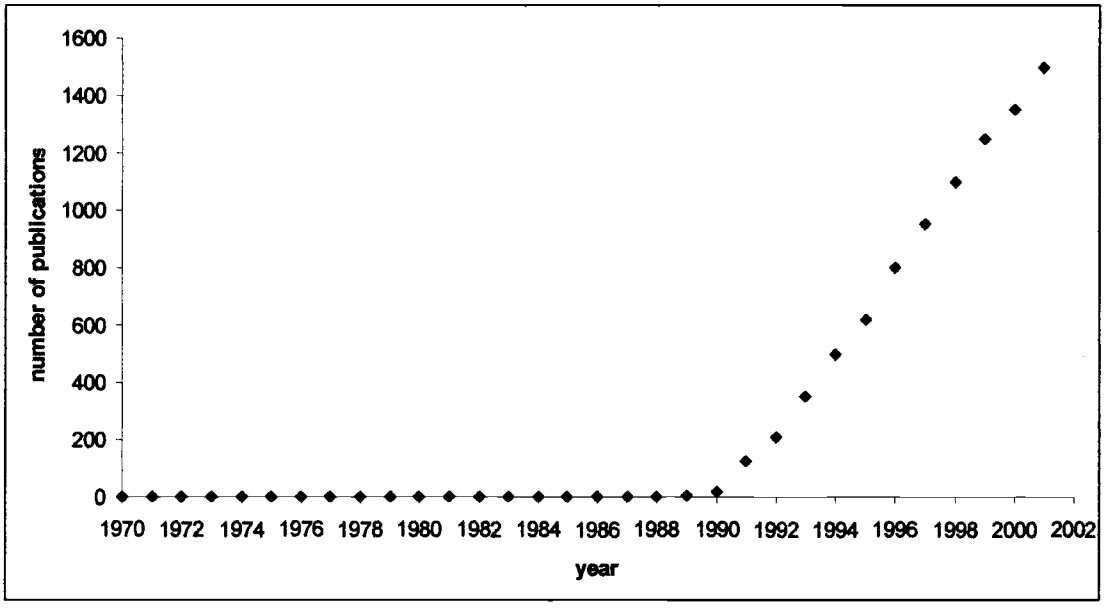

Figure 1.5. The graph shows the increasing number of publications that incorporate LC ESI-MS/MS, a reproduction of the figure shown in John Fenn's Nobel lecture (23).

The Advancement of LC ESI-MS/MS and Its Impact on Fluorochemicals

After Taves's initial discovery of organofluorine in the blood, the 3M Company continued to monitor workers' fluorochemical exposure; however, they were never able to unequivocally identify what organofluorine species were present. Although the workers' levels remained higher than the general population, medical records indicated no unusual illnesses or deaths in the

exposed workers (24); thus, concerns were held to a minimum. In the early 1990s, analytical methods, using the "new" LC ESI-MS/MS technology, were developed to unambiguously detect specific fluorochemicals in workers' blood down to 0.5 parts per million levels. In 1997, this methodology was used in an international study examining samples collected from blood banks in the United States, Europe, and Asia (25). Unexpected results emerged. Fluorochemicals, primarily perfluorooctane sulfonate (PFOS), were observed in every blood sample, except in old, stored samples from the Korean War, which predates Scotchgard™ production. Continued research showed PFOS to be a persistent, bioaccumulative, and toxic chemical (26,27). Global sampling revealed selected fluorinated alkyl substances in animal tissues from not only densely populated regions, but also in remotely populated regions where no local commercial, municipal or industrial sources exist (26). Fluorinated alkyl substances have since been detected in air (28-30), surface waters (31-39), groundwater (40-42), biota (43-48), and human serum (49-55), including non-occupationally-exposed humans (56). From these observations, concerns have once again been raised about the risks that fluorinated alkyl substances may pose towards humans and other organisms. In response to these concerns, the 3M Company voluntarily announced the "phase-out" of its C_8-based fluorochemistry in May of 2000 (57).

By contrast, fluorotelomerized products continue to be manufactured despite a preliminary risk assessment issued by the Environmental Protection Agency in 2003 for PFOA, a chemical still used by fluorotelomer manufacturers (58). Research continues to examine the risks PFOA and other fluorinated alkyl

substances may pose to humans, including children whose levels may be more prevalent than in nonoccupationally exposed adults (Figure 1.6) (59).

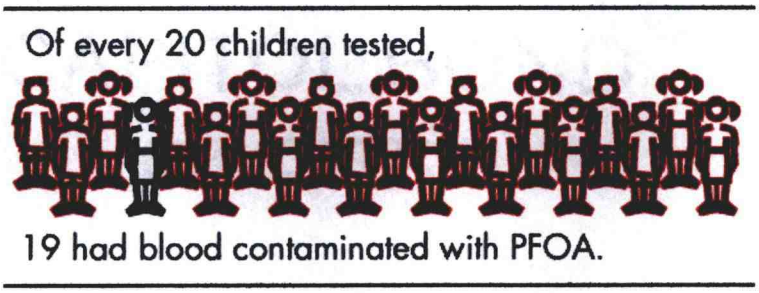

Figure 1.6. Cartoon that raises concern over the potential risk to children posed PFOA, a chemical still in use (60). All children and adults tested have some combination of fluorinated alkyl substances present in their blood. Preliminary studies suggest that the levels are higher in children than in adults.

Prior to the present study, environmental analysis of fluorotelomer sulfonates had received little attention by comparison to that developed and carried out for the electrochemically fluorinated chemicals. The development of an LC ESI-MS/MS procedure for the analysis of fluorinated alkyl substances in groundwater samples and its application to the quantitative analysis of fluorotelomer sulfonates, which were unexpectedly detected in groundwater contaminated with aqueous film-forming foam (AFFF), is described and discussed in Chapter 3.

Wastewater Treatment

As mentioned in the preceding sections, fluorinated alkyl substances have been quantitated in many different matrices, including air, surface waters, groundwater, and biota. Little, however, is known about their transport or behavior in the environment. Wastewater effluent is one of the principal routes for introducing environmental contaminants into aquatic environments. For example, a recent study examined the influence of wastewater discharge on environmental levels of pharmaceuticals and hormones in U. S. streams. Of these streams, 80% contained at least one of the organic wastewater contaminants (61), and as many as 38 organic wastewater contaminants were observed in one sample, suggesting that secondary treatment is unsuccessfully removing pharmaceuticals, hormones, and other organic contaminants from wastewater.

Previous studies suggest that fluoroalkyl sulfonamides may undergo microbial degradation to PFOS or PFOA (62-65). This degradation could take place in a wastewater treatment plant. Therefore, wastewater discharge may contain enhanced levels of PFOS, PFOA, and other fluorinated alkyl substances and may act as a potential environmental source of these analytes. There has been no comprehensive study of the fate and transport of fluorinated alkyl substances through a wastewater treatment plant prior to that presented in this dissertation. The development of an LC ESI MS/MS method, which exploits a high-volume sample injection loop (500 µL) to achieve added sensitivity, and the

application of this method to the analysis of fluorinated alkyl substances in the influents and effluents of a nationwide set of municipal wastewater treatment plant, is described and discussed in Chapter 4. Finally, the use of this method to monitor the mass flows of fluorinated alkyl substances through a municipal wastewater treatment plant is described and discussed in Chapter 5.

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2. FLUORINATED ALKYL SUBSTANCES

Melissa M. Schultz, Douglas F. Barofsky, and Jennifer A. Field

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Abstract

Fluorinated alkyl substances, which include perfluorooctane sulfonate (PFOS), constitute a diverse class of chemicals that occur in a wide range of products. Concern about the presence of fluorinated alkyl substances in the environment has increased since PFOS and related fluoroalkyl substances were detected in blood plasma of nonoccupationally-exposed humans and in animal tissues collected from around the globe, including sparsely populated regions that have no apparent sources. PFOS was also found to exhibit both bioaccumulative and toxic properties. This review focuses on the analysis and occurrence of fluorinated alkyl substances that have been observed in the environment. Although, fluorinated alkyl substances were identified and quantified in groundwaters, surface waters, wastewaters, and air samples, little is known about their transport or behavior in the environment. Numerous laboratory and field experiments are still needed to elucidate these processes. Additionally, techniques for efficiently treating wastewaters containing fluorinated alkyl substances must be found in order to prevent their release into the environment.

Introduction

Fluorinated alkyl substances compose a diverse class of chemicals that are constituents in a wide range of products including fluoropolymers (i.e. polymers like PTFE and PVDF that are largely fluorinated along the polymer's backbone); liquid repellants for paper, packaging, textile, leather, and carpet goods; industrial surfactants, additives, and coatings; and firefighting foams (1). The majority of fluorinated alkyl substances are synthesized from either perfluorinated sulfonyl fluoride and carbonyl fluoride intermediates by electrochemical fluorination (ECF) or perfluoroalkyl iodide intermediates by telomerization. Some of the intermediates used in the manufacturing processes were detected in blood plasma of nonoccupationally-exposed humans (2), and recently, some of them were discovered in various tissues of animals from less densely populated regions of the world where there are no local commercial, municipal, or industrial sources of fluorinated alkyl substances (3). Among these chemicals are perfluorooctane sulfonate (PFOS, Figure 2.1) and perfluorooctanoate (PFOA, Figure 2.2), arguably the two most studied fluorinated alkyl substances. These two compounds do not biodegrade under aerobic or anaerobic conditions and, thus, could persist in the environment (4-6). Furthermore, PFOS was found to exhibit both bioaccumulative (3) and toxic (7) properties. Taken together, the preceding observations raise concerns about the occurrence and behavior of the entire class of fluorinated alkyl substances in the environment and the risk they may pose toward humans and other organisms. In a decision that reflected this concern and anticipated

increasing public attention to the use and management of its perfluorooctane sulfonyl fluoride-based products, the 3M Company announced in the Spring of 2000 that it was "phasing out of the perfluorooctanyl chemistry used to produce certain repellents and surfactant products" (6). In light of this announcement and the scientific findings that antedated it, the present review of fluorinated alkyl surfactants in the environment is timely.

This review focuses on the analysis, occurrence, and remediation of fluorinated alkyl surfactants that have been found in the environment. Environmental studies of fluorinated alkyl substances to date have concentrated almost exclusively on fluorinated surface active agents (surfactants), in particular the sulfonates and carboxylates. Despite the fact that fluoropolymers are produced in far greater volume than fluorinated surfactants, virtually nothing has been published on fate and behavior of fluoropolymers in the environment. There are numerous other nonsurfactant forms of fluorinated alkyl substances that similarly merit interest, but for which very little environmental information exists. These circumstances dictate that fluorinated surfactants, which compose a subset of fluorinated alkyl substances, be the focal point of this paper.

Synopses of the properties, synthesis, production, applications, and toxicology of fluorinated alkyl substances are presented in the remainder of this introduction. While obviously important, these topics are far too extensive in their own rights to be treated adequately within a review of the present length. The interested reader is referred to books by *Banks et al.* (8) and by Kissa (1)

for excellent treatises on the chemistry, production, and applications of fluorinated alkyl substances and to the recent overview by Giesy and Kannan (7) for a guide to the literature on the toxicology of fluorinated alkyl substances.

Properties. Perfluoroalkyl sulfonates and carboxylates, can resist degradation by acids, bases, oxidants, reductants, photolytic processes, microbes, and metabolic processes because of the strength of the carbon-fluorine bond (≥ 450 kJ/mole), the presence of three pairs of nonbonding electrons around each fluorine atom, and the effective shielding of carbon by the fluorine atoms (1). Consequently, these fluorosurfactants as well as other fluorinated alkyl substances are stable under conditions that degrade their hydrocarbon analogues (1). Unfortunately, little is currently known about the physicochemical properties, e.g. Henry's Law constant, vapor pressure, water solubility, and octanol-water partition coefficient that are required to predict the behavior of fluorinated alkyl substances under various environmental conditions. Meaningful modeling studies on the fate of fluorinated alkyl substances in general and fluorosurfactants in particular will require that these physicochemical properties be determined.

Synthesis. Commercial synthesis of fluorinated alkyl substances originated in the late 1940's when 3M Company licensed and began developing an electrochemical fluorination (ECF) process (8) invented by Joseph Simons and DuPont Company began developing a telomerization fluorination process (1).

ECF, which refers to the fluorination of organic compounds in anhydrous hydrogen fluoride, primarily yields perfluorinated sulfonyl (Reaction 1) and carbonyl fluorides (Reaction 2) (1).

$$C_nH_{2n+1}SO_2Cl + (2n + 2)\ HF \rightarrow C_nF_{2n+1}SO_2F + HCl + \text{byproducts;} \quad (1)$$

$$C_nH_{2n+1}COCl + (2n + 2)\ HF \rightarrow C_nF_{2n+1}COF + HCl + \text{byproducts.} \quad (2)$$

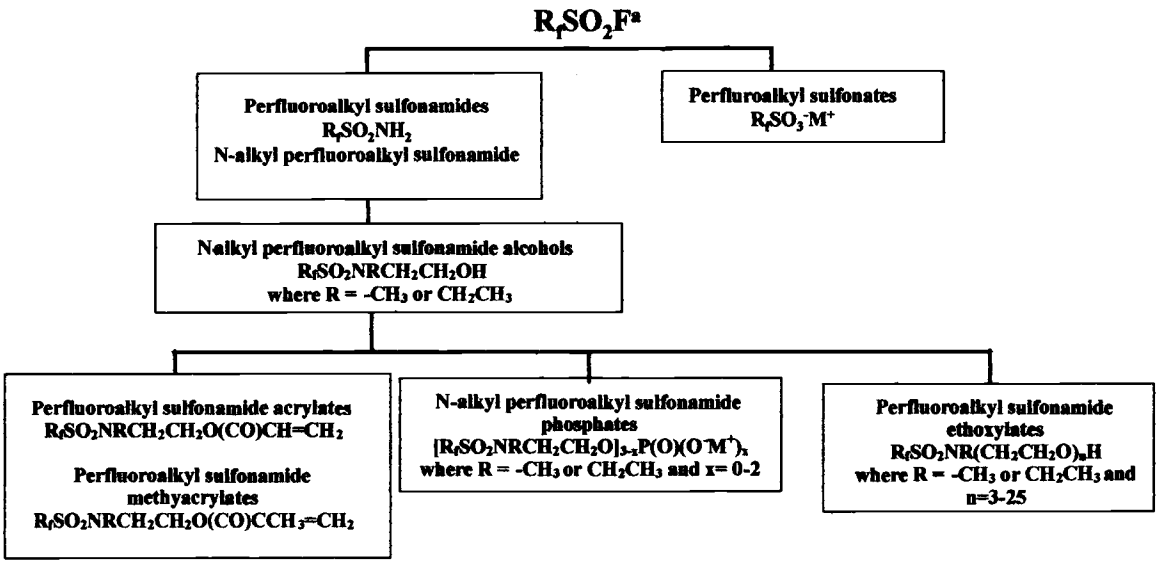
Since the process is neither efficient nor selective, it yields numerous byproducts. The perfluoroalkyl chains on the sulfonyl and carbonyl fluorides form in homologous series of odd- and even-numbered carbons (1). Most of the chains have eight carbons, but they typically range in length from four to thirteen carbons. All molecules with the same number of carbons compose an isomeric set in which the forms of the fluorocarbon tails are distributed between linear and branched respectively in a ratio of approximately 70:30 (7).

The perfluoroalkyl sulfonyl and carbonyl fluorides obtained from Reactions 1 and 2 are not themselves end-products but rather are intermediates in the synthesis of the various fluorinated alkyl substances that end up as industrial and commercial products. For example, hydrolysis of the sulfonyl and carbonyl products of Reactions 1 and 2 readily converts them respectively into perfluoroalkyl sulfonates and carboxylates[†] or their corresponding acids (1). More specifically, perfluorooctane sulfonyl fluoride and perfluorooctanecarbonyl fluoride, the most abundant products of Reactions 1 and 2, are the precursors respectively for the fluorosurfactants PFOS (Figure 2.1) and PFOA (Figure 2.2). Perfluorooctane sulfonamide (FOSA, Figure 2.1), which is volatile and thus

[†]Throughout this text, anionic fluorinated alkyl surfactants will be referred to as salts rather than acids since their low pK_a-values dictate that they exist in their salt forms at all environmentally relevant pHs.[8]

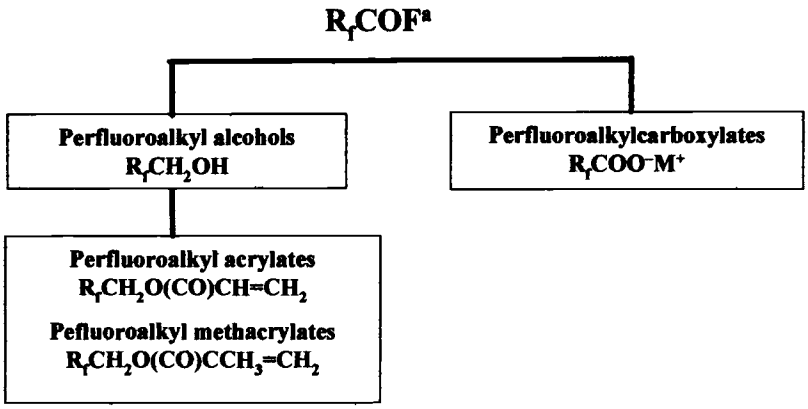
possibly mobile in the environment, is produced by reacting a primary amine with perfluorooctane sulfonyl fluoride (R_8SO_2F) (1). FOSA can in turn be converted to sulfonamido alcohols, such as *N*-methyl perfluorooctane sulfonamide (*N*-MeFOSE, Figure 2.1) and *N*-ethyl perfluorooctane sulfonamide[‡] (*N*-EtFOSE, Figure 2.1). These two sulfonamide alcohols, both of which were detected in the environment (9), are themselves building blocks for acrylates and methacrylate polymer intermediates, phosphates, ethoxylates, and other perfluoroalkyl substances (1). Instead of subjecting perfluoroalkyl carbonyl fluorides to hydrolysis, they can be reacted with other reagents to create the alcohols used to synthesize acrylate and methacrylate polymer-intermediates (Figure 2.2).

[‡]*N*-EtFOSE can also be synthesized directly by reacting R_8SO_2F with a secondary amine.[8]



[a] $R_f = F_3C(CF_2)_n$ where typically n = 3-11 and 30% is terminally branched and M^+ = cation

Figure 2.1. Selected production pathways for chemicals based upon sulfonyl fluoride intermediates obtained by electrochemical fluorination.



[a] $R_f = F_3C(CF_2)_n$ where typically n = 3-11 and 30% is terminally branched and M^+ = cation

Figure 2.2. Selected production pathways for chemicals based upon carbonyl fluoride intermediates obtained by electrochemical fluorination.

Telomerization, the other commercially important process used for synthesizing perfluoroalkyl substances, begins with fluoroiodination of tetrafluoroethylene (TFE) to produce pentafluoroiodoethane (Reaction 3) and ends with reaction of the pentafluoroiodoethane with a varying number (*n*) of TFEs to yield a mixture of perfluoroalkyl iodide telomers (Reaction 4):

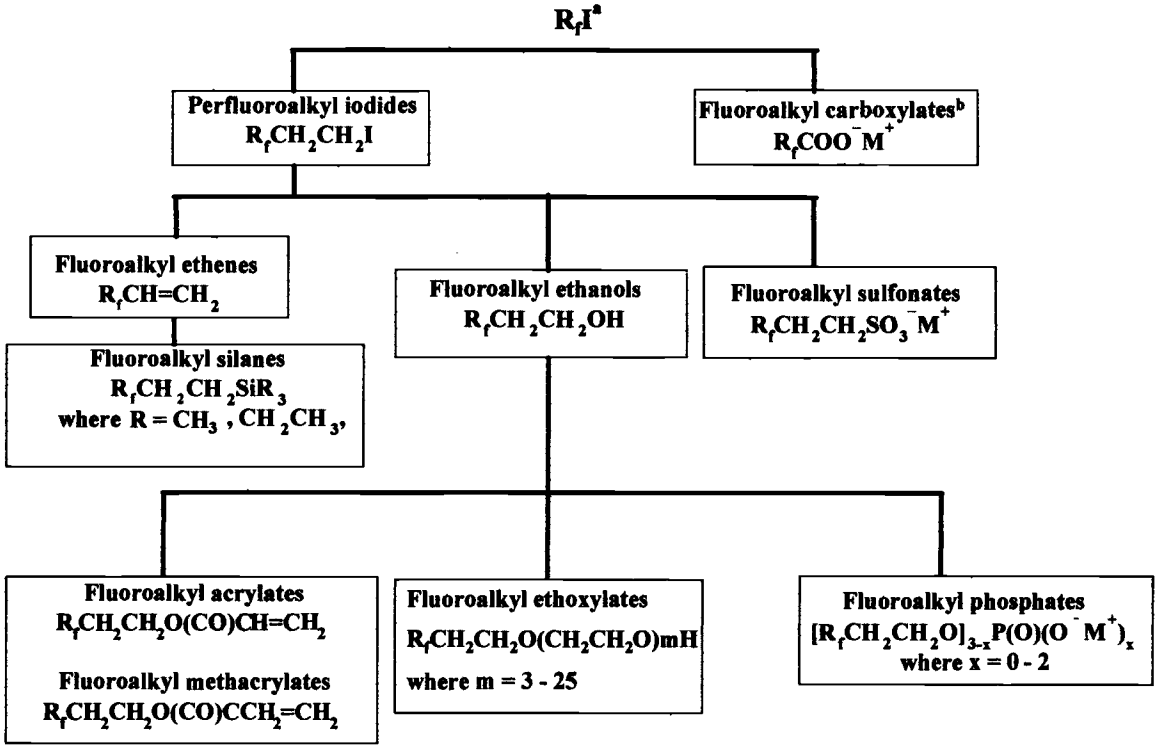
$$5CF_2{=}CF_2 + 2I_2 + IF_5 \rightarrow 5F(CF_2CF_2)I; \quad (3)$$

$$F(CF_2CF_2)I + nCF_2{=}CF_2 \rightarrow F(CF_2CF_2)_{n+1}I. \quad (4)$$

The homologous fluoroalkyl chains generated by the telomerization process are all linear and contain only even numbers of fluorinated carbons in contrast to ECF, which produces a mix of linear and branched chains with both odd and even numbers of fluorinated carbons. The perfluoroalkyl iodides resulting from Reaction 4 are commonly reacted with ethylene to produce intermediate perfluoroalkylethyl iodides (Reaction 5):

$$F(CF_2CF_2)_nI + CH_2{=}CH_2 \rightarrow F(CF_2CF_2)_{n+1}CH_2CH_2I. \quad (5)$$

These iodides can be easily converted to yet other intermediates, such as olefins, alcohols, thiocyanates, sulfonyl chlorides, and thiols (Figure 2.3). The olefins can be used to produce fluoroalkyl silanes while the thiocyanate-, sulfonyl chloride-, and thiol-intermediates can be converted to telomer sulfonates (Figure 2.3). Oxidation of pentafluoroiodoethane is used to form carboxylates (Figure 2.3). In addition, perfluoroalkylethyl iodide can be hydrolyzed to an alcohol that acts in turn as an intermediate for end products like acrylate and methacrylate polymers, ethoxylates, and phosphates (Figure 2.3) (1).



[a] $R_f = F(CF_2CF_2)n$ and n= 3 -7 with no terminal branching and M^+ = cation

Figure 2.3. Selected production pathways for chemicals based on fluoroalkyl iodide intermediates obtained by telomerization.

The presence of an ethyl group in the alkyl chains of the perfluoroalkylethyl iodide intermediates further distinguishes the fluorinated chemicals derived from telomerization from their ECF-counterparts. This C_2H_4 is reflected in telomeric terminology. For example, $F(CF_2CF_2)_3CH_2CH_2SO_3^-NH_4^+$ (1-Octanesulfonic acid, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-,

ammonium salt – Figure 2.3) is referred to as 6:2 telomer sulfonate† because it has six fluorinated carbons and two hydrocarbons in the fluoroalkyl chain.

Production. The total global production of fluorinated alkyl substances by ECF and telomerization is not currently known (10). It is known, however, that nearly all industrial output of such substances derives from the production either of perfluorinated sulfonyl and carbonyl fluoride intermediates by ECF or of perfluoroalkyl iodide intermediates by telomerization. While intermediates are not marketed as products themselves, they are nevertheless likely to be present as impurities in finished products, and as was stated earlier, it is largely intermediates or byproducts like PFOS that have been detected in humans (2) and the environment (3). In the United States, commercial synthesis of PFOS and PFOA is based entirely on ECF; outside the United States, some production of PFOA is based on oxidation of perfluorooctyl iodide, $C_8F_{17}I$. Inasmuch as 3M Company is the sole manufacturer of fluorinated alkyl substances by ECF in the United States, the approximately three million kilograms of materials produced by 3M from perfluorinated sulfonyl fluoride intermediates in the year 2000 (10) give some sense of the scale of sulfonyl fluoride production in the United States. Regrettably, it is impossible from this number for perfluorinated sulfonyl fluoride intermediates† to estimate the quantity of these compounds that might eventually find their way into the environment. Fluorinated alkyl substances based on

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†The 6:2 telomer sulfonate has also been referred to in some literature by the acronym H-PFOS; however, this term is misleading because it implies a structural parallel with PFOS that does not exist.

†No comparable production number is available for the perfluorinated carbonyl fluoride intermediates.

telomerization are produced by a number of companies including DuPont, Asahi Glass, Atofina, Clariant, and Daikin, but no production numbers are available for the telomer-based fluorinated alkyl substances.

Applications. The unique physical and chemical properties of fluorinated alkyl substances make them useful in a wide range of industrial and commercial applications. These properties are particularly manifest in fluorosurfactants. Surfactants are a class of chemicals that, at low concentrations, reduce the interfacial tension between the liquid in which they are dissolved and the gas, liquid, or solid phase with which the surfactant solution is in contact. Fluorosurfactants have alkyl tails that are both hydrophobic and oleophobic (i.e. oil repelling), and fluorosurfactants reduce interfacial tension to a greater degree than do hydrocarbon surfactants (1,11). Consequently, fluorocarbon surfactants are more versatile wetting agents than their hydrocarbon analogues (1). This versatility is exploited, for example, in aqueous film foaming foams (AFFF), which use a mixture of fluorinated surfactants and hydrocarbon surfactants to extinguish hydrocarbon-fueled fires (1,12). The dual hydrophobic/oleophobic nature of the fluorinated surfactants in the AFFF formulation enables them to act both as the principal fire-extinguishing chemicals and as the vapor sealants that prevent reignition of fuel (1). The oleophobic property of fluorosurfactants is applied in other ways, for example in the use of fluoroalkyl sulfonamide phosphates as a US Food and Drug Administration-approved grease-proofing agent for paper products that come into contact with food (1).

3M reported in the year 2000 that 41% of its American production of perfluorooctane sulfonyl fluoride-based fluorinated alkyl substances was coated onto paper and packaging products; 37% was impregnated into textile, leather, and carpet goods; 10% was used as ingredients in industrial surfactants, additives, and coatings; and 3% was incorporated into firefighting foams (10). 3M further reported that higher percentages of its perfluorooctane sulfonyl fluoride-based substances were used in Europe for textile, leather, and carpet goods (49%) and for industrial surfactants, additives, and coatings (15%) than in the United States whereas a lower percentage was used for paper and packaging products (33%) (10). The same percentage of 3M's ECF-products was used in Europe for firefighting foams (3%) as in the United States during the period covered by 3M's report (10). A similar breakdown of the uses for carbonyl fluoride-based fluorinated alkyl substances or telomerization-based fluorinated alkyl substances has not been found in the literature by the present authors. Unfortunately, without supporting numbers on the production of the respective products and the percentages of intermediates and byproducts contained in each, knowledge of the relative production of the various categories of products in and of itself cannot be used to estimate the quantities of these fluorinated alkyl substances that might be introduced into the environment.

Toxicology. A wide range of studies on the toxicological effects of PFOS, PFOA, and other fluorinated alkyl substances were performed during the past fifteen years. For example, PFOS was shown (13-18) to produce a

cumulative toxicity in rats and primates that might be caused by changes in fatty acid transport and metabolism, membrane function, peroxisome proliferation, and mitochondrial bioenergetics while PFOA was shown (7) to produce hepatomegaly, focal hepatocyte necrosis, hypolipidemia, alteration of hepatic lipid metabolism, peroxisome proliferation, induction of the cytochrome P450 superfamily, and uncoupling of oxidative phosphorylation in laboratory-exposed animals.

In 1968, organic forms of fluorine were detected in human blood serum(19); analyses by nonspecific analytical techniques tentatively identified the major constituent of these compounds as PFOA (20). More recent liquid chromatographic/mass spectrometric analyses, which can identify specific fluorinated alkyl substances, have indicated that PFOS, rather than PFOA, accounts for most of the total organic fluorine levels measured in human blood sera (2). Despite finding PFOS, PFOA, and possibly other fluorinated alkyl substances in human blood, adverse health-effects in humans have not yet been connected to these chemicals (18). The present authors have found no literature on the occurrence of telomer-based fluorinated substances in human blood.

Analytical Methods

Nonvolatility and absence of chromophores limited early efforts to analyze fluorinated alkyl substances (21). Neutron activation and X-ray fluorescence were among the first techniques used to determine total organofluorine content (Table 2.1) (1). Unfortunately, these nondestructive

methods lack sensitivity and do not yield structure-specific information. Oxyhydrogen combustion was also used to determine total organic fluorine in environmental and biological samples; however, the explosive mixture of oxygen and hydrogen used in this nonspecific method poses a possible laboratory safety hazard (1,22). The methylene blue active substances (MBAS) test, another nonspecific method, was used to monitor groundwater samples for anionic surfactants in AFFF-contaminated groundwater (23). ^{19}F-NMR was also used for the quantitative determination of PFOS, PFHxS, PFOA, and PFHxA in surface waters (24). This method requires preconcentration of the analytes prior to analysis in order to exceed the technique's limit of detection (10 μg/L). It is unclear whether or not individual perfluorinated compounds can be distinguished in a mixture by ^{19}F-NMR since quantification is based on the terminal CF_3 group common to all fluoroalkyl substances. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, a more recently reported spectroscopic method that does not require preconcentration, has potential for being used to analyze specifically for PFOS (25).

Table 2.1. Analytical methods used for the determination of fluorinated alkyl substances.

Method	Sample matrix	Target compounds	Preconcentration required	Derivatization required	Detection Limit Reported
Neutron activation (1)	None specified	Nonspecific organofluorine	No	No	None
X-ray fluorescence (1)	None specified	Nonspecific organofluorine	No	No	None
Oxyhydrogen combustion (1)	None specified	Nonspecific organofluorine	Optional	No	1 ppm
Methylene blue active substances test (MBAS) (1)	Groundwater	Anionic surfactants	No	No	200 µg/L
^{19}F-NMR (24)*	Surface water	PFOS, PFHxS, PFOA, PFHxS	Yes	No	10 g/L
GC/ECD (26)	Plasma, urine, and liver tissue	PFOA	Yes	Yes	15 µg/L†, 1.5 µg/L‡, 60 µg/L**
GC/MS (27)	Plasma and liver tissue	PFOA	Yes	Yes	1 µg/mL†, 0.1 µg/mL‡
GC/MS (30)	Groundwater	PFHxA, PFHpA, PFOA	Yes	Yes	18 g/L
GC/MS (50)	Groundwater	PFHxA, PFHpA, PFOA	Yes	Yes	3 µg/L
GC/MS (9)	Air	*N*-MeFOSE, *N*-EtFOSE, *N*-EtFOSA, PFOSF, fluorotelomer alcohols	Yes	No	0.4-6.2 pg/m^3††
HPLC with fluorescence detection (32)	Liver homogenate	PFHxA, PFHpA, PFOA, PFNA, PFDA	Yes	Yes	50 pmol/50 mg

Table 2.1. (Continued)

Direct injection MS (31)	Surface water	Perfluorinated (C3-C8) sulfonates	No	No	5 g/L
Direct injection MS (50)	Groundwater	PFOS, PFHxS	No	No	3 µg/L
HPLC/NESI/MS/MS	Sera	PFOA, PFOS, PFHxS, PFOSA	Yes	No	1-2 g/L
HPLC/NESI/MS/MS (24)	Surface water	PFPeA, PFHxA, PFHpA, PFOA, PFDoA, PFBS, PFHxS, PFOS	Yes	No	0.01-0.02 µg/L
HLPC/NESI/MS/MS (40)	Groundwater	6:2 telomer sulfonate, 8:2 telomer sulfonate	No	No	0.5 µg/L

* Unable to distinguish individual perfluorochemicals in a mixture
† Plasma
‡ Urine
** Liver tissue
†† Range for individual analytes

Gas chromatography (GC) with electron capture detection (26) or mass spectrometric (MS) detection (27,28) can be used to sensitively and selectively measure derivatized fluorinated carboxylates. Fluoroalkyl carboxylates have been derivatized by means of diazomethane (26,29), a liquid/liquid extractive alkylation method that utilizes benzyl bromide (27), and by a strong anion exchange extraction method coupled with methyl iodide derivatization (30). By contrast, perfluorinated sulfonates like PFOS, which do not form stable, volatile derivatives, can not be analyzed by GC/MS. To the best of our knowledge, derivatization of telomer sulfonates has not been reported even though it may be possible due to the C_2H_4 group between the perfluoroalkyl chain and the sulfonate group. *N*-EtFOSE, *N*-MeFOSE, FOSA, and fluorotelomer alcohols are sufficiently volatile to be analyzed without derivatization prior to GC/MS.(9)

Recently, a quantitative, mass spectrometric analysis of perfluoroalkyl sulfonates was reported in which samples with no preparation were injected directly into the mass spectrometer (31). Although this technique is less time consuming, its developers do not recommend it for the analysis of blood serums, whole tissues, or wastewaters because of possible, interfering matrix effects.

High performance liquid chromatography (HPLC) followed by fluorescence detection (32) was used to quantitatively determine perfluorinated carboxylates in biological samples. Fluorescence detection has superb sensitivity (LOD = 1-10 pg); unfortunately, this advantage is offset by

limited specificity and excessive susceptibility to interference from sample matrices. HPLC coupled to a single quadrupole mass spectrometer (HPLC/MS) was used to analyze for perfluorochemicals, including PFOS, in surface water and fish (33). Regrettably, biological and environmental samples are so complex that many of the substances contained in such samples (analytes and nonanalytes alike) unavoidably coelute in the chromatographic stage of this technique, and as often as not, these coeluting species cannot be resolved in the single stage of mass spectrometric detection that follows. Consequently, use of HPLC/MS for quantitative analysis still requires considerable sample clean-up in order to sufficiently reduce such interferences. This limitation can be largely overcome, albeit with a substantial increase in cost, by using tandem mass spectrometry (MS/MS) to increase the molecular specificity of detection by one to two orders of magnitude (34). Analytical methods based on HPLC negative electrospray ionization (NESI) MS/MS were developed to analyze perfluoroalkyl carboxylates in human serum (35); sulfonates, carboxylates, and sulfonamides in biological matrices (2); and sulfonates and carboxylates in surface waters (24). A HPLC/NESI/MS/MS method was used to survey the global distribution of PFOS (3) and its accumulation in marine mammals (36,37), fish-eating water birds (38), and oysters (39). Because of its sensitivity and selectivity, HPLC/NESI/MS/MS is, at present, the analytical method of choice for fluorinated alkyl surfactants in biological and environmental samples.

One of the primary obstacles to the analysis of fluorinated alkyl substances is the lack of authentic fluorinated standards available for use as internal standards or surrogates in quantitative analyses. Rigorous quantitative analyses require reference materials that do not overlap with compounds that are used commercially. For example, the recent observation of 6:2 telomer sulfonate in AFFF-contaminated groundwater (40,41) renders this compound unsuitable for use as an internal standard with samples that may contain telomer-based products. Intensive measures to synthesize or otherwise find new internal standards in the near term are strongly indicated if future studies of the occurrence and fate of fluorosurfactants in the environment are to be quantitatively accurate.

Scrupulous care must be taken in the laboratory to avoid sample contamination because these compounds appear to be ubiquitous in analytical laboratory products. In a recent study of PFOS and PFOA in river water, PFOS and PFOA were detected in the background blanks that included extracts from bottled drinking water and field blanks (2). With only one exception, the analytes were at levels below the limit of quantitation (PFOS-LOQ = 10-25 ng/L; PFOA-LOQ = 25-50 ng/L). It is the authors' experiences that the levels of background contamination vary from one instrument to another. When analyzing fluorinated alkyl substances, background interferences can be minimized by not storing aqueous samples in glass and PTFE containers; if glassware, including LC vials, must be used, it should be thoroughly rinsed with deionized water and methanol prior to use (2).

Aluminum foil should not be used to seal or cover containers because fluorinated alkyl substances are used to lubricate the mill-rollers used in foil manufacturing. Because paper food containers and wrappings are treated with fluorinated alkyl substances, packaged, stored, and wrapped foods must be kept out of the laboratory, and analysts who handle food supplied in such items must take appropriate care to clean up before entering the laboratory (42). New clothes may be treated with fluorinated alkyl substances, thus impurities, such as PFOS, may be present; consequently, they must be washed thoroughly before personnel in the field or in the laboratory wear them. Recently, the present authors analyzed methanol extracts of two fabric samples cut from finished clothing products (data unpublished). The combined concentration of the perfluoroalkyl sulfonates and carboxylates was 13 ng/g of fabric in one sample and 882 ng/g of fabric in the other sample. Fluorinated alkyl substances used as standards should be stored in a laboratory or facility away from that in which the analyses are conducted. Similarly, syntheses of these compounds should be conducted well away from where analyses are performed.

Occurrence Data

A surprising result kept resurfacing in the 1990s when researchers were searching for human serum samples they could use as quantitative blanks in HPLC MS/MS assays of PFOS levels in the blood sera of 3M factory workers (43). PFOS was found in all of the serum samples purchased from sources in

the United States (18 blood banks), Europe, and Asia (20,43). The only stored blood samples they could find that were free of PFOS were some that had been drawn from U.S. military recruits during the Korean War (1950-1953), which preceded the industrial production of fluorochemicals (20,43). The mean PFOS concentration obtained from the 18 American blood banks for nonoccupationally exposed people was 29.7 µg/L (Table 2.2) with a low of 14 µg/L (Santa Barbara, California, USA) and a high of 52 µg/L (Greenville, South Carolina, USA) (20). Levels of PFOS examined in occupationally exposed people employed by 3M Company were substantially higher than in the general population (levels ranged from 250 to 12,800 µg/L in 1994 and from 100 to 9,930 µg/L in 1997; Table 2.2) (17,20). PFOA was also detected in human sera, but at lower levels than PFOS (concentrations due to nonoccupational exposure ranged from 3 to 17 µg/L while concentrations due to occupational exposure ranged from 840 to 6,400 µg/L in 1994 and from 100 to 982 in 1997; Table 2.2) (44). The presence of PFOS and PFOA in workers' blood sera is not surprising; 3M's Material Data Safety Sheet (MSDS) for AFFF states that AFFF comprises "one or more organic fluorochemicals that have the potential to be absorbed and remain in the body for a long period of time, either as the parent molecule or as metabolites, and may accumulate with repeated exposures"(45). The presence of these two compounds in nonoccupationally-exposed people, however, is at the moment subject to conjecture. In the remainder of this section, those occurrences of fluorochemicals in biological and physical matrices that have been reported to

date (Tables 2.2 and 2.3) are summarized. It will become apparent to the reader that little is known yet about the fate and transport of fluorinated chemicals in the environment.

Recent surveys found PFOS in a variety of organs and tissues taken from wildlife found in urbanized regions of North America (Great Lakes region and coastal waters) and Europe (Mediterranean and Baltic Seas) as well as in remote regions of the Arctic and North Pacific Oceans (3,36-39). In general, PFOS concentrations were found to be several times greater in samples from urban areas than in those from remote areas. This is seen in the data shown in Table 2.2 by comparing, for example, the concentrations of PFOS found in the livers of Alaskan fur seals (<10-122 ng/g wet wt.) with the concentration detected in the livers of minks that inhabit populated regions within the continental United States (20-5140 ng/g wet wt.) (36,37). This comparison is but a small sampling of the quantitative data that has been collected from wildlife around the globe. For a complete summary of the global distribution of PFOS in wildlife tissues, the reader is referred to recent articles (3,36-39).

Table 2.2. Concentrations of fluorinated alkyl substances in biological matrices.*

Chemical	Nonoccupationally exposed human sera (g/L)	Occupationally exposed human sera (g/L)	Animal livers remote locations[a] (ng/g wet wt)	Animal livers populated locations[b] (ng/g wet wt)
PFOS	14-52[c,d,e] 6.7-81.5[f]	250-12,800[c,d] 100-9930[c,d]	<DL (10)-122†	20-5140‡
PFHxS	<DL (1.5)-21.4[f]	NA	NA	<DL (4.5)-85‡
PFOA	3-17[c,d] <DL(5)-35.2[f]	840-6400[g] 100-982[h]	NA	<DL (4.5)-27‡
FOSA	<DL (1.5)-2.2[f]	NA	NA	<DL(37)-590‡

NA = not analyzed; <DL = below detection limit

* PFHpA, PFHxA, N-MEFOSE, N-EtFOSE, N-EtFOSA, 4:2-10:2 FtOH, and telomer sulfonates were not analyzed for in any these matrices.

[a] (36)

[b] (37)

[c] (13,17,20)

d (13,17,20)

e (13,17,20)

[f] (2)

[g] (44)

[h] (35)

† Detected in livers of northern fur seals from Alaska

‡ Detected in livers of minks from the United States (IL, MA, SC, & LA)

Table 2.3. Concentrations of fluorinated alkyl substances in physical matrices.*

	Surface Water		Groundwater			Air	
	Etobicoke Ck.[a] (g/L)	**Tennessee R.[b] (g/L)**	**Wurtsmith AFB (g/L)**	**NAS Fallon[c] (g/L)**	**Tyndall AFB[c] (g/L)**	**Urban[d] (pg/m^3)**	**Rural[d] (pg/m^3)**
PFOS	<DL(0.017)-2220	0.032 ± 0.011†, 0.114 ± 0.019‡	<DL (3)-130[e]	NA	NA	NA	NA
PFHxS	<DL (0.017)-49.6	NA	<DL (3) -120[e]	NA	NA	NA	NA
6:2 telomer sulfonate	NA	NA	<DL(0.5)-173[f]	NA	NA	NA	NA
PFOA	<DL (0.009)-11.3	<DL (0.025)†, 0.394 ± 0.128‡	<DL (3)[e]	<DL (18)-6720	<DL (18)-116	NA	NA
PFHpA	<LOQ**	NA	NA	<DL (18)-154	<DL (18)-38	NA	NA
PFHxA	<LOQ**	NA	<DL (3)-8[e]	<DL (18)-376	<DL (18)-144	NA	NA
N-MeFOSE	NA	NA	NA	NA	NA	86-123	34, 36
N-EtFOSE	NA	NA	NA	NA	NA	51-393	68, 85
N-EtFOSA	NA	NA	NA	NA	NA	14	NA
4:2 FtOH	NA	NA	NA	NA	NA	<DL(0.4)	<DL(0.4)
6:2 FtOH	NA	NA	NA	NA	NA	30-196	16,41
8:2 FtOH	NA	NA	NA	NA	NA	9-123	40,25
10:2 FtOH	NA	NA	NA	NA	NA	7-46	20,15

NA = not analyzed; <DL = below detection limit

*FOSA not analyzed for in any of these matrices.

[a] (24,47),[b] (53),[c] (30),[d] (9),[e] (50),[f] (40)

Table 2.3. (Continued)
† Upstream of the Decatur, AL fluorochemical manufacturing facility.
‡ Downstream of the Decatur, AL fluorochemical manufacturing facility.
** Observed but not quantifiable; detection limits not given.

PFOS also appears to bioaccumulate. PFOS concentrations in animals high in the food chain, for example minks and bald eagles, were greater than the concentrations found in their diets (3). In a laboratory feeding study, PFOS concentrations in mink livers were found to be dose-related in the range 1120 – 3250 ng/g, wet wt. The mean biomagnification factor of 18 obtained from this study is similar to the values observed for PCBs and PCDDs/DFs in mink livers (46). Despite finding convincing evidence for bioaccumulation of PFOS in minks, the investigators who conducted these studies emphasized that the toxic effects of PFOS on minks are unknown and would be subjects of future investigation (37). In contrast to PFOS in minks, studies of PFOA in fish provided no evidence for bioaccumulation of this compound (44).

Higher chained perfluorocarboxylate homologues (10, 11, and 14 carbon chains) were observed in livers of fish whose living environments were contaminated by an accidental spill of AFFF (24,47). The higher chain perfluorocarboxylate homologues partitioned to a greater extent into fish liver tissues than did the lower chain perfluorocarboxylate homologues (5, 6, 7, and 8 carbon chains). This is consistent with results from previous studies of the partitioning of hydrocarbon surfactants in fish liver tissues (48). Higher chained perfluorocarboxylates were also observed in polar bears from the Canadian Arctic and Greenland (49). At this time, the route by which these chemicals enter the arctic food chain is unknown.

PFOS also was detected in groundwater and in surface waters (24,28,47,50,51). Repeated use of AFFF at military bases for firefighting

activities has led to AFFF-contaminated groundwater (28,50,51). In AFFF-contaminated groundwater collected from firefighting facilities at Fallon Naval Air Station, Nevada (USA) and Tyndall Air Force Base, Florida (USA), perfluoroalkyl carboxylates containing six to eight carbons were detected with total concentrations ranging from 125 to 7096 μg/L (Table 2.3) (28,30). PFOS was also detected in groundwater collected from Wurtsmith Air Force Base in Oscoda, Michigan (USA), where firefighting activities occurred; concentrations of total perfluoroalkyl sulfonate (containing six to eight perfluorinated carbons) ranged from below detection (3 μg/L) to 235 μg/L (Table 2.3) (40,50). Telomer sulfonates (4:2, 6:2 and 8:2) were also detected in the Wurtsmith groundwater, but the 10:2 telomer sulfonate was not (40,41). The concentration of the 6:2 telomer sulfonate ranged from below detection (0.5 g/L) to 173 g/L (40). The presence of fluorinated surfactants derived from both ECF- and telomerization-based AFFF formulations is qualitatively consistent with the history of the US military's AFFF procurement over the past three or more decades (52). Up to 1983, 3M Company held the contract for supplying AFFF to the US military; from 1984 through 1988, the AFFF-contract was awarded to Ansul Incorporated, Marionette, Wisconsin, USA (an AFFF supplier that uses telomerization-based fluorinated alkyl substances); and from 1989 to the present, the contract has regularly gone to 3M Company. During a contract period, approximately 90% of the military's demand for AFFF is supplied by the contract-holder, and the remaining 10% is supplied by other manufacturers whose AFFF-product meets military specifications (52). During the Gulf War

(August 1990 to March 1991), the US military's demand for AFFF was met by suppliers of both ECF- and telomerization-based formulations.

A unique occurrence allowed for the analysis of PFOS and PFOA in surface water from Etobicoke Creek (Toronto, Ontario) after an accidental spill of 22,000 L of AFFF in June 2000 (47). The total surface water concentrations of perfluoroalkyl sulfonates (6 and 8 carbons) ranged from below detection (< 0.017 μg/L) to 2270 μg/L (Table 2.3) and the PFOA concentrations ranged from below detection (< 0.009 μg/L) to 11.3 μg/L (Table 2.3). The highest measured concentration of PFOS in the liver of a fish taken out of Etobicoke Creek after the AFFF-spill was 72.9 μg/g (47). After the AFFF spill into Etobicoke Creek, the levels of PFOS in livers of fish taken out of the creek were found to be significantly higher (in some cases 1000 times) than had been previously reported for PFOS present in fish liver tissues (47).

Analyses for PFOS and PFOA were conducted along a 50 km stretch of the Tennessee River centered about the location from 3M's fluorochemical manufacturing facility in Decatur, Alabama (USA) (53). Low levels of PFOS (0.032 ± 0.011 g/L; Table 2.3) were observed upstream of the fluorochemical manufacturing facility while PFOA was below detection (< 0.025 g/L)(Table 2.3). Downstream of the facility, the concentrations of PFOS and PFOA increased to 0.114 ± 0.019 g/L and 0.394 ± 0.128 g/L (Table 2.3), respectively. The PFOS level was found to be relatively constant along the upstream of the manufacturing plant and the levels of both PFOS and PFOA were found to be relatively uniform downstream of the plant. These observations indicate that,

over the 50 km distance tested along the river, removal mechanisms such as volatilization or sorption did not affect analyte concentrations (53).

Little is known about the occurrence of volatile fluorinated organics, such as *N*-EtFOSE, *N*-MeFOSE, FOSA, and fluorotelomer alcohols, in the environment. In air sampled from both urban and rural sites, analyses found these compounds to be present at pg/m^3 concentrations (Table 2.3) (9). To date, no analyses have been reported for *N*-EtFOSE, *N*-MeFOSE, FOSA, and fluorotelomer alcohols in groundwater, wastewater, surface water, or human sera. Moreover, to date there has been no mention of sulfonamide derivatives, ethoxylates, silanes, phosphates, or betaines in any environmental context.

Treatment

Although a large number of fluorinated alkyl substances exist, very few biodegradation studies have been reported. Hence, detailed biodegradation studies are needed to establish the mechanisms of transformation and the products formed. Biodegradation of PFOS and the 6:2 telomer sulfonate, which have identical numbers of carbon atoms but different numbers of fluorines, were studied under aerobic and sulfur-limiting conditions in microcosms containing a *Pseudomonas* species of bacteria (5,6). The 6:2 telomer sulfonate degraded into six volatile, nonsulfur, oxygen and fluorine containing products, but the fully fluorinated PFOS did not biodegrade. In the first of three studies conducted by a contract lab for 3M Company, no

measurable biotic or abiotic degradation of PFOS was observed after 35 days of exposure to a municipal-wastewater-treatment activated sludge (54). In the second study, PFOA was found to be resistant to biodegradation under both aerobic and anaerobic conditions. However, *N*-EtFOSE was degraded under activated sludge conditions with only 15.9% remaining after 18 days, and FOSA was degraded under the same conditions with 90% of the parent remaining after 18 days (55). In the third study, it was found that only 10% of the *N*-EtFOSE remained after 35 days (56). Based on the observation that PFOS and PFOA appeared as metabolites in this series of studies, they investigators suggested that these two compounds may be end-products in the biological degradation of perfluoroalkyl sulfonamide derivatives (54-56).

The alkyl substituents of telomeric sulfonates undergo biodegradation to stable perfluorinated products when ingested by rats. *In vivo* studies with male rats observed the formation of PFOA from the 8:2 telomer alcohol, which involves the removal of two fluorine atoms from the carbon atom bonded to the CH_2 group (29) Because this study was performed with a mammalian system, it is not clear whether defluorination reactions will be carried out by microbial populations in the environment.

The present authors recently initiated a systematic investigation of the fate of fluorinated alkyl substances during municipal wastewater treatment. Municipal wastewater treatment is one of the principal routes for disposing of aqueous-borne surfactant wastes; therefore, it is particularly important to understand how the mass-flow and composition of mixtures of fluorinated alkyl

substances change as they pass through wastewater treatment facilities and how different types of treatment affect their removal. Preliminary data indicates that 98.4% of PFOS is removed during wastewater treatment in a municipal plant (51). This estimate is based on a comparison between PFOS levels in 24 hr composites of the primary and secondary sewage effluents. Inasmuch as no biodegradation pathways have been identified for PFOS, it is likely that PFOS is removed onto sludge; however, detailed analyses, including mass flow studies at the scale of functioning wastewater treatment plants, must be conducted in order to determine the fate of PFOS and other fluoroalkyl substances.

Since perfluorinated sulfonates and carboxylates (i.e. PFOS and PFOA) do not appear to biodegrade under aerobic or anaerobic conditions and are persistent in the environment (3-6), alternative treatments and removal techniques for wastewaters containing perfluorinated surfactants should be found. Perfluorinated surfactants present in AFFF mixtures, emulsified oil, fuel, and grease can be removed from wastewater using the Air-Sparged Hydrocyclone (ASH) reactor technology developed by the United States Air Force in conjunction with Advanced Processing Technologies, Inc. ASH uses a centrifuge to achieve fast flotation of fine particles, thus separating fuel, oil and grease from water for subsequent removal (57). Pilot-scale tests were performed with a 76 L/min mobile ASH unit at five different Air Force bases, each possessing unique wastewater streams. The foam-forming properties of AFFF were exploited in the pilot tests to remove between 70 and 90% of the

surfactant from the wastewater (57). Unfortunately, information was not given in this study on the subsequent disposal of the recovered fluorinated surfactants. Some of the advantages of ASH technology include system mobility, small physical space requirement, small waste disposal volume, and low operational costs (57).

Activated carbon was used to extract 98% of fluorinated surfactants from water at the laboratory scale (58). Once the activated carbon is saturated, the sorbed fluorinated surfactants can be destroyed by incinerating the activated carbon at 1200 °C for 20 min, burning the gas generated at 1200 °C for 2 s, and treating the burned gas to produce solid CaF_2 (59). Activated carbon treatment has not yet been assessed in terms of recovering fluorinated surfactants from wastewater.

Future Research Needs

The surveys of biological samples summarized herein show unquestionably that PFOS and PFOA are present in the blood plasma of both occupationally and nonoccupationally-exposed humans (2) and that PFOS is present in the tissues of terrestrial and aquatic animals found in both urban and remote locations around the globe (3). The water and air surveys show that PFOS, PFOA, and certain telomeric sulfonates can to varying degrees be identified and quantified in groundwaters, surface waters, and waste-treatment waters and that some fluoroalcohols and fluoroalkyl sulfonamide derivatives can be identified and quantified in urban and rural air samples. When all of

these findings are viewed as a whole, two comprehensive questions about the presence of fluorinated alkyl substances in the environment emerge as overriding.

The first question is, why, despite finding significant levels of fluorosurfactants in the tissues of wild animals and significant concentrations of fluorosurfactants in the blood of humans, has no evidence of toxicity yet been observed in those animals whose tissues contain significant levels of fluorosurfactants or in those humans who have significant blood-levels of fluorosurfactants? Toxicological studies have shown that there are numerous possible pathways between exposure to fluorinated alkyl substances and toxicity in animals (and, thus, by analogy in humans), but toxicity has not yet been reported in any of the wild animals or humans that have been shown to have substantial levels of fluorosurfactants somewhere in their systems. Clearly, extensive toxicological research is needed to determine functional levels of exposure to a wide variety of fluorinated alkyl substances and to relate those levels to critical mechanisms of toxicity.

The second question is, how do nonvolatile fluorosurfactants find their way from sites that have been heavily exposed to fluorinated alkyl substances to locations far removed from obvious sources of these compounds? At the moment, the best estimate for the vapor pressure of PFOS is on the order of 10^{-4} Pa (60). According to one global transport model, a compound with a vapor pressure in this range is not likely to migrate far from its source (61). Nonetheless, PFOS was found in the tissues of animals captured in remote

regions far from any of PFOS's sources. At least two intriguing possibilities have been postulated that could account for the presence of PFOS in far away places: either volatile precursors of PFOS, such as *N*-EtFOSE, may escape into the atmosphere, migrate in the vapor phase, and then breakdown into PFOS in remote locations (61); or PFOS might condense onto atmospherically-mobile aerosol particles that are then transported over long distances and eventually deposited in remote locations (61). In order to test models such as these, it will be necessary to carry out experiments in atmospheric chambers as well as extensive analyses of air and water samples collected from monitoring sites around the globe.

This review has provided several examples of fluorinated alkyl substances released from point sources into the hydrosphere, i.e. discharges from fluorinated alkyl substances plants like 3M's facility on the Tennessee River (53), heavy product-usage like AFFF at firefighting training facilities on military bases (28,30,40,50,51), and accidental spills like that on Etobicoke Creek in Ontario (24,47). Industrial and municipal wastewater effluents, sewage sludges, and landfill leachates are examples of potential point sources that have received little attention. For example, fluorinated alkyl substances contained in sludge applied as fertilizer to agricultural lands might possibly be passed on to humans through the farm crops grown on those lands or livestock feeding on such crops.

Altogether, the number of field studies performed to date has been small in number and, for the most part, limited in scope to determining

distribution and range of concentrations. Field surveys of water and air samples have produced benchmarks for the identities and concentration ranges of those fluorinated alkyl substances most likely to be present in the environment, and analyses of animal tissues have provided compelling evidence for global distribution and bioaccumulation of fluorosurfactants. Field and laboratory studies now need to be designed and conducted to elucidate physical, chemical, and biological mechanisms of transformation, partitioning between phases, and transport within and between physical and bionomical systems. Systematic, simultaneous collection and analysis of proximate physical and biological samples are needed to determine how animals living in remote locations are exposed and how fluorinated alkyl substances bioaccumulate in food chains. This entire enterprise will entail increasing the number of field trials and performing complementary laboratory experiments aimed at, for example, finding ways to increase the number of species within the family of fluorinated alkyl substances that can be quantitatively detected by mass spectrometry or other techniques, developing more rigorous protocols for quantitative analyses, measuring physicochemical properties, and determining pathways for biotic and abiotic degradation.

In summary, there is still very much to be learned about the distribution and behavior of fluorinated alkyl substances in the environment. The overriding, comprehensive questions that remain to be answered pose a large number of experimental and intellectual challenges and, thus, present numerous opportunities for established and entering investigators alike to

make significant contributions to this important, timely field of environmental research.

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3. QUANTITATIVE DETERMINATION OF FLUOROTELOMER SULFONATES IN GROUNDWATER BY LC MS/MS

Melissa M. Schultz, Douglas F. Barofsky, and Jennifer A. Field

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Abstract

Aqueous film forming foams (AFFF) are complex mixtures containing fluorocarbon and hydrocarbon-based surfactants that are used to fight hydrocarbon-fueled fires. The military is the largest consumer of AFFF in the United States and fire-training activities conducted at military bases have led to groundwater contamination by unspent fuels and AFFF chemicals. A direct-injection, liquid-chromatography tandem mass spectrometry (LC MS/MS) method was developed to quantify a suite of fluorotelomer sulfonate surfactants in groundwater collected from military bases where fire-training activities were conducted. The 4:2, 6:2, and 8:2 fluorotelomer sulfonates were detected and quantified in groundwater from two of the three military bases. The total fluorotelomer sulfonate concentrations observed at Wurtsmith AFB, MI, and Tyndall AFB, FL, ranged respectively from below quantitation (≤ 0.60 µg/L) to 182 µg/L and from 1,100 µg/L to 14,600 µg/L. Analyses of a fluorotelomer-based AFFF concentrate by negative ion fast atom bombardment/mass spectrometry (FAB MS) and LC MS/MS analyses indicate that the AFFF concentrate contains only a small amount of fluorotelomer sulfonates and that fluoroalkylthioamido sulfonates are the main anionic fluorosurfactant in the mixtures. More research is needed to determine the fate of fluoroalkylthioamido sulfonates in the environment.

Introduction

Environmental concern over fluorinated alkyl surfactants is emerging due to their occurence in humans and wildlife and their bioaccumulative, nondegradative, and toxic properties (1-4). Fluorinated alkyl surfactants, as well as hydrocarbon surfactants, are used as 'active ingredients' in aqueous film forming foams (AFFFs), which are used to fight hydrocarbon-fueled fires. The fluorinated alkyl substances present in AFFF lower the surface tension (15 to 20 dynes/cm), thus smothering the flames, preventing air from reaching flammable materials, and therefore, suppressing re-ignition of the fire (5). Formulations of AFFF contain mixtures of fluorosurfactants produced by either electrochemical fluorination or fluorotelomerization as well as hydrocarbon surfactants, cosolvents, and solvents (6). Although AFFFs compose only a small percent of the total fluorosurfactant production, repeated applications of AFFF during fire-training activities conducted at military bases has led to groundwater contamination by AFFF chemicals, unspent fuels, and solvents. AFFFs have been commercially available for fire-fighting applications since their development by the United States Navy and 3M Company in the mid-1960s. Up to 1982, the 3M Company, an electrochemical fluorinated-based AFFF manufacturer, was the sole supplier of AFFF to the U.S. military. From 1983 to 1988, both Ansul Incorporated and 3M were awarded military AFFF-contracts. Ansul Incorporated purchased their fluorochemicals, which were fluorotelomer-based, from the former Ciba-Geigy Corporation. From 1989 to

2001, the contract was again held solely by 3M, and since 2002, the contract has gone to Kidde National Foam (7).

The fluorotelomerization process yields products characterized by homologous fluoroalkyl chains that are linear and contain only even numbers of fluorinated carbons. In contrast, electrochemical fluorination produces mixtures of linear and branched chains with both odd and even numbers of fluorinated carbons (8,9). In addition, the fluorotelomerization synthesis process inserts an ethyl group between the fluoroalkyl chain and the end-group that determines the compounds functionality; this ethyl moiety distinguishes fluorotelomer-based chemicals from those produced by electrochemical fluorination. In referring to fluorotelomer sulfonates, the numbers of fluorocarbons (X) and hydrocarbons (Y) are designated in a ratio X:Y. For example, the compound $F(CF_2CF_2)_3CH_2CH_2SO_3^-NH_4^+$ (1-octanesulfonic acid, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-, ammonium salt) is referred to as 6:2 fluorotelomer sulfonate (6:2 FtS) since it has six fluorinated carbons and two hydrocarbons in the fluoroalkyl chain.

Liquid chromatography (LC) in conjunction with tandem mass spectrometry (MS/MS) was employed in various investigations to analyze perfluoroalkyl carboxylates and sulfonates in human sera (10), animal tissues (11-15), and in surface water (16,17). LC MS/MS was the approach of choice for analyzing fluorosurfactants in these studies because it is sensitive and selective. To date, fluorotelomer-based surfactants have received little attention. In at least one previous study, 6:2 FtS was indicated as an internal

standard (10). However, careful reading of this manuscript suggests that it was not used as an internal standard, since "quantitation of the analytes was based on comparison of a single product ion peak area to the response of two standard curves" (10). Initial attempts to use the 6:2 FtS as an internal standard in the authors' laboratory produced confounding results because it was detected in unspiked groundwater samples (7,18-20).

In this report, the development and application of a quantitative, direct-injection LC MS/MS method for identifying fluorotelomer sulfonates in groundwater is reported. To the best of the authors' knowledge, this is the first instance in which fluorotelomer sulfonates have been observed in groundwater and the first description of a methodology for their quantitative analyses. This methodology was applied to groundwater collected from Naval Air Station (NAS) Fallon, NV, USA, Tyndall Air Force Base (AFB), FL, USA, and Wurtsmith AFB, MI, USA. The concentrations of perfluoroalkyl carboxylates at NAS Fallon, Tyndall AFB, and Wurtsmith AFB were determined in previous studies (21,22). Data for perfluoroalkyl sulfonates, however, had only been obtained in these earlier investigations for Wurtsmith AFB (22). Therefore in order to complete the data sets for all three classes of fluorosurfactants, the same method described in this paper for the fluorotelomer sulfonates was used to quantify the perfluoroalkyl sulfonates in samples obtained from NAS Fallon and Tyndall AFB. The concentrations of the three classes of fluorosurfactants were compared. Finally, high resolution fast atom bombardment mass spectrometry (FAB MS) was used to examine AFFF

formulations in an attempt to identify possible sources of fluorotelomer sulfonates in groundwater.

Experimental Section

Standards and Reagents. A standard of 6:2 FtS (98%) was purchased from Apollo Scientific Limited (Derbyshire, UK). Potassium perfluorobutane sulfonate (PFBS), potassium perfluorohexane sulfonate (PFHxS), and potassium perfluorooctane sulfonate (PFOS) standards were provided by the 3M Company (St. Paul, MN). It was determined by LC MS/MS analysis that this mixture of salts did not contain any major impurities (≤3%). The minor impurities present (of perfluorosulfonate homologs) were accounted for in the overall determination of each perfluorosulfonate concentration. The internal standard, hexafluoroglutaric acid (97%), was obtained from Acros Organics (New Jersey, USA). Zonyl® TBS was provided by the DuPont Company and was used as a reference material known to contain a mixture of fluorotelomer sulfonates; it is important to point out that Zonyl® TBS is not used in AFFF formulations (23).

The solvents used for the LC separations were Milli-Q water (Bedford, MA) and optima grade methanol (Fisher Scientific, Pittsburgh, PA), and both were filtered with anion exchange cartridges (BioBasix Ax cartridges, Thermo Hypersil-Keystone, Bellefonte, PA) prior to use. The aqueous phase was 2mM ammonium acetate (98%) (Aldrich Chemical, Milwaukee, WI) buffer.

The LC column rinse consisted of 10% (v/v) formic acid (97%) (Sigma-Aldrich, St. Louis, MO) in optima grade isopropanol (Fisher Scientific, Pittsburgh, PA).

Field Sites and Sample Collection. Samples for this study were collected in 1999 from sites associated with fire-training activities at Wurtsmith AFB, Tyndall AFB, and NAS Fallon. Although the exact history of fire-training activities at each site is not known, it is known that AFFF was used at each site. The bases were in operation as follows: NAS Fallon (1950s-93), Tyndall AFB (1980-92), and Wurtsmith AFB (1952-93). Unfortunately, the specific history of AFFF usage (years, formulation type, etc.) is unknown. The physical characteristics of each site and methods by which samples were collected have been detailed in previous publications describing the studies performed at these locations (21,22). The groundwater samples were stored in high-density polyethylene brown bottles at 4°C. Formalin was not used to preserve samples because it suppresses analyte signals (unpublished data); however, visual inspection did not reveal any bacterial growth in the vessels. A total of 18 groundwater samples were obtained from wells surrounding the FTA-02 fire pad at Wurtsmith AFB (Figure 3.1). One set of four groundwater samples, all from within 50 m of the fire-training pit, were each collected from Tyndall AFB and NAS Fallon (21).

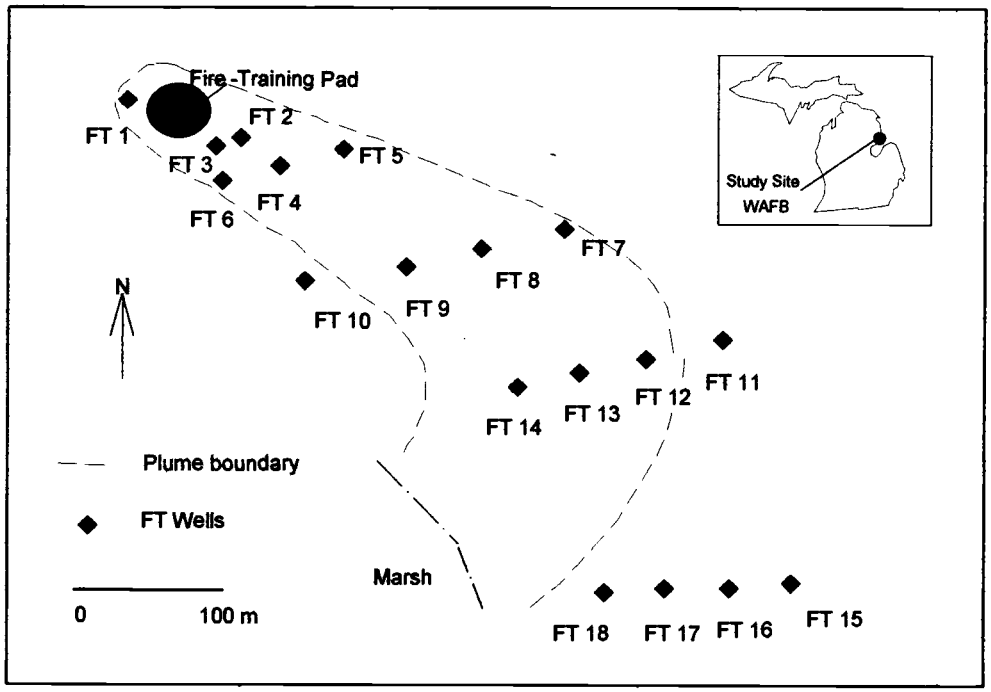

Figure 3.1. Map showing the FTA-02 groundwater plume at Wurtsmith AFB, MI, USA.

Spike and Recovery. Spike and recovery experiments were performed to determine the precision and accuracy of the direct injection LC MS/MS method for fluorotelomer sulfonates and perfluoroalkyl sulfonates. Water drawn from Wurtsmith AFB background well (FT-D2 MW 2), in which no fluorotelomer sulfonates were detected, was used as blank groundwater for spike and recovery experiments. A single aliquot of blank groundwater was spiked with the 6:2 FtS standard to a final concentration of 5.0 µg/L and analyzed 5 times. Another aliquot of blank groundwater was spiked with authentic standards of PFBS, PFHxS, and PFOS to final concentrations each of 3.5 µg/L, and then analyzed five times.

Standard addition experiments also were performed on two different well samples from each site (Wurtsmith AFB well FT-13, Wurtsmith AFB well FT-2, Tyndall AFB well TY22FTA, and Tyndall AFB well PW-10). The levels (above the linear range) of fluorotelomer sulfonates observed in Wurtsmith AFB well FT-2 (total concentration = 88 μg/L), Tyndall AFB well TY22FTA (total concentration = 1,100 μg/L), and Tyndall AFB well PW-10 (total concentration = 14,600 μg/L) were all beyond the upper limit of the linear range of calibration. Consequently, the groundwaters from these wells were diluted by factors of 10, 180, and 2250, respectively. The diluted groundwater samples were spiked to final concentration of 17.7 μg/L, 12.2 μg/L, and 13.0 μg/L, respectively. The undiluted Wurtsmith AFB well FT-13 was spiked to contain a final concentration of 31.7 μg/L. One standard addition experiment was performed with perfluoroalkyl sulfonates on a sample from Tyndall AFB well TY22FTA; after diluting this sample by a factor of 100, PFBS, PFHxS, and PFOS standards were spiked to achieve final concentrations of 3.6 μg/L, 4.6 μg/L, and 5.0 μg/L, respectively.

The method detection limit was determined as outlined by Grant *et al.* (24) where a blank groundwater sample was spiked to a concentration one to five times the estimated detection limit (approximately 1.00 μg/L); seven replicate aliquots of this sample were then analyzed. The method detection limit was calculated by multiplying the standard deviation of the replicate analyses by the one-sided *t*-value corresponding to six degrees of freedom and a 99% confidence level. The quantitation limit was defined as the

concentration needed to produce a signal-to-noise of 10:1 within the groundwater matrix.

Liquid Chromatography/Mass Spectrometry. All separations were performed on a Waters 2690 liquid chromatograph (LC) (Milford, MA) equipped with a reverse-phase Betasil C-18 150 mm x 2 mm column (Thermo Hypersil-Keystone, Bellefonte, PA) that was heated to 35°C. The gradient consisted of increasing methanol from 30-90% over five minutes followed by a five-minute hold at 90% methanol and 5-minutes of equilibration at 30% methanol. All accessible polytetrafluoroethylene (PTFE) lines were replaced with polyetheretherketone (PEEK) tubing (Upchurch Scientific, Oak Harbor, WA). The LC was directly interfaced to the electrospray ionization (ESI) source of a Micromass Quattro Micro triple quadrupole mass spectrometer (Beverly, MA). The triple quadrupole was operated in the negative ESI mode, and multiple reaction monitoring was used for quantitation. The capillary voltage was 3.05 kV and the cone potential was set at a value between 20 and 65 V depending on the compound of interest. The temperatures of the source block and desolvation capillary were 125°C and 250°C, respectively. The flow rates of the nebulizer and desolvation gases were 80 and 575 L/hr, respectively. Argon was used as the collision gas, and the collision energy was set at a value between 15 eV and 40 eV depending on the compound being analyzed.

To obtain ions suitable for quantitation, standards and Zonyl® TBS were first infused with a syringe pump into the Micromass Quattro Micro triple

quadrupole mass spectrometer (Beverly, MA) at a flow rate of 30 µL/min, and the mass spectrometer was tuned to optimum. Quantitation of the fluorotelomer sulfonates was based on the loss of HSO_3^- from the fluorotelomer sulfonate precursor ($[M]^- \rightarrow [M-81] + HSO_3^-$), which is characterized by the appearance of a signal in the precursor's product ion spectrum at *m/z* 81 (HSO_3^-). The transition monitored for the internal standard, hexafluoroglutaric acid, was *m/z* 239 → *m/z* 131, which corresponds to the loss of $C_3F_5^-$ from the precursor $[M-1]^-$. Hexafluoroglutaric acid was chosen as the internal standard for the quantification of the fluorotelomer sulfonates because to the best of the authors' knowledge it has no industrial uses, and it was not observed in the NAS Fallon, Tyndall AFB, or Wurtsmith AFB groundwater samples. Quantitation was based on the ratio of the peak area of the analyte to that of the internal standard. Six point calibration curves (not including the origin) were constructed for 6:2 FtS between the limits 0.60 µg/L and 30.9 µg/L. All calibration curves were plotted using linear regression, weighted 1/X, and $r^2 > 0.99$. Authentic standards of 4:2 and 8:2 FtS were commercially unavailable. Therefore, for the quantification of these compounds, response factors were assumed equal to that of an equimolar amount of 6:2 FtS.

The transitions monitored for the perfluoroalkyl sulfonates were based on the loss of the sulfonate ion ($[M]^- \rightarrow [M-80] + SO_3^-$), which is characterized by the appearance of a signal at *m/z* 80 (SO_3^-) in the product ion spectrum. Calibration curves of PFBS, PFHxS, and PFOS were produced between the

limits 0.58 µg/L and 31.3 µg/L, 0.47 µg/L and 31.8 µg/L, and 0.62 µg/L and 31.5 µg/L, respectively. Because authentic standards of perfluoropentane sulfonate (PFPS) and perfluoroheptane sulfonate (PFHpS) were not commercially available, their response factors were assumed equal to that of an equimolar amount of PFHxS.

The greatest reduction in background was achieved by replacing the accessible PTFE tubing in the Waters LC system with PEEK lines. Despite this extreme measure, persistently high backgrounds were still observed occasionally, especially after the analysis of highly concentrated samples. In such cases, the entire LC, including the column, was rinsed with 10% formic acid in IPA. This procedure would be performed as necessary overnight or for a few hours depending on the strength of observed background. After a formic/IPA rinse, the column was re-equilibrated, a procedure that takes up to a couple of hours.

Fast Atom Bombardment/Mass Spectrometry. Fast atom bombardment mass spectrometry (FAB MS) experiments were performed on a Kratos MS-50TC (Manchester, England, United Kingdom) double focusing instrument. The analyte of interest was mixed on the probe tip with a 3-nitrobenzyl alcohol (98%, Sigma-Aldrich) matrix. Xenon gas was used to generate the primary ionizing beam from an Ion-Tech gun operated at 7-8 kV. For high mass accuracy measurements, a mixture of polyethylene glycols (PEGs) was used as the reference compound; the mixture consisted of three PEGs with masses 600, 800, and 1000 Da in the ratio 4:2:1, respectively.

Results and Discussion

Liquid Chromatography/Mass Spectrometry. The negative ESI mass spectrum (Figure 3.2) of the 6:2 FtS (*m/z* 427) exhibits peaks corresponding to the neutral losses of HF (*m/z* 407) and 2 HF (*m/z* 387), loss of SO_3^- (*m/z* 80), and the loss of HSO_3^- (*m/z* 81), which produces the most abundant peak. No evidence for spectral or background interferences in the *m/z* 427 → *m/z* 81 transition was found; therefore, this highly favored reaction was used for quantitation of the 6:2 FtS. For the quantitation of the perfluoroalkyl sulfonates, the $[M]^-$ → *m/z* 80 transition was monitored, where M equals the mass of the perfluoroalkyl sulfonate Although other investigators have reported interferences for the *m/z* 499 (PFOS) → *m/z* 80 transition in biological matrices (10), this interference was not observed for the AFFF-contaminated groundwater analyzed in the present study. The split chromatographic peaks shown in Figure 3.3 indicate the presence of both branched and linear isomers of the electrochemically-fluorinated PFOS and PFHxS. Quantitation of PFOS and PFHxS were based on the integration of both peaks. Chromatograms of the 6:2-10:2 FtS in Zonyl® TBS and of authentic standards of PFBS, PFHxS, and PFOS indicated the following overall elution order: PFBS < 4:2 FtS (not shown) < PFHxS < 6:2 FtS < PFOS < 8:2 FtS < 10:2 FtS (Figure 3.3).

The Zonyl® TBS was analyzed to establish that homologous fluorotelomer sulfonates could be detected without interference. This test was

necessary because only a single authentic standard of a fluorotelomer sulfonate, 6:2 FtS, could be obtained. Higher chained fluorotelomer sulfonates (12:2-16:2) were detected in the Zonyl® TBS, but are not shown. The Zonyl® TBS mixture contained less than 1% 4:2 FtS. Due to complications in carryover when analyzing the Zonyl® TBS, the mixture was not used for calibration.

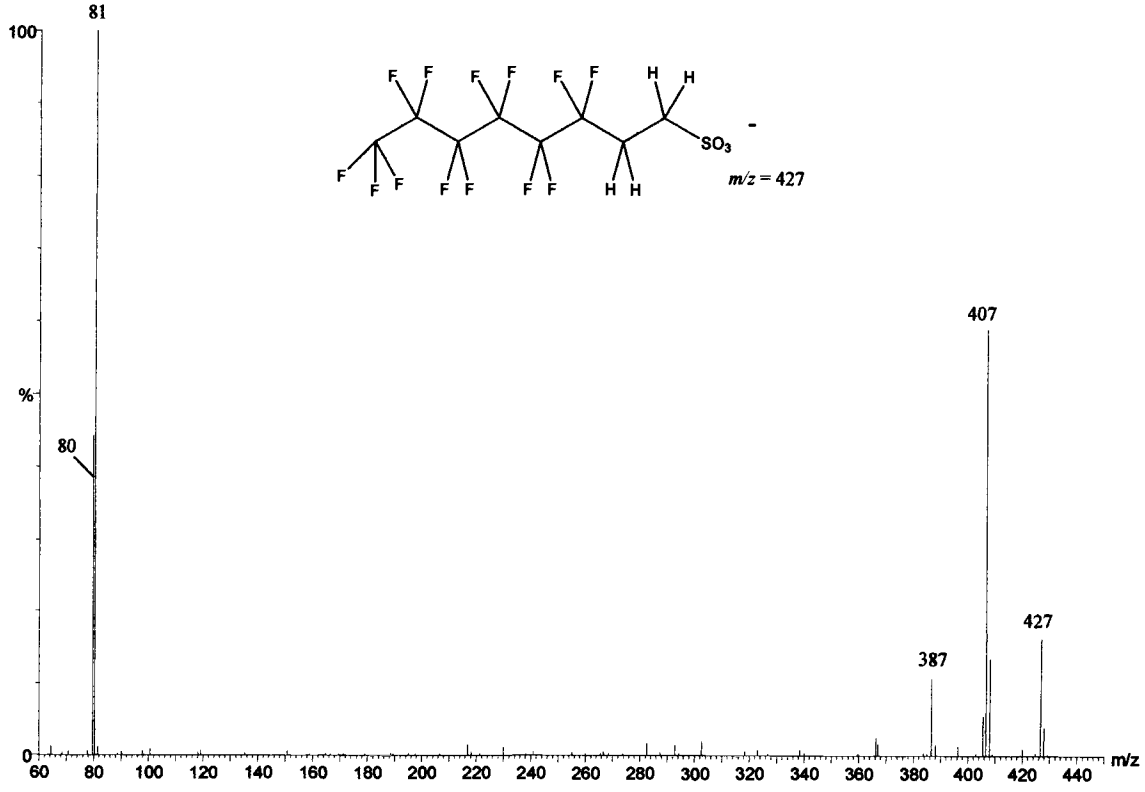

Figure 3.2. Negative ESI MS/MS mass spectrum of 6:2 FtS.

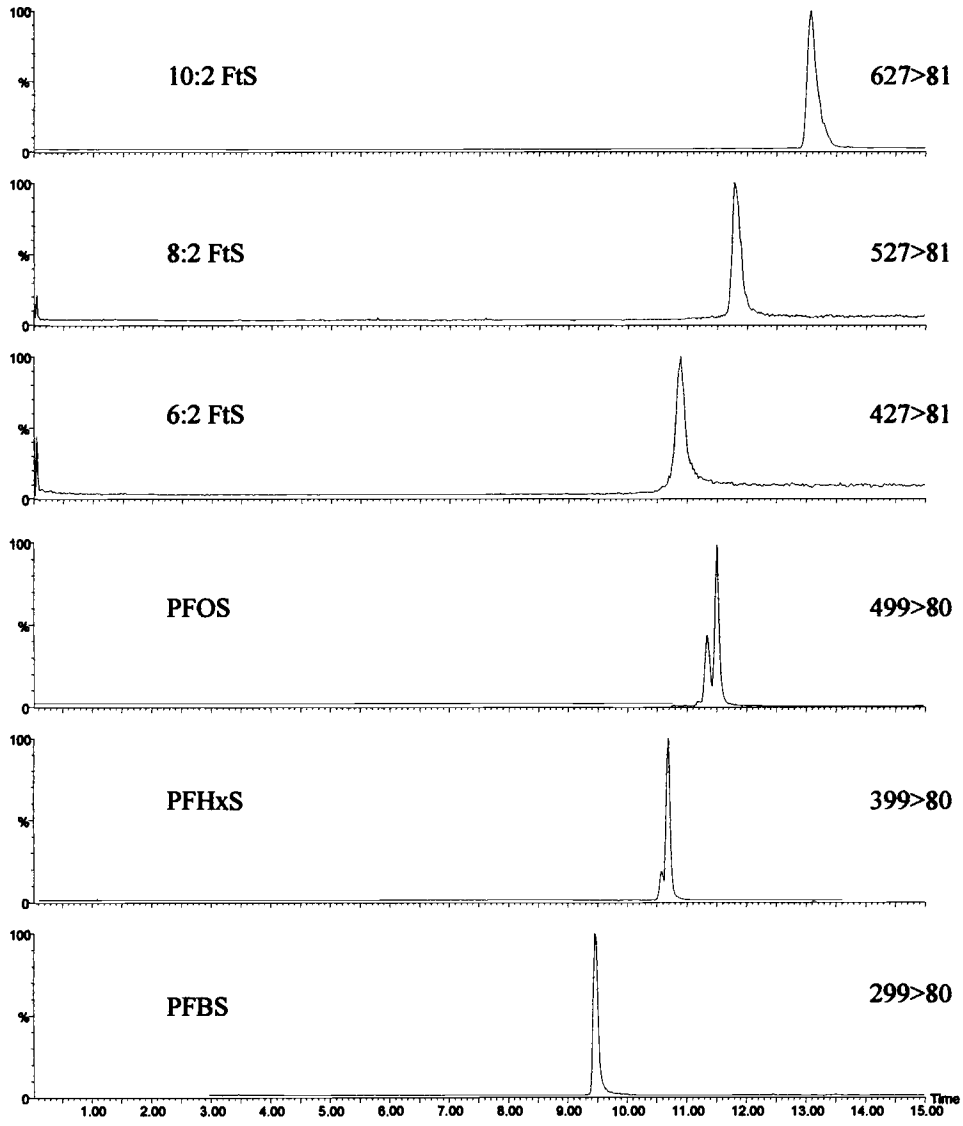

Figure 3.3. LC MS/MS chromatograms of 6:2 FtS, 8:2 FtS, and 10:2 FtS (in Zonyl® TBS) and authentic standards of PFBS, PFHxS, and PFOS. The product ion transition used for quantitation in each case is indicated in the upper right-hand corner of each chromatogram.

Accuracy, Precision, and Detection Limits for LC MS/MS. The average percent-recovery of the 6:2 FtS spiked into background well groundwater was 104, and the precision associated with this recovery, as indicated by the relative standard error (RSE), was 3% (Table 3.1). No fluorotelomer sulfonates were detected at NAS Fallon, and no blank groundwater was available from Tyndall AFB. Thus, no spike and recovery experiments were performed for the fluorotelomer sulfonates on samples from these two bases.

To validate the LC MS/MS method for quantification of perfluoroalkyl sulfonates, spike recoveries were performed with groundwater drawn from NAS Fallon well MW 50U, which was previously found not to contain perfluoroalkyl sulfonates. The average percent recoveries (and RSEs) for PFBS, PFHxS, and PFOS were 111 (±1%), 117 (± 2%) and 89.2 (±3%) (Table 3.1), respectively.

Standard addition experiments were performed with groundwater samples out of two wells from Wurtsmith AFB and two wells from Tyndall AFB, from all of which fluorotelomer sulfonates were detected. No standard additions were performed with samples from NAS Fallon since no fluorotelomer sulfonates were detected in them. The percent recoveries ranged from 96 - 101 at Tyndall AFB samples and 95 – 108 in the Wurtsmith AFB samples (Table 3.1). The percents of PFBS, PFHxS, and PFOS recovered from aliquots of groundwater drawn out of Tyndall AFB well TY22FTA ranged from 82 (± 2%) to 120 (± 3%) (Table 3.1).

Table 3.1. Recoveries of 6:2 FtS, PFBS, PFHxS, and PFOS spiked into groundwater samples from Wurtsmith Air Force Base and Tyndall Air Force Base[a].

Compound	Sample well	*n*	Initial added concentration µg/L	Final measured concentration µg/L	% Recovery
6:2 FtS	WAFB FT-D2 MW 2 (background well)	5	Nd	5.0	104±(3%)
6:2 FtS	WAFB FT-13	2	16	31.7	108±(5%)
6:2 FtS	WAFB FT-2	2	8.8[b]	17.7	95±(4%)
6:2 FtS	TAFB TY22FTA	2	6.0[c]	12.2	96±(2%)
6:2 FtS	TAFB PW-10	2	6.5[d]	13.0	101±(8%)
PFBS	NASF MW 50U	5	Nd	3.5	111±(1%)
PFBS	TAFB TY22FTA	4	0.10[e]	3.6	82±(2%)
PFHxS	NASF MW 50U	5	≤LOQ	3.5	117±(2%)
PFHxS	TAFB TY22FTA	5	1.1[e]	4.6	120±(3%)
PFOS	NASF MW 50U	5	≤LOQ	3.5	89±(3%)
PFOS	TAFB TY22FTA	5	1.5[e]	5.0	93±(2%)

[a]%RSEs are given in the parentheses.
[b]Diluted by a factor of 10.
[c]Diluted by a factor of 180.
[d]Diluted by a factor of 2250.
[e]Diluted by a factor of 100.
nd = not detected; ≤LOQ= less than or equal to the quantitation limit

Table 3.2. Concentrations of fluorotelomer sulfonates, perfluoroalkyl sulfonates, and perfluoroalkyl carboxylates in groundwater samples from NAS Fallon (NASF), Tyndall AFB (TAFB), and Wurtsmith AFB (WAFB) (µg/L)[a].

Sample	4:2 FtS	6:2 FtS	8:2 FtS	PFBS	PFPS	PFHxS	PFHpS	PFOS	PFHxA	PFHpA	PFOA
NASF MW 51U	nd	nd	nd	210	216	876	nd	380	372±(1%)[b]	149±(2%)[b]	6570±(1%)[b]
NASF MW 16	nd	nd	nd	54	38	115	nd	≤LOQ	57±(6%)[b]	18±(5%)[b]	460±(2%)[b]
NASF MW 50U	nd	nd	nd	nd	nd	≤LOQ	nd	≤LOQ	nd[b]	nd[b]	nd[b]
NASF MW 17	nd	nd	nd	≤LOQ	≤LOQ	≤LOQ	nd	≤LOQ	nd[b]	nd[b]	nd[b]
TAFB PW-10	7.3	14,600	3.3	144	134	920	nd	2300	144[b]	38[b]	116[b]
TAFB PW-07	5.7	7100	0.70	82	73	540	nd	270	73[b]	22[b]	64[b]
TAFB T11-2	4.2	4630±(8%)	≤LOQ	58	70	360	nd	210	64±(3%)[b]	19±(2%)[b]	42±(2%)[b]
TAFB TY22FTA	1.1	1080	17	10	8.3	107	nd	147	nd[b]	nd[b]	nd[b]
WAFB FT 1	nd	2.9	1.5	na[c]	na[c]	36[c]	na[c]	8[c]	5[c]	na[c]	5[c]
WAFB FT 2	nd	88±(7%)	≤LOQ	na[c]	na[c]	120[c]	na[c]	14[c]	5[c]	na[c]	98[c]
WAFB FT 3	nd	95	0.78	na[c]	na[c]	104[c]	na[c]	110[c]	5[c]	na[c]	105[c]
WAFB FT 4	nd	2.0	nd	na[c]	na[c]	5[c]	na[c]	9[c]	nd[c]	na[c]	nd[c]
WAFB FT 6	nd	nd	nd	na[c]	na[c]	5[c]	na[c]	9[c]	nd[c]	na[c]	nd[c]
WAFB FT 5	nd	42	nd	na[c]	na[c]	18[c]	na[c]	16[c]	nd[c]	na[c]	3[c]
WAFB FT 10	nd	≤LOQ	≤LOQ	na[c]	na[c]	9[c]	na[c]	7[c]	nd[c]	na[c]	nd[c]
WAFB FT 7	nd	≤LOQ	nd	na[c]	na[c]	na[c]	na[c]	na[c]	nd[c]	na[c]	nd[c]
WAFB FT 8	nd	53	2.7	na[c]	na[c]	30[c]	na[c]	8[c]	nd[c]	na[c]	20[c]
WAFB FT 9	nd	66	3.7	na[c]	na[c]	46[c]	na[c]	40[c]	nd[c]	na[c]	19[c]
WAFB FT 11	nd	7.2	≤LOQ	na[c]	na[c]	na[c]	na[c]	na[c]	nd[c]	na[c]	nd[c]
WAFB FT 12	1.24	27	1.3	na[c]	na[c]	23[c]	na[c]	6[c]	8[c]	na[c]	15[c]
WAFB FT 13	nd	16±(3%)	6.5	na[c]	na[c]	26[c]	na[c]	30[c]	nd[c]	na[c]	24[c]
WAFB FT 14	nd	173	8.7	na[c]	na[c]	27[c]	na[c]	16[c]	nd[c]	na[c]	8[c]
WAFB FT 18	nd	139	1.6	na[c]	na[c]	33[c]	na[c]	20[c]	nd[c]	na[c]	10[c]
WAFB FT 17	nd	8.7	1.1	na[c]	na[c]	9[c]	na[c]	4[c]	nd[c]	na[c]	nd[c]
WAFB FT 15	nd	0.98	nd	na[c]	na[c]	na[c]	na[c]	na[c]	nd[c]	na[c]	nd[c]
WAFB FT 16	nd	0.90	nd	na[c]	na[c]	na[c]	na[c]	na[c]	nd[c]	na[c]	nd[c]

[a]The %RSEs is given in parentheses.
In data shown by *Moody et al.*[21], %RSDs were converted to %RSEs by dividing by $n^{1/2}$.

Table 3.2. (Continued)
[b]Determined by *Moody et al* (21).
[c]Determined by *Moody et al.*(22)
PFHpA = Perfluoroheptanoic acid
nd = not detected above the detection limit, na = sample well not analyzed

Table 3.3. Limits of detection (LOD) and limits of quantitation (LOQ) for fluorotelomer sulfonates, perfluoroalkyl sulfonates and perfluoroalkyl carboxylates[a].

		6:2 FtS	PFBS	PFHxS	PFOS	PFOA
NAS Fallon	LOD	0.33	0.32	0.15	0.36	18[b]
	LOQ	0.60	0.58	0.47	0.62	36[b]
Tyndall AFB	LOD	0.33	0.32	0.15	0.36	18[b]
	LOQ	0.60	0.58	0.47	0.62	36[b]
Wurtsmith AFB	LOD	0.33	nd[c]	nd[c]	3[d]	3[d]
	LOQ	0.60	nd[c]	nd[c]	5[d]	13[d]

[a]All concentrations are reported as µg/L.
[b]Determined by *Moody et a.l*(21)
[c]Quantification of PFBS and PFHxS were based on the assumption that response factors were equal to an equimolar amount of PFOS.
[d]Determined by *Moody et al.*(22)

The precision of the direct injection LC MS/MS method was estimated by calculating the relative standard error of five replicate analyses of unspiked groundwater samples collected from Wurtsmith AFB and Tyndall AFB. Since a wide range of fluorotelomer sulfonate concentrations were observed at Wurtsmith AFB, estimates of analytical precision were obtained from a set of five replicate analyses performed on groundwater from well FT-2 and a similar set from well FT-13. The RSEs calculated for unspiked groundwater from the FT-2 and FT-13 wells were 7% and 3%, respectively (Table 3.2). The sample taken from Tyndall AFB well T11-2 was also analyzed five times; the RSE from this set of measurements was estimated to be 8% (Table 3.2).

The method detection limit, defined as the minimum concentration of an analyte that can be reported with 99% confidence to be greater than zero, was 0.33 µg/L for the 6:2 FtS (Table 3.3). The quantitation limit, defined as the concentration required to produce a signal-to-noise of 10:1, was 0.60 µg/L for the 6:2 FtS (Table 3). For LC MS/MS quantitation of perfluoroalkyl sulfonates taken from NAS Fallon and Tyndall AFB, the method detection limits and quantitation limits were 0.32 µg/L and 0.58 µg/L for PFBS, 0.15 µg/L and 0.47 µg/L for PFHxS, and 0.36 µg/L and 0.62 µg/L for PFOS (Table 3.3). The method detection limits and quantitation limits estimated in a previous study by direct injection mass spectrometry (no LC) for the perfluoroalkyl sulfonates at Wurtsmith AFB were 3 and 5 µg/L, respectively (Table 3.3) (22); these values are an order of magnitude higher than those estimated in the present study from the NAS Fallon and Tyndall AFB samples (Table 3.3). The perfluorinated

carboxylate method detection limits and quantitation limits were determined by GC/MS in previous studies to be 18 µg/L and 36 µg/L (21), respectively, for samples collected from sites at NAS Fallon and Tyndall AFB and 3 µg/L and 13 µg/L (22), respectively, for samples from Wurtsmith AFB (Table 3.3).

Application to Groundwater Samples. The LC MS/MS method was applied to groundwater samples from NAS Fallon, Tyndall AFB, and Wurtmsith AFB to quantitatively determine the levels of 4:2 – 12:2 fluorotelomer sulfonates. The 4:2 FtS, 6:2 FtS, and 8:2 FtS were all present in varying concentrations at Wurtsmith AFB and Tyndall AFB. No fluorotelomer sulfonates were detected (detection limit 0.33 µg/L) at NAS Fallon. Odd-numbered (e.g. 5:2 or 7:2 fluorotelomer sulfonates) were not detected, this being consistent with the fluorotelomerization process for which only even-numbered homologues are produced (8,9).

Naval Air Station Fallon, Nevada, USA. At this site, the total µM-distribution of fluorosurfactants was 0% fluorotelomer sulfonates, 25% perfluoroalkyl sulfonates, and 75% perlfuoroalkyl carboxylates (Table 3.4, Figure 3.4a). No fluorotelomer sulfonates were observed above the detection limit at NAS Fallon. Although, NAS Fallon was in operation from the 1950's through 1988, it appears that no fluorotelomer-based AFFF was used at this site. However, relatively high concentrations of PFBS, PFPS, PFHxS, and PFOS were detected in the NAS Fallon wells MW 51U and MW 16; the total perfluorinated sulfonate concentrations in these two wells were 1,680 µg/L and 206 µg/L (Table 3.4), respectively. No perfluorinated sulfonates were

observed above the quantitation limit in wells MW 50U and MW 17. These findings for the perfluoroalkyl sulfonates mimic the levels of perfluorinated carboxylates at NAS Fallon obtained from a previous study (21) (Table 3.4).

Table 3.4. Total concentrations of fluorotelomer sulfonates (FtS), perfluoroalkyl sulfonates (PFS), and perfluoroalkyl carboxylates (PFC) in groundwater samples from NAS Fallon (NASF), Tyndall AFB (TAFB), and Wurtsmith AFB (WAFB) (µg/L).

Sample	Total [FtS]	Total [PFS]	Total [PFC]
NASF MW 51U	0	1680	7090[a]
NASF MW 16	0	206	535[a]
NASF MW 50U	0	0	0[a]
NASF MW MW 17	0	0	0[a]
TAFB PW-10	14,600	3500	298[a]
TAFB PW-07	7100	960	159[a]
TAFB T11-2	4600	700	125[a]
TAFB TY22FTA	1100	273	0[a]
WAFB FT 1	4.4	44[b]	10[b]
WAFB FT 2	88	134[b]	103[b]
WAFB FT 3	96	213[b]	110[b]
WAFB FT 4	2.1	14[b]	0[b]
WAFB FT 6	0	14[b]	0[b]
WAFB FT 5	42	34[b]	3[b]
WAFB FT 10	0	16[b]	0[b]
WAFB FT 7	0	na[b]	0[b]
WAFB FT 8	56	38[b]	20[b]
WAFB FT 9	70	86[b]	19[b]
WAFB FT 11	7.2	na[b]	0[b]
WAFB FT 12	29.7	29[b]	23[b]
WAFB FT 13	22.4	56[b]	24[b]
WAFB FT 14	182	43[b]	8[b]
WAFB FT 18	141	53[b]	10[b]
WAFB FT 17	9.8	13[b]	0[b]
WAFB FT 15	0.98	na[b]	0[b]
WAFB FT 16	0.90	na[b]	0[b]

[a]Determined by *Moody et al.*(21).
[b]Determined by *Moody et a.l*(22)
na = sample well not analyzed

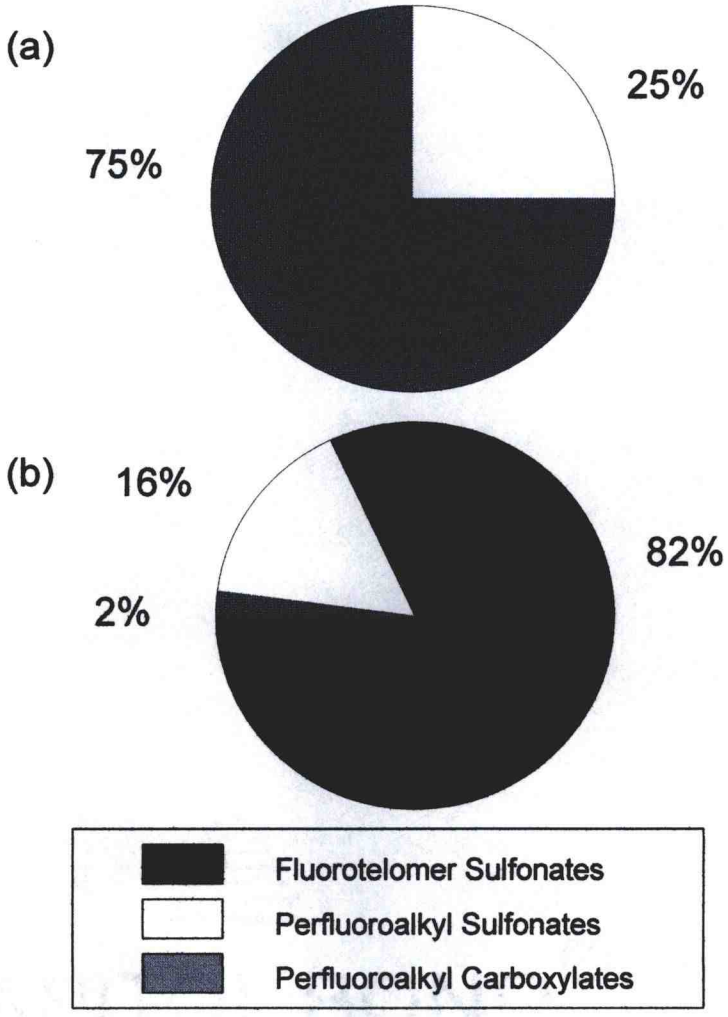

Figure 3.4. Distribution of fluorosurfactants on a µM basis (a) at NAS Fallon and (b) at Tyndall AFB.

Tyndall Air Force Base, Florida, USA. The total µM-distribution of fluorosurfactants quantified at Tyndall AFB is represented in a pie graph (Figure 3.4b). The fluorotelomer sulfonates were the most abundant (82%) fluorosurfactant observed at this site. The highest fluorotelomer sulfonate

concentrations measured in this study were in samples collected from Tyndall AFB; total FtS concentrations ranged from 1,100 µg/L to 14,600 µg/L (Table 3.4). The predominant fluorotelomer sulfonate was the 6:2 FtS, which accounted for 99% of fluorotelomer sulfonate present (Table 3.2). The second most abundant fluorotelomer sulfonate, 4:2 FtS, was present in all four wells, ranging in concentration from 1 to 8 µg/L (Table 3.2). The 8:2 FtS was quantified in three out of the four wells, ranging in concentration from 0.7 µg/L to 17 µg/L (Table 3.2). The highest total concentrations of fluorotelomers were observed for wells within 15 m of the fire-training pad, PW-10 (14,600 µg/L) and PW-7 (7,100 µg/L), and the lowest concentrations were detected 30 m down gradient from the pad, T11-2 (4,600 µg/L), and 40 m north of the pad, TY22FTA (1,100 µg/L)

The total perfluoroalkyl sulfonate concentrations, which ranged from 273 µg/L to 3,500 µg/L (Table 3.4), accounted for 16% of the total content of fluorosurfactants observed at Tyndall AFB (Figure 3.4b). The perfluoroalkyl carboxylates, whose total concentrations ranged from below detection to 298 µg/L (Table 3.4), accounted for only 2% of the total fluorosurfactants detected at this site (Figure 3.4b).

The presence of fluorotelomer sulfonates, perfluoroalkyl carboxylates, and perfluoroalkyl sulfonates in the groundwater collected from this site is consistent with the facts that military contracts for both fluorotelomer-based and electrochemical fluorinated-based AFFF formulations had been issued during the period, 1983 to 1988, and that these years fall in the middle of the

time (1980-92) during which Tyndall AFB had been in operation. The composition of fluorotelomer sulfonates observed at Tyndall suggests that the fluorotelomer products supplied to the U.S. military from 1983-1988 through Ansul Incorporated, contained predominantly 6:2 FtS as the feedstock or other fluorotelomer chemicals that degraded primarily to 6:2 FtS.

Wurtsmith Air Force Base, Michigan, USA. Fluorotelomer sulfonates, perfluoroalkyl sulfonates and perfuoroalkyl carboxylates were all observed at Wurtsmith AFB. The total concentrations ranged from below detection (0.33 µg/L) to 182 µg/L for fluorotelomer sulfonates, 13 µg/L to 213 µg/L for perfluoroalkyl sulfonates (not all wells were analyzed), and below detection (3 µg/L) to 105 µg/L for perfluoroalkyl carboxylates (Table 3.4).

The 6:2 FtS concentrations were the highest among the fluorotelomer sulfonates, ranging from 0.90 µg/L to 173 µg/L (Table 3.2). The second most abundant fluorotelomer sulfonates was the 8:2 FtS, ranging in concentration from 0.78 µg/L to 8.66 µg/L (Table 3.2); the 4:2 FtS was only found in the FT-12 well at a concentration of 1.24 µg/L. The composition of these three homologs at Wurtsmith contrasts with that observed at Tyndall AFB, where the 4:2 FtS was the second most abundant fluorotelomer after the 6:2 FtS. For purposes of comparison, total perfluoroalkyl sulfonate concentrations (PFOS and PFHxS) detected in Wurtsmith AFB groundwater ranged from 13 µg/L (FT-17) to 213 µg/L (FT-3) (22), while total perfluorocarboxylate concentrations, perfluorooctanoic acid (PFOA) and perfluorohexanoic acid (PFHxA), were from below the detection limit (3 µg/L) to 110 µg/L (FT-3) (21).

The spatial distribution of concentrations among the different classes of chemicals differed (Figure 3.5). High concentrations of 6:2 FtS were observed in wells close in proximity to the fire-training pad; wells FT-2 (17 m) and FT-3 (18 m) contained 6:2 FtS concentrations of 88 µg/L and 95 µg/L, respectively. However, the highest 6:2 FtS concentrations measured in the plume were in wells FT-14 (173 µg/L) and FT-18 (139 µg/L) located downgradient of the fire pad at 305 m and 518m, respectively (Figure 3.5). By contrast, the concentrations of perfluoroalkyl sulfonates and carboxylates were highest near the fire-training pad and lower in wells downgradient of the training pad (Figure 3.5).

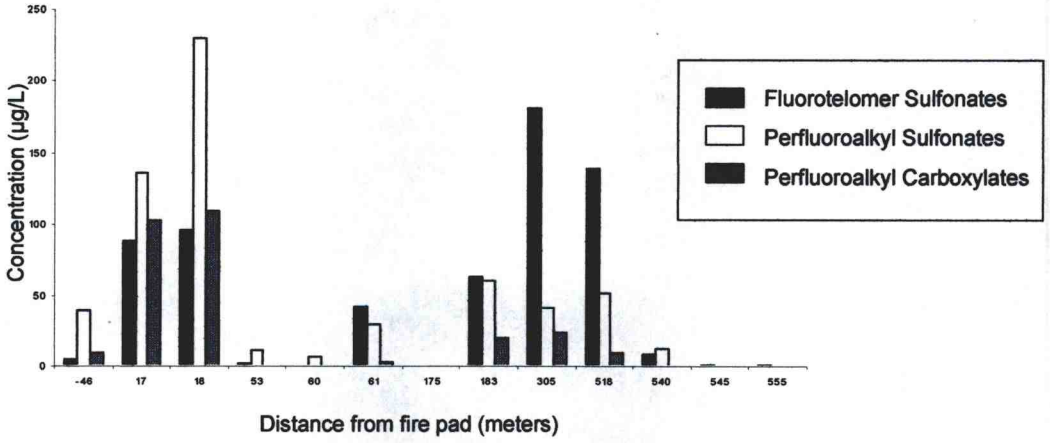

Figure 3.5. A distance versus concentration plot for fluorosurfactants at Wurtsmith AFB.

It is conceivable that the peak in fluorotelomer sulfonate concentration that occurred downstream in 1999 (Figure 3.5), the year the samples were collected from this site, might be the result, in part, of conservative chemical transport in the plume illustrated in Figure 3.1. Wurtsmith AFB's long period of operation (1952-93) spans the years (1983-88) during which AFFF formulations were based on chemicals produced by both electrochemical fluorination and fluorotelomerization. Thus, chemicals released between 1983-88, transported at a flow rate on the order of 0.1 m/day (25-27), could, by the year 1999, very likely have become distributed in the vicinity of wells FT-14 and FT-18 (approximately 300 m – 500 m) downstream, respectively. Simplistic reasoning of this sort cannot, however, account for the relatively high concentrations of fluorotelomer sulfonates concentrations near the fire-training pad nor the uneven distribution of the electrochemically-based perfluoroalkyl sulfonates and perfluoroalkyl carboxylates, which were used in AFFF throughout the entire period Wurtsmith AFB was in operation.

AFFF and Fluorotelomer Sulfonates. In an effort to gain knowledge of the composition of an AFFF mixture based on fluorotelomer chemicals, negative ion FAB MS as well as the LC MS/MS method described in this paper were used to analyze a single fluorotelomer-based AFFF. The identities of the fluorosurfactants used in fluorotelomer-based AFFF formulations are proprietary and, therefore, not listed in a material safety data sheet. The AFFF product was provided by Tyndall AFB and was presumably sold by Ansul Incorporated to the military on contract sometime during the years of 1983 to

1988. AFFF is transported and stored as a concentrate; prior to application, it is diluted to three or six% (by volume) with fresh water, salt water, or hard water. Thus, the AFFF from Tyndall AFB was far more concentrated than what would have been applied in the field. For this reason, it was diluted approximately 200,000-fold prior to LC MS/MS analysis. The 6:2 FtS was detected in the AFFF concentrate, but the 4:2 FtS and 8:2 FtS were not. A standard of the 6:2 FtS yielded a signal at *m/z* 427 by negative ion FAB MS (28); however, no fluorotelomer sulfonates were detected by FAB MS in the AFFF concentrate. Instead 3 higher molecular weight molecules were observed at masses, *m/z* 486, 586, and 686 (28). The mass difference of 100 corresponds to a $[CF_2CF_2]$ group, which is consistent with the chemicals produced by fluorotelomerization where the fluoroalkyl group is $F(CF_2CF_2)_nCH_2CH_2$-R. FAB MS measurements performed at high mass accuracy and ESI MS/MS analyses of the product ion fragmentation patterns of the three higher molecular weight precursors identified the latter as fluoroalkylthioamido sulfonates with a structural formula that is consistent with R_f-$SCH_2CH_2CONHC(CH_3)_2CH_2SO_3^-$, where $R_f = F(CF_2CF_2)_nCH_2CH_2^-$ and n = 2 (*m/z* 486), 3 (*m/z* 586), or 4 (*m/z* 686).

Given the presence of fluoroalkylthioamido sulfonates in the AFFF concentrate, those groundwater samples containing the highest concentrations of fluorotelomer sulfonates were reanalyzed semi-quantitatively for the fluoroalkylthioamido sulfonates by LC MS/MS (Figure 3.6). The transition $[M]^- \rightarrow [M\text{-}135] + C(CH_3)_2CHSO_3^-$ (*m/z* 135) was used for the semi-

quantitation. Analysis of the AFFF concentrate supplied to Tyndall AFB yielded an estimate 1,600 µg/L for the concentration of 6:2 FtS (Figure 3.6a). Lack of an authentic standard precluded rigorous quantitation of the fluoroalkylthioamido sulfonates; however, assuming response factors equal to those of equimolar amounts of 6:2 FtS, the concentrations of the 6:2 and 8:2 fluoroalkylthioamido sulfonates in the AFFF concentrate were estimated to be 12,000 µg/L and 6,000 µg/L, respectively (Figure 3.6a). Provided that the preceeding assumption is valid, the fluoroalkylthioamido sulfonates are much more abundant in the AFFF concentrate than the fluorotelomer sulfonates.

None of the fluoroalkylthioamido sulfonates were observed in any of the groundwater samples, including the Tyndall AFB well PW-10 that contained such exceptionally high fluorotelomer sulfonates concentrations (Figure 3.6b). This raises interesting questions about the fate of the fluoroalkylthioamido sulfonates in groundwater. Based on the concentration of 6:2 FtS in the AFFF concentrate at hand (1,600 µg/L), the concentration of 6:2 FtS in an AFFF formulation (i.e. after dilution of the concentrate to 3% by volume) used in fire-training activities would only be ~50 µg/L. Since the concentrations of 6:2 FtS in the four wells at Tyndall AFB all exceeded 1,000 µg/L, it is difficult to imagine how they arose strictly in terms of a direct application of AFFF containing something on the order of 50 µg/L of 6:2 FtS. Therefore, it seems plausible that degradation of the fluoroalkylthioamido sulfonates (and cationic and nonionic fluorosurfactants also present in the AFFF products) into fluorotelomer sulfonates could have occurred.

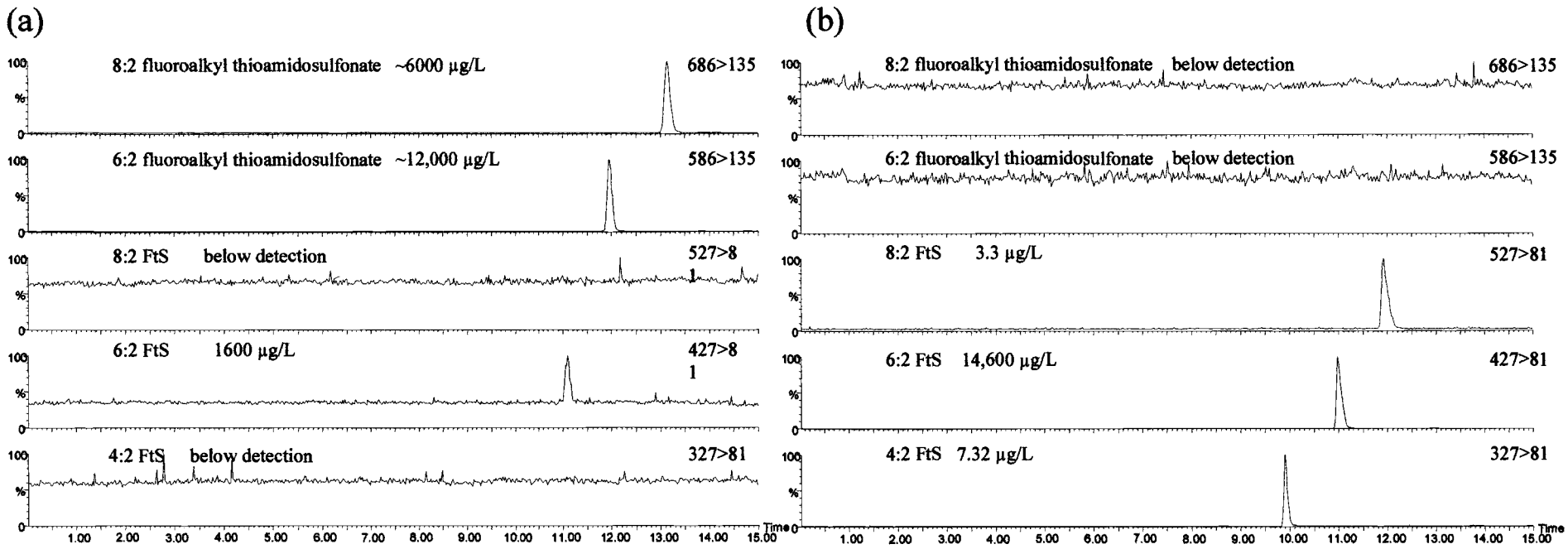

Figure 3.6. A comparative LC MS/MS analysis of (a) fluorotelomer-based AFFF and (b) Tyndall AFB well PW-10 with fluoroalkylthioamido sulfonates and fluorotelomer sulfonates indicated.

The present study emphasizes how little is known concerning the fate and transport of fluorotelomer sulfonates in groundwater. Previous studies have shown that the 6:2 FtS is susceptible to biodegradation under sulfur-limiting and aerobic conditions (29); however, it is certainly not known if these transformations could have occurred under the conditions that existed at Tyndall AFB or Wurtsmith AFB. Studies are needed to better understand the transport and biodegradation of fluorotelomer sulfonates, fluoroalkylthioamido sulfonates, other AFFF fluorochemicals (e.g. cationic and nonionic), and their precursors under various aerobic and anaerobic conditions.

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4. QUANTITATIVE DETERMINATION OF FLUORINATED ALKYL SUBSTANCES IN MUNICIPAL WASTEWATER BY HIGH-VOLUME-INJECTION LC ESI-MS/MS

Melissa M. Schultz, Douglas F. Barofsky, and Jennifer A. Field

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Abstract

Wastewater effluent is a potential environmental point source of fluorinated alkyl substances. A high-volume-injection liquid chromatography with electrospray ionization tandem mass spectrometry (LC ESI-MS/MS) quantitative method was developed for the determination of trace levels of fluorinated alkyl substances in municipal wastewater influents and effluents. Recoveries from standard addition experiments ranged from 77.0% - 95.1% (± 2.4%) and 85.3% - 95.5% (± 2.3%) in the raw influent and final effluent, respectively. The limit of quantitation for the fluorinated alkyl substances is analyte dependent and ranged from 0.14 ng/L for 1H, 1H, 2H, 2H-perfluorooctane sulfonate to 3.0 ng/L for *N*-methyl perfluorooctanesulfonamidoacetate. The method was applied to 24 h composites of raw influent and final effluent samples collected from 10 wastewater treatment plants (WWTPs) nationwide. Fluorinated alkyl substances were observed in wastewater at all treatment plants sampled and each plant exhibited a unique fingerprint of fluorinated alkyl substances, despite similar treatment processes. In 9 out of the 10 plants sampled, at least one class of fluorinated alkyl substances exhibited increased concentrations in the effluent as compared to the influent concentrations. Detection of these analytes in final effluents at the ng/L level indicates that treated wastewater is a point source of fluorinated alkyl substances.

Introduction

Liquid chromatography (LC) tandem mass spectrometry (MS/MS) applications have boomed as a result of the invention of electrospray ionization (ESI) in the mid-1980s, an ionization technique for polar molecules that couples an LC to a MS (1). ESI disperses liquid into small droplets by the application of an electric field (2). One application of the "new" LC ESI-MS/MS technology in the early 1990s was the unambiguous determination of fluorinated alkyl substances in blood drawn from occupationally-exposed workers. Previous analytical methods were able to detect organic fluorine (3), however the methods were nonspecific, thus unequivocal identification was difficult. From the developed LC ESI-MS/MS methodology, data indicated that fluorinated alkyl substances, primarily perfluorooctane sulfonate (PFOS), were observed in every blood sample collected from blood banks in the United States, Europe, and Asia (4). Continued research showed PFOS to be a persistent, bioaccumulative, and toxic chemical (4,5). Global sampling revealed selected fluorinated alkyl substances in animal tissues from not only densely populated regions, but also in remotely populated regions where no commercial, municipal or industrial sources exist (6). Thus, fluorochemicals have ignited widespread interest due to their ubiquitous, worldwide presence in the environment and analytical methods have been developed for their quantitative determination in air (7-9), surface waters (10-18), groundwater (19-21), biota (22-27), and human serum (28-35), including

nonoccupationally-exposed humans (36). At present time, few methods exist for the determination of fluorinated alkyl substances in wastewater matrices.

Wastewater effluent, one of the principal routes for introducing environmental contaminants into aquatic environments, may be contributing to the levels of fluorinated alkyl substances in the environment. Few studies have examined fluorinated alkyl substances fate in wastewater treatment plants. In a multi-city study (6 cities) conducted by the 3M Company, PFOS and PFOA were observed in all sampled wastewater treatment plant (WWTP) effluents at low to sub parts-per-billion concentrations (37). Similar concentration levels of PFOA and PFDA were also observed by Alzaga and Bayona in two urban WWTP effluents (38).

The objective of this study was to develop a quantitative analytical method for the determination of fluorinated alkyl substances in aqueous municipal wastewater matrices. The fluorinated alkyl substances studied include the perfluoroalkyl sulfonates, fluorotelomer sulfonates, perfluoroalkyl carboxylates, as well as selected fluorinated alkyl sulfonamides (Table 1). The validated methodology was applied to wastewater raw influents and final effluents collected from ten wastewater treatment plants located nationwide. Comparison of 24 h composite influent and effluent concentrations provide a snapshot of behavior of fluorinated alkyl substances during wastewater treatment.

Table 4.1. Fluorinated Alkyl Substance Analytes

Analyte	Acronym	Precursor Ion (*m/z*)	Product Ion(s) (*m/z*)	LOQ[a]
Perfluorobutane sulfonate	PFBS	299	80/99	0.70
Perfluorohexane sulfonate	PFHxS	399	80/99	0.82
Perfluorooctane sulfonate	PFOS	499	80/99	0.40
Perfluorodecane sulfonate	PFDS	599	80/99	0.47
1H, 1H, 2H, 2H-perfluorooctane sulfonate	6:2 FtS	427	81/407	0.14
Perfluorohexanoate	PFHxA	313	269/119	0.28
Perfluoroheptanoate	PFHpA	363	319/169	0.48
Perfluorooctanoate	PFOA	413	369/169	0.33
Perfluorononoate	PFNA	463	419/219	0.35
Perfluorodecanoate	PFDA	513	469/219	0.32
Perfluorooctanesulfonamide	FOSA	498	78/169	0.34
N- ethyl perfluorooctanesulfonylamido acetate[b]	*N*-EtFOSAA	584	526/483	2.78
Perfluorooctanesulfonamido acetate[b]	FOSAA	556	498/419	2.01
N-methyl perfluorooctanesulfonamido acetate[b]	*N*-MeFOSAA	570	512/483	3.0
Perfluoro(2-ethoxyethane)sulfonic acid	PFEES (internal std)	315	135	
[1,2-$^{13}C_2$]perfluorooctanoate	[$^{13}C_2$]PFOA (recovery & internal std)	415	370	

[a]All concentrations are reported as ng/L
[b]Analytes were scanned for in samples, but were not detected.

Experimental Section

Standards and Reagents. A standard of 1H, 1H, 2H, 2H, perfluorooctane sulfonate (6:2 FtS, 98%) was purchased from Apollo Scientific

Limited (Derbyshire, UK). Potassium perfluorobutane sulfonate (PFBS, 99%), potassium perfluorohexane sulfonate (PFHxS, 99%), potassium perfluorooctane sulfonate (PFOS, 98%), perfluorooctanesulfonamide (FOSA, 99%), *N*- ethyl perfluorooctanesulfonylamidoacetate (*N*-EtFOSAA, 53.82%), perfluorooctanesulfonamidoacetate (FOSAA, 99.6%), and *N*-methyl perfluorooctanesulfonamidoacetate (MeFOSAA, 100%) standards were all donated by the 3M Company (St. Paul, MN). A standard of perfluorodecane sulfonate (ammonium form in water/butoxyethanol) (PFDS, 25% wt.), perfluoroheptanoic acid (PFHpA, 99%), perfluorooctanoic acid (PFOA, 96%), perfluorononanoic acid (PFNA, 97%), and perfluorodecanoic acid (PFDA, 98%) were acquired from Aldrich Chemical (Milwaukee, WI). Perfluorohexanoic acid (PFHxA, 99%) and the internal standard, perfluoro(2-ethoxyethane)sulfonic acid (PFEES, 97%), were obtained from Oakwood Research Chemicals (West Columbia, SC). The recovery standard, [1,2-$^{13}C_2$]perfluorooctanoic acid ([$^{13}C_2$]PFOA, 97.5%) was acquired from PerkinElmer (Wellesley, MA).

Solvents for LC separations included Milli-Q water (Bedford, MA) that contained 2 mM ammonium acetate (98%) (Aldrich Chemical, Milwaukee, WI) and optima grade methanol (Fisher Scientific, Pittsburgh, PA). The LC column rinse solvent mixture consisted of 10% (v/v) formic acid (97%) (Sigma-Aldrich, St. Louis, MO) in optima grade isopropanol (Fisher Scientific, Pittsburgh, PA).

Field Sites and Sample Collection. 24-h composite samples of raw influents and final effluents were collected nationwide from 10 municipal

wastewater treatments in the spring of 2004. Characteristic details of each wastewater treatment plant are located in Table 4.2. Samples were collected and shipped on ice overnight to the laboratory in 125 mL high density polyethylene bottles that were cleaned according to EPA wash procedures (EaglePicher, Joplin, MO). Formalin was not used to inhibit biological activity because it was found to suppress the response of fluorinated alkyl substances in wastewater matrices (unpublished data). Samples that were run within 48 hrs after arrival to the laboratory were refrigerated at 4°C until analysis. Wastewater samples that were not analyzed within 48 hrs were kept frozen at -20° C and then thawed prior to analysis. Subsequent analyses run over 6 months showed no signs of loss or enhancement of the analyte's concentration.

Table 4.2. Wastewater Treatment Plant Characteristics[a]

WWTP ID	U.S. region	Treatment type	Sample dates	Plant flow[b]	Population	% Breakdown of waste treated
WWTP 1	Pacific Northwest	P + TF + AS	April 2004	12.02	50,000	90% domestic, 10% light industry
WWTP 2	Pacific Northwest	P + AS	May 2004	56	600, 000	93% domestic, 7% industrial
WWTP 3	Pacific Northwest	P + TF + AS	May 2004	28	130,000	90% domestic/commercial, 10% industrial
WWTP 4	Southeast	AS	June 2004	14	240,000	97% domestic, 3% industrial
WWTP 5	West	P + TF + AS	June 2004	17	202,000	60% domestic, 10% industrial, 30% business
WWTP 6	West South Central	P + AS	June 2004	7	65,000	99% domestic, 1% light industry
WWTP 7	West North Central	AS + MMF	June 2004	11	110,000	50% papermill effluent, 50% domestic/commercial
WWTP 8	West	P + AS + MMF	June 2004	26	220,000	99% domestic 1% industry
WWTP 9	West North Central	P + TF + AS	June 2004	63	415,000	85% domestic, 10% light industry, 5% heavy industry
WWTP 10	Northeast	P + AS	June 2004	3	17,000	80% domestic, 10% leachate, 10% industrial

[a]P = primary gravitational settling, TF = trickling filter, AS = activated sludge, MMF = mixed media filters
[b] Plant flow reported in million gallons per day (MGD)

Accuracy and Precision. A set of QC samples were also included for each sampling site. Each cooler contained a field blank, a capped bottle filled with only Milli-Q water. The field blank stayed in the cooler that was used for sample storage and shipping; thus, it received the same exposure as the collected wastewater samples. Spike control samples and matrix spike samples were included with the QC protocol. They consisted of preweighed bottles, marked with a line indicating 100 mL, which were spiked with a fixed amount of fluorinated alkyl substances. For WWTPs 1 and 2, each fluorinated alkyl substance, with the exception of FOSA, was spiked to a final concentration of 49.5 ng/L. FOSA was spiked to a final concentration of 99 ng/L. After the initial analysis of these wastewater samples from these two plants, it was discovered that the overall analyte concentrations were significantly lower than the initial spiked amounts. Thus, for WWTPs 3-10, each fluorinated alkyl substance was spiked to a final concentration of 23 ng/L. The recovery standard or surrogate used with the field spikes was [$^{13}C_2$]PFOA, and it was spiked to the same final concentrations as the other fluorinated alkyl substances. The spike control samples were filled to the premarked line with Milli-Q water and were shipped in the sample cooler that was used for storage and shipping. The two matrix spikes were collected for both the raw influent and the final effluent at each wastewater treatment plant. The operator who collected the wastewater samples was asked to fill these two bottles one with raw influent and the other with final effluent to the premarked line that indicated 100 mL. After the return shipment to the laboratory, each

spike control and matrix spike bottle was weighed so that the exact volume of water added could be determined by density.

Standard addition experiments in municipal wastewater influents and effluents were performed to determine the precision and accuracy of fluorinated alkyl substances with the high-volume-injection LC MS/MS method. Spike and recovery experiments were not performed because a blank wastewater sample, a sample containing no analytes of interest, was not found. For the standard addition experiments, sixteen preweighed high density polyethylene bottles were spiked to the following final concentrations of fluorinated alkyl substances: 26 ng/L of PFHpA, PFOA, [$^{13}C_2$]PFOA, PFNA, PFDA, 49.5 ng/L of PFBS, PFHxS, PFOS, PFDS, 6:2 FtS, and 100 ng/L of PFHxA, FOSA, *N*-EtFOSAA, FOSAA, MeFOSAA. 8 spiked bottles were filled with wastewater raw influent, and wastewater final effluent was collected in the remaining 8 spiked bottles. Additionally, samples of unspiked wastewater influent and effluent were collected to determine the background concentrations of fluorinated alkyl substances present in the native waters.

The precision of the method was determined by performing analysis of variance (ANOVA) calculations. ANOVA addresses the sources of error, such as sample collection, preparation, and analysis, which can each contribute to the overall cumulative error of the method. The ANOVA calculations were executed with an Excel ANOVA (two factor with replication) program. The sampling sets used for these calculations were the QC samples, the spike

control and matrix spike samples, collected (n = 20) in the wastewater campaign.

Liquid Chromatography/Mass Spectrometry. Liquid chromatography with electrospray ionization tandem mass spectrometry (LC ESI-MS/MS) was used to identify and quantitate all fluoroalkyl substances in municipal wastewater. Sample clean-up involved centrifugation at 13,200 rpm for ten minutes; the supernatant was decanted, spiked with the internal standards (PFEES and [$^{13}C_2$]PFOA). Sample concentration was achieved by placing a 500 µL polyetheretherketone (PEEK) (Upchurch Scientific, Oak Harbor, WA) sample loop in the LC, and method conditions were optimized accordingly including the sample gradient and injection volume (500 µL). All separations were performed on a Waters 2690 LC (Milford, MA) equipped with a 4 mm x 3 mm C-18 guard cartridge (Phenomenex, Torrance, CA) followed by a reverse-phase Betasil C-18 150 mm x 2 mm column (Thermo Hypersil-Keystone, Bellefonte, PA) that was heated to 35°C. The gradient consisted of an initial 2 minute hold at 50% methanol, then increasing from 50-90% methanol over five minutes followed by a five-minute hold at 90% methanol and 5-minutes of equilibration at 50% methanol. All accessible polytetrafluoroethylene (PTFE) lines were replaced with PEEK tubing (Upchurch Scientific, Oak Harbor, WA) to reduce background levels. The LC was directly interfaced to the ESI source of a Micromass Quattro Micro triple quadrupole mass spectrometer (Beverly, MA). The triple quadrupole was operated in the negative ESI mode and multiple reaction monitoring was used for quantitation. The capillary voltage

was 3.05 kV and the cone potential was set at a value between 10 and 70 V depending on the compound of interest. The temperatures of the source block and desolvation capillary were 125°C and 250°C, respectively. The flow rates of the nebulizer and desolvation gases were 80 and 575 L/hr, respectively. Argon was used as the collision gas, and the collision energy was set at a value between 10 eV and 45 eV depending on the compound being analyzed.

Quantitation of the fluorinated alkyl substances was based on the ratio of the analyte's peak area to that of the internal standard. The product ion(s) chosen for quantitation were determined by infusion of the analytes with a syringe pump directly into the triple quadrupole mass spectrometer (Table 4.1). Two product ions were selected for quantitation of perfluoroalkyl sulfonates and fluorotelomer sulfonates because previous work reported known interferences in biological matrices (32) (unpublished data). For the perfluoroalkyl carboxylates and fluorinated alkyl sulfonamides, one transition was used for quantitation and a second transition was used for qualitative validation. There have been no reported observed interferences for the perfluoroalkyl carboxylates or fluorinated alkyl sulfonamides. The two product ions monitored for the perfluoroalkyl sulfonates were *m/z* 80 and *m/z* 99, which correspond to the following transitions: $[M]^- \rightarrow [M-80] + SO_3^-$ and $[M]^- \rightarrow [M-99] + FSO_3^-$. Quantitation of the fluorotelomer sulfonates was based on the losses of HSO_3^- ($[M]^- \rightarrow [M-81] + HSO_3^-$) and HF ($[M]^- \rightarrow [M-20]^- + HF$). The transitions monitored for the perfluoroalkyl carboxylates were based on the loss of carbon dioxide ($[M]^- \rightarrow [M-44]^- + CO_2$). The qualitative confirmation product ions for

the perfluoroalkyl carboxylates were fragments of the perfluoroalkyl chain, including $C_2F_5^-$ (m/z 119), $C_3F_7^-$ (m/z 169), and $C_4F_9^-$ (m/z 219) (Table 1). The fluorinated alkyl sulfonamides exhibited more structure specific transitions. The precursor ions were all $[M]^-$. The product ions monitored for quantitation of FOSA, *N*-EtFOSAA, FOSAA, MeFOSAA were SO_2N^- (*m/z* 78), $C_8F_{17}SO_2N(CH_2CH_3)^-$ (*m/z* 526), $C_8F_{17}SO_2^-$ (*m/z* 483), and $C_8F_{17}^-$ (*m/z* 419), respectively. The qualitative confirmation product ions for the fluoroalkyl sulfonamides are identified in Table 4.1. The internal standards used for quantitation were PFEES and $[^{13}C_2]$PFOA. The product ions monitored were $C_2F_5O^-$ (*m/z* 135) and $1\text{-}^{13}C_1C_6F_{15}^-$ (*m/z* 370), respectively. The location of the isotopically-labeled carbons on $[^{13}C_2]$PFOA were opportune because one of the isotope labeled carbons carried through to its product ion, adding additional selectivity for that transition. Both PFEES and $[^{13}C_2]$PFOA were used as internal standards for quantitation, except for the samples where $[^{13}C_2]$PFOA was used as a recovery standard. In these cases, only PFEES was used as an internal standard.

Calibration curves were prepared by spiking known quantities of target analytes into Milli-Q water. The six-to eight-point curves spanned concentrations of 0.5 ng/L to 125 ng/L, were plotted using linear regression, weighted 1/X, and the intercept was not forced through zero. Points included in the calibration curves were required to be within 30% of the theoretical concentration. Determination of the uncertainty of x (s_x) established the accuracy of the calibration curves. Calibration curves were run at the

beginning and end of each sample set with blanks and check standards run within the set.

Results and Discussion

Method Optimization Early attempts to analyze municipal wastewaters by direct injection LC ESI-MS/MS proved unsuccessful because the levels of fluorinated alkyl substances were at or below the detection limits, which were around 0.3 µg/L (21). High-volume-injection, instead of solid-phase-extraction (SPE), was chosen as a sample concentration step because high-volume-injection requires less time and resources for method development and analysis, and there is potentially no loss of analytes in the concentration process. In addition, initial studies conducted in the authors' laboratory that utilized SPE yielded low and variable analyte recovery (50-90%) (data not shown). A 500 µL PEEK sample loop was placed in the LC and methods conditions were optimized accordingly, including the sample gradient and injection volume. The sample gradient was modified to include a 2 minute hold before beginning the solvent gradient to account for the larger dead volume resulting from the larger sample loop. The injection volume was optimized by injecting different volumes, 300, 350, 400, 450, and 500 µL, and plotting it versus their respective response signals to insure that as the injection volume increase, the response responded accordingly. From the plotted data, a 500 µL injection was determined to deliver the most sensitive response.

Filtration was initially explored as a means for reducing the heavy particle load present in the wastewater, which can produce matrix effects or reduce the LC column life. Various filter medias were examined, including glass, nylon, cellulose acetate, and polyethersulfone filters. The percent of analyte that passed through each filter ranged from 0% to 350%. The absence of analyte indicates that the fluorinated alkyl substances sorbed to the filter media. The high percentage suggests that fluorinated alkyl substances may be present in some commercial filters. Since no one filter was capable of passing all the fluorinated alkyl substances in wastewater, centrifugation was used as a sample clean-up step. Experiments were performed to determine whether analytes were lost during centrifugation and ≥ 90% of the fluorinated alkyl substances remained after centrifugation for 10 minutes at 13,200 rpm.

The four different classes of fluorinated alkyl substances screened for in this study are shown in a chromatogram in Figure 4.1, the final effluent collected at WWTP 6. The split chromatographic peaks of PFHxS, PFOS, and FOSA, shown in Figure 4.1, suggest the presence of branched and linear isomers, indicative of electrochemical fluorination. Quantitation of these analytes was based on the integration of both peaks.

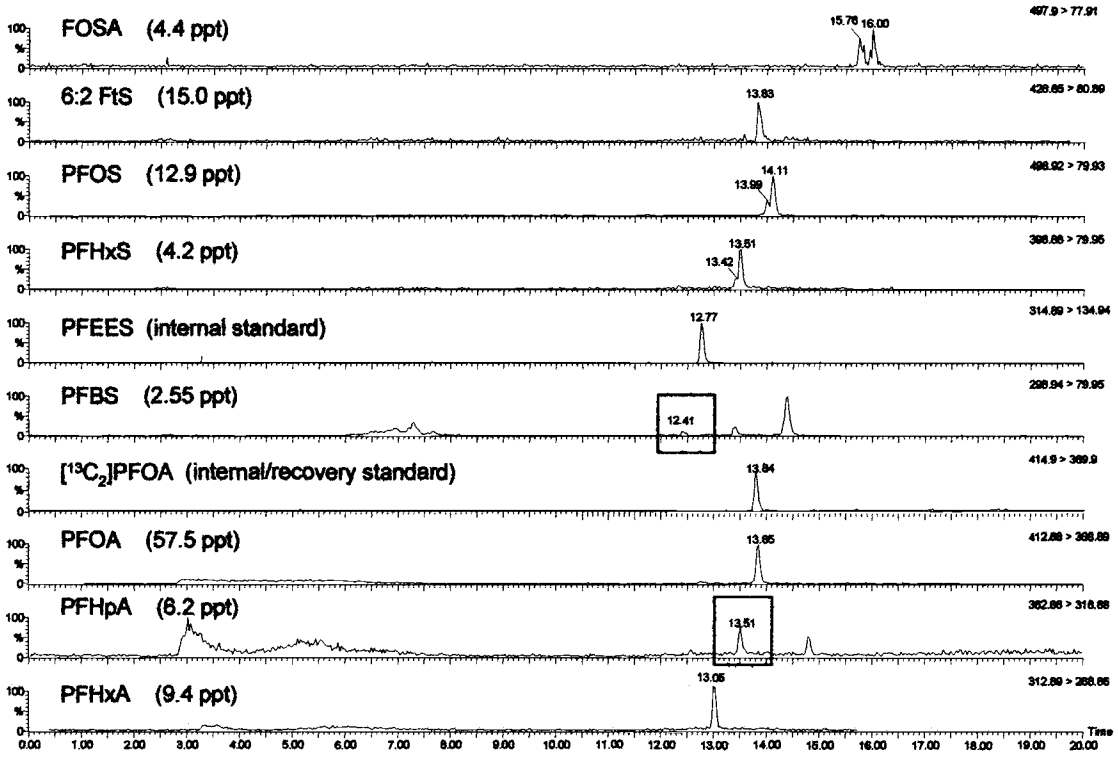

Figure 4.1. LC MS/MS chromatograms for target analytes observed in effluent collected from WWTP 6. The concentrations of each analyte are presented in parentheses. If multiple peaks are present for a specific transition, the peak at the correct retention time is depicted in the box.

Method Accuracy and Precision. The high-volume-injection LC MS/MS method was validated for the analysis of fluorinated alkyl substances in municipal wastewater raw influents and final effluents. The accuracy of the method for wastewater was established by 1) determining the propagation of uncertainty (s_x) with the calibration curve and 2) performing standard addition experiments. To determine the s_x, a subset of calibration curves was evaluated using an Excel program, and a repeated trend appeared illustrating

that the largest uncertainty was located at the low end of the curve and was ± 8.1%.

Since a blank wastewater matrix was not available, standard addition experiments with raw influent and final effluent were performed to assess the accuracy of the quantitative method for actual wastewater samples. Measured recoveries were corrected for background levels of the fluorinated alkyl substances. The recovery results for all the analytes in the influent and effluent are summarized in the first two lines in Table 4.3. Recoveries were satisfactory and ranged from 77% - 95.1 % (± 2.4%) and 85.3% - 95.5% (± 2.3%) in the raw influent and final effluent, respectively (Table 4.3). The only exceptions were *N*-EtFOSAA and *N*-MeFOSAA where their recoveries ranged from no recovery (NR) – 51.4% in the raw influent and 55.6% - 59.3% in the final effluent. This issue will be addressed later in the paper.

Table 4.3. Recoveries (x ± se) of Sample Collection QC from each WWTP: Spike Control Samples (SPC) and Matrix Spikes Samples (MSS)[a]

	n	PFBS	PFHxS	PFOS	PFDS	6:2 FtS	PFHxA	PFHpA	PFOA	[$^{13}C_2$] PFOA	PFNA	PFDA	FOSA	*N*-Et FOSAA	FOSAA	*N*-Me FOSAA
MSS/SA (I) Recovery[b]	**8**	**86.4**	**86.6**	**90.1**	**94.2**	**85.0**	**82.4**	**95.0**	**87.6**	**95.1**	**87.2**	**82.6**	**77**	**51.4**	**79.5**	**NR**
MSS/SA (E) Recovery[c]	**8**	**85.3**	**92.0**	**85.3**	**86.2**	**87.2**	**87.8**	**95.5**	**92.3**	**87.5**	**91.4**	**91.1**	**98.6**	**55.6**	**92.4**	**59.3**
Average SPC Recovery[d]	**10**	**97.5**	**87.8**	**90.7**	**91.5**	**96.8**	**91**	**94.1**	**96.8**	**93.1**	**88.3**	**91.2**	**87.4**	**NA**	**NA**	**NA**
Average WWTP (I) MSS Recovery[e]	**10**	**103**	**97.6**	**95.0**	**94.1**	**97.3**	**88.4**	**91.9**	**92.3**	**88.1**	**92.2**	**90.0**	**82.3**	**NA**	**NA**	**NA**
Average WWTP (E) MSS Recovery[f]	**10**	**100**	**95.3**	**88**	**92.4**	**95.2**	**93.1**	**89.0**	**94.8**	**88.6**	**89.0**	**85.8**	**91.7**	**NA**	**NA**	**NA**

[a] x = average, se = standard error, (I) and (E) denotes raw influent and final effluent, respectively. NA = not analyzed, SA=standard addition experiments

[b]The standard error is 2.4% as determined by nested ANOVA. [c]The standard error is 2.3% as determined by nested ANOVA. [d]The standard error is 1.6% as determined by nested ANOVA. [e]The standard error is 2.1% as determined by nested ANOVA. [f]The standard error is 2.0% as determined by nested ANOVA.

ANOVA calculations were performed to determine the precision of the wastewater method. From the ANOVA calculations, the standard errors of the influent (se_i) and of the effluent (se_e) were determined to be 2.1% and 2.0%, respectively. The standard error in Milli-Q water was 1.6%; thus, the wastewater matrix does appear to contribute in part to the method's error.

The limit of quantitation (LOQ) was defined as the higher of either the analyte concentration required to produce a signal-to-noise of 10:1 in the wastewater matrix or the lowest point on the calibration curve. The LOQs for the fluorinated alkyl substances range from 0.14 (6:2 FtS) to 3 ng/L (*N*-MeFOSAA) (Table 4.1).

Quality Control Samples. The field blanks were analyzed upon return to the laboratory, and in all 10 cases, no fluorinated alkyl substances were detected above quantitation in the field blanks. A limited number of duplicate samples were collected at the wastewater treatment plants. Typically, the 24 h composites are an accumulation of automated flow-dependent aliquots that collect in a refrigerated jug. Field duplicates, two samples collected from the same 24 h composite jug, stored and analyzed separately, were collected at WWTPs 1-3. The average duplicate (n = 2) precision was within ±5%. The average recoveries of each fluorinated alkyl substance in the spike controls and matrix spike samples are summarized in Table 4.3. The recoveries for all analytes ranged from 87.4% - 97.5% (± 1.6%) for the spike controls, 82.3%-103% (± 2.1%) for the raw influent matrix spikes, and 85.8%-100% (± 2.0%) for the final effluent matrix spikes. These recovery results indicate the stability

of the samples over the period of time in which the samples were collected, shipped, stored (in some cases frozen at -20°C), and then finally analyzed.

Application to Wastewater Samples. The high-volume-injection LC ESI-MS/MS method was applied to municipal wastewater raw influents and final effluents collected from 10 plants nationwide. Fluorinated alkyl substances were observed at each wastewater treatment plant, and at each plant, there was a different distribution of analytes (Table 4.4). PFBS, PFHxS, PFOS, 6:2 FtS, PFHxA, PFHpA, and PFOA were all present in varying concentrations at all 10 WWTPs. In addition, PFDS, PFNA, PFDA, and FOSA were observed at some, but not all WWTPs. To assure confidence in these observations, *t*-tests (two sample assuming equal variances) at the 95% confidence level or higher were employed to determine whether the differences, both increases and decreases, observed between the influent and effluent concentrations were significant. The differences in influent and effluent analyte concentrations for each fluorochemical class are shown in Figure 4.2, and the observed differences were only included if they were found to be significant as determined by the t-test. Homogeneity of variance was assumed, and this assumption is justified because all measurements were made the same way on the same instrument. The differences between influent and effluent concentrations deemed significant by the t-test are denoted in Table 4.4 as a footnote.

Table 4.4. Concentrations of Fluorinated Alkyl Substances in Wastewater Treatment Influents and Effluents (average ± standard error)

	PFBS	PFHxS	PFOS	PFDS	6:2 FtS	PFHxA	PFHpA	PFOA	PFNA	PFDA	FOSA
WWTP1 I	4.8±0.34	8.55±0.77	20.8±0.9	nd	10.7±0.1	30.5±0.6	6.6±0.4	13.0±0.4	1.0±0.1	nd	nd
WWTP1 E	‹0.70[a]	4.5±0.7[a]	11.0±1.6[a]	nd	3.9±0.5[a]	‹0.28[a]	nd[a]	2.45±0.15[a]	0.70±0.30	nd	1.0±0.2[a]
WWTP2 I	27.2±1.8	9.25±0.46	400±1.8	6.1±0.1	38.1±0.74	nd	6.45±0.87	16.3±1.2	5.3±0.1	1.0±0.4	nd
WWTP2 E	19.9±1.4[a]	5.0±0.5[a]	132±5[a]	nd[a]	372±8[a]	3.35±0.43[a]	7.5±0.31	27.5±0.8[a]	2.3±0.9[a]	3.3±0.9[a]	nd
WWTP3 I	5.15±0.70	2.3±0.9	20.4±1.3	9.25±0.90	2.1±0.3	10.6±0.04	nd	7.35±0.88	7.3±0.4	nd	nd
WWTP3 E	5.45±0.92	2.4±0.2	6.2±1.78[a]	‹0.47[a]	4.4±0.51[a]	15.8±0.4[a]	1.75±0.88	6.6±0.6	5.74±0.98	nd	4.4±0.2[a]
WWTP4 I	6.93±0.58	10.6±0.6	25.9±1.5	nd	56.8±1.2	9.0±0.1	14.6±1.1	88.7±1.2	5.05±0.36	nd	nd
WWTP4 E	9.93±0.24[a]	17.4±0.6[a]	24.4±1.9	nd	15.3±0.6[a]	16.7±0.7[a]	14.9±1.6	96.9±3.4[a]	6.06±0.59	2.12±0.64[a]	1.6±0.2[a]
WWTP5 I	3.25±0.73	11.5±0.7	11.6±1.3	nd	11.5±1.9	8.3±0.1	0.65±0.13	4.9±0.4	nd	nd	5.5±0.9
WWTP5 E	3.06±0.29	5.34±0.29[a]	5.3±0.23[a]	nd	6.4±0.6[a]	7.2±0.2[a]	3.7±0.2[a]	14.9±0.6[a]	0.70±0.30	nd	10.0±0.3[a]

Table 4.4. (Continued)

WWTP6 I	‹0.70	5.95± 1.24	11.5± 0.39	nd	1.7±.8	10.7± 0.7	7.2±0.9	28.9± 0.8	nd	1.7± 0.7	nd
WWTP6 E	2.55± 0.15[a]	4.2±0.4	12.9± 0.7	nd	15.0±1.9[a]	9.4±1.8	6.2±0.5	57.5±4[a]	nd	27.6± 3.0[a]	4.9± 0.3[a]
WWTP7 I	nd	4.2±1.2	14.4± 1.1	10.4± 1.2	9.0±0.2	22.8± 0.2	0.80±0.34	1.65± 0.32	nd	nd	nd
WWTP7 E	nd	nd[a]	10.9± 0.9	0.65± 0.36[a]	6.4±0.2[a]	8.3±0.3	2.35±0.29[a]	7.65± 0.43[a]	nd	nd	nd
WWTP8 I	nd	7.73± 0.37	12.5± 0.77	8.8±1.1	9.4±1.2	13.1± 0.1	nd	8.53± 0.18	nd	nd	nd
WWTP8 E	nd	7.07± 0.29	7.12± 0.66[a]	nd[a]	11.1± 0.85	16.8±1[a]	1.0±0.34	12.3± 0.71[a]	nd	nd	nd
WWTP9 I	0.45± 0.17	5.7±0.5	27.1± 2.3	nd	15.6±2	17.0± 0.8	1.6±0.1	13.1± 0.7	nd	‹0.32	nd
WWTP9 E	1.8± 0.3[a]	4.9±0.9	25.3± 2.5	nd	24.4±3.4[a]	19.9± 0.1[a]	nd[a]	10.6± 0.8	nd	‹0.32	2.4± 0.4[a]
WWTP10 I	5.52± 0.33	12.4± 0.76	1.4± 0.4	nd	5.8±0.8	19.8±1	25.2±1.4	48.9± 1.7	7.2±0.6	‹0.32	nd
WWTP10 E	‹0.70[a]	5.7± 0.72[a]	1.05± 0.27	nd	nd[a]	17.7± 1.9	23±1.5	64.6± 0.3[a]	0.667± 0.56[a]	‹0.32	nd

nd denotes not detected

[a]The effluent concentration was determined to be significantly different than the influent's concentration as determined by the t-test at the 95% confidence level or higher.

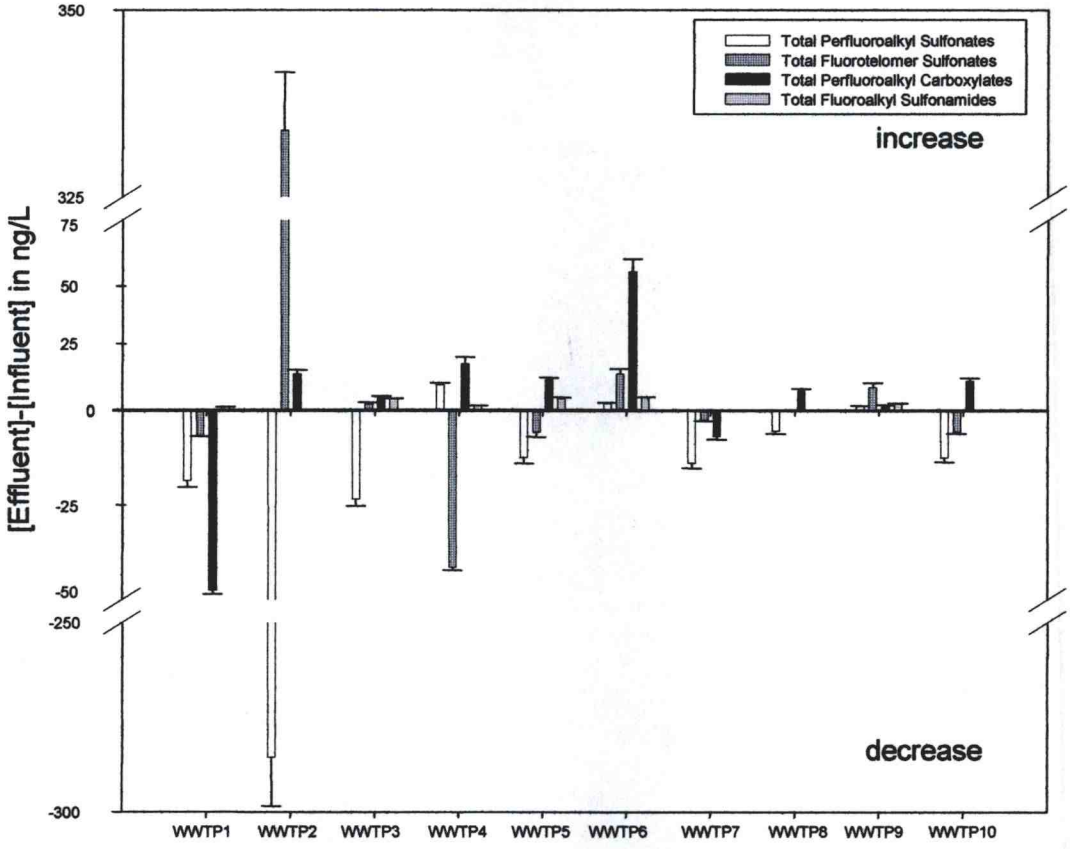

Figure 4.2. Overall significant removals and enhancements in the final effluents are presented for the total concentrations of the different fluorinated alkyl substance classes at each WWTP.

Three overall trends were observed for fluorinated alkyl substances in the wastewater treatment plants. The final effluent concentrations were observed to significantly decrease, significantly increase, or remain statistically unchanged. In the following sections the observed behavioral trends during wastewater treatment for each class of fluorinated alkyl substance will be described and discussed.

Perfluoroalkyl Sulfonates. PFBS, PFHxS, PFOS, and PFDS were each observed in municipal wastewater. PFOS was the most abundant

perfluoroalkyl sulfonate quantitated at each WWTP, except for at WWTP 10 where PFHxS had the highest levels. The largest concentration of perfluroalkyl sulfonates was observed at WWTP 2, where a PFOS concentration of 400 (±11.8) ng/L was quantitated in the influent, and the highest of all fluorinated alkyl substance concentrations observed in this wastewater study. The large presence of PFOS in wastewater treatment is an interesting observation because, as already stated, the 3M Company began their phase-out of their C_8-based chemistry in 2000. The full phase-out has been in effect since 2002 (39). The PFOS levels still seen in wastewater may be a result of products bought before its discontinuance and are now being used or indicative of PFOS' persistent properties. In a parallel study performed by *Higgins et al.* that examined the presence of perfluorochemicals in sludge and sediment, a large difference was observed in the levels of PFOS in sludge collected from the same WWTP (WWTP 1) in 1998 (2610 ng/g) and 2004 (167 ng/g) (40). Lower levels were also observed for PFDS and N-EtFOSAA in the 2004 sludge. It is unclear from the small sampling set in the sludge study if the decrease is a result of the 3M phase-out because only a single sample was obtained for each year.

In 7 out of 10 WWTPs, total perfluoroalkyl sulfonates were significantly removed by wastewater treatment (Figure 4.2). A likely removal process of the perfluoroalkyl sulfonates in WWTPs is sorption onto sludge. For example in WWTP 1, a significant decrease in PFOS concentration was observed in the effluent (11.0 ± 1.6 ng/L) as compared to the influent concentration

(20.8 ± 0.9 ng/L, Table 4.4). Using the total plant plow for the day (12.02 MGD, Table 4.1), it was calculated that 0.948 g/day PFOS entered the plant and 0.501 g/day PFOS was discharged from WWTP 1. Therefore, 0.447 g/day PFOS was removed from the wastewater stream. *Higgins et al.* observed 167 ng/g (dry wt.) PFOS in the anaerobically-digested sludge collected from WWTP 1 on the same day as the aqueous samples (40). Using the sludge hydraulic flow for that day (27,300 gallons/day), the dry to wet conversion factor for WWTP 1 (0.021555134 g (dry)/ g (wet)), and assuming a density of 1 g/mL, 83% of the PFOS calculated to be removed from the wastewater stream can be accounted for on the digested sludge (0.372 g/day PFOS). This is assuming that the concentrations of PFOS in digested sludge are representative of what is observed in the primary sludge and remain relatively constant over time.

The increases of the total perfluoroalkyl sulfonate concentrations observed in WWTPs 4, 6, and 9 (Figure 4.2) may suggest the degradation of precursor molecules, such as the fluoroalkyl sulfonamides, to the perfluoroalkyl sulfonates. Preliminary results have shown the formation of PFOS from *N*-ethyl-*N*-(2-hydroxyethyl)perfluorooctanesulfonamide (*N*-EtFOSE) by microbial activity present in municipal wastewater treatment sludge (41).

Fluorotelomer Sulfonates. The 6:2 FtS was the only fluorotelomer sulfonate observed in the wastewater influents or effluents. Previous work that examined the levels of fluorotelomer sulfonates in contaminated groundwater

impacted by fire-fighting, found that the 6:2 FtS was also the most dominant fluorotelomer sulfonate observed, with 4:2 FtS and 8:2 FtS concentrations combined comprised at the most 9% of the total fluorotelomer sulfonates detected (21).

As with the perfluoroalkyl sulfonates, the highest level of the 6:2 FtS was detected at WWTP 2; however, in contrast, this high 6:2 FtS concentration (372±8 ng/L, Table 4.4), was observed in the effluent, not in the influent as it was for PFOS. When the large effluent concentration (372 ng/L) is compared to the influent concentration (38.1 ng/L), the result is approximately a 900% increase of 6:2 FtS in the effluent. In previous analyses of WWTP 2 (from water samples collected in March 2003), 110 ng/L of 6:2 FtS was observed in the primary effluent compared to 200 ng/L of 6:2 FtS in the final effluent (unpublished data). In previous work, it was proposed that 6:2 FtS may be a degradation product of fluoroalkylthioamido sulfonates, active ingredient in fire-fighting foams (21). More research is needed to better understand the sources of fluorotelomer sulfonates because other chemistries exist that could potentially degrade to form fluorotelomer sulfonates.

In 5 of the 10 WWTPs, 6:2 FtS effluent concentrations were found to significantly decrease relative to influent concentrations (Table 4.4), which indicate that 6:2 FtS likely is being removed either by sorption onto sludge or by biodegradation. An earlier study has shown that 6:2 FtS is susceptible to biodegradation under sulfur-limiting and aerobic conditions (42). Therefore, the significant removal of 6:2 FtS observed in the 5 plants could be a result of

biodegradation. To date, no methodologies exist for the determination of fluorotelomer sulfonates in sludge; thus, more research is required to ascertain whether sorption onto sludge is a likely removal process for fluorotelomer sulfonates in wastewater treatment plants.

Perfluoroalkyl Carboxylates. PFHxA, PFHpA, PFOA, PFNA, and PFDA were all observed in municipal wastewater. The highest concentration of a perfluoroalkyl carboxylate was PFOA in the effluent of WWTP 4 at 96.9±3.4 ng/L (Table 4.4). No apparent patterns emerged of either even- or odd- numbered perfluoroalkyl carboxylates, except for the observation that PFHxA and PFOA were the most abundant perfluoroalkyl carboxylates detected in wastewater.

The perfluoroalkyl carboxylates significantly increased in 8 out of the 10 plants sampled, which contrasts to what was observed for the perfluoroalkyl sulfonate class. In WWTP 6, there was a significant increase in perfluoroalkyl carboxylates concentrations. For example, the PFOA influent concentration was 28.9±0.8 ng/L and the effluent concentration was 57.5±4 ng/L (Table 4.4), a 99% increase in concentration. Additionally, *Higgins et al.* observed 29.4 ng/g (dry wt.) of PFOA in digested sludge (40). These observations not only suggest that perfluoroalkyl carboxylates are biotransformed degradation products in the wastewater stream, but may also be produced during sludge digestion. Perfluoroalkyl carboxylates are known degradation products of FtOHs (43-45); thus, it is possible that the observed

increases of perfluoroalkyl carboxylates in the final effluents and digested sludge are a result FtOH degradation.

Perfluoroalkyl carboxylates were significantly removed in WWTPs 1 and 7 (Figure 4.2, Table 4.4). A possible explanation of this removal of perfluoroalkyl carboxylates may be explained by sorption onto sludge. Both the *Higgins et al.* perfluorochemical sludge study at the 3M Company multi-city study, found that there was sorption of perfluorocarboxylates onto sludge; however, their concentrations were generally lower than the perfluoroalkyl sulfonates levels. At WWTP 1, *Higgins et al.* found that the total perfluoroalkyl sulfonate concentrations (PFHxS, PFOS, and PFDS) in sludge were 16 times more abundant than the total perfluoroalkyl carboxylate concentrations (PFOA, PFNA, PFDA, and perfluorododecanoic acid) observed in sludge (40). These results suggests that sorption onto sludge may not play a large role in the removal of perfluoroalkyl carboxylates from the wastewater stream as it does with the perfluoroalkyl sulfonates.

Fluoroalkyl Sulfonamides. FOSA was the only fluoroalkyl sulfonamide observed in the wastewater samples, and it was observed in six WWTPs (Table 4.4). In all six locations, the concentration of FOSA significantly increased in the effluent (Figure 4.2), implying degradation of fluorochemical precursor compounds to FOSA during wastewater treatment. Previous research has shown that FOSA biotransforms as a degradation intermediate from fluoroalkyl sulfonamides precursor compounds, such as *N*-EtFOSE and *N*-ethylperfluorooctanesulfonamide, and that FOSA further degrades to form

PFOS (41,46-48). Further research is needed to identify where in the wastewater treatment plant FOSA is formed by sampling wastewater after each treatment step.

The results of this study do not necessarily suggest that the other fluoroalkyl sulfonamides, such as *N*-EtFOSAA, FOSAA, and MeFOSAA are not present in a WWTP; they just may not be present in aqueous wastewater matrices. *Higgins et al.* observed concentrations of *N*-EtFOSAA, FOSAA, and MeFOSAA in sludge at all WWTPS sampled, including *N*-EtFOSAA and MeFOSAA concentrations that often exceeded detected PFOS levels (40); thus, indicating that *N*-EtFOSAA, FOSAA, and MeFOSAA may prefer to sorb to suspended solids in wastewater than partition into the aqueous matrix. The standard addition experiments performed for this study further support this assumption. The recoveries of N-EtFOSAA, FOSAA, and MeFOSAA in the spiked raw influent were 51.4 ± 2.4 %, 79.5 ± 2.4 %, and no recovery, respectively, and the recoveries improved to 55.6 ± 2.3%, 92.4 ± 2.3%, and 59.3± 2.3%, respectively, in the spiked final effluent (Table 4.3). The observed improvement of the recoveries of the fluoroalkyl sulfonamides in the final effluent may be correlated to the reduced particle load in wastewater discharge. FOSA recoveries also improved from 77.55 ± 2.4% in the spiked influent to 98.6 ± 2.3% in the spiked effluent, suggesting that it, like the other fluoroalkyl sulfonamides, may be somewhat influenced by suspended solids; however there is little supporting evidence that FOSA prefers to partition into the solid phase as compared to the aqueous phase. *Higgins et al.* did not

report any observations of FOSA in sludge. The 3M Company, detected FOSA in the sludge, but at low levels and at few locations that were sampled (37). Thus, suggesting that FOSA, a transformation intermediate, does not have a long half-life on sludge or inferring that partitioning onto sludge is not major removal process from wastewater as it is for the other fluoroalkyl sulfonamides and perfluoroalkyl sulfonates. However, sorption studies would be required to fully confirm the partitioning behavior of fluoroalkyl sulfonamides between aqueous and solid phases.

Behaviors of Fluorinated Alkyl Substance in WWTPs. Each of the WWTPs sampled exhibited a unique fingerprint of fluorinated alkyl substances. With the exception of FOSA, the fluorinated alkyl substance classes did not exhibit the similar removal and/or enhancement trends at each WWTP. Sources of fluorinated alkyl substances present in this study remain largely unknown. Domestic waste comprised the largest type of waste treated, ranging from 50% - 99% in each of the raw influents (Table 4.1). As previously mentioned, fluorinated alkyl substances are largely used as repellants or coatings in many domestic products such as clothing, furniture, and carpets (49) and, thus, could wear off with repeated washings. As an example, the present authors analyzed methanol extracts of fabric cut from finished clothing for fluorinated alkyl substances (50). The combined perfluoroalkyl sulfonates and carboxylates concentrations detected in the two different fabric samples were 13 ng/g and 882 ng/g. Fluorinated alkyl substances are also widely employed in industrial applications as industrial

surfactants, coatings, additives, and electroplating (49). The industrial waste inputs ranged from 1% - 15% per plant. WWTPs 7 and 10 provided a unique opportunity to analyze wastewater that contained papermill effluent (WWTP 7), from a mill that applies paper coatings and landfill leachate (WWTP 10). Coatings onto paper and packaging products are a predominant application of fluorinated alkyl substances. Thus, papermill effluent has the potential to be a substantial source of fluorinated alkyl substances; however, relatively low levels (≤22 ng/L, Table 4.4) were observed at WWTP 7, where 50% of the waste treated was papermill effluent, indicating that this mill did not likely use fluorinated coatings. Landfill leachate is also a potential source of fluorinated alkyl substances in that old furniture, carpets, and other textiles coated with fluorinated repellants could be disposed of at landfills. 10% of WWTP 10's waste was from landfill leachate (Table 4.2). The second highest levels of PFOA and th highest levels of PFHpA were observed at WWTP 10; however, the lowest levels of PFOS were also detected there. Additional studies on landfill leachate are required before a conclusive statement can be made as to whether it is a source of fluorinated alkyl substances in wastewater.

Treatment processes present at a WWTP did not appear to influence the fate of fluorinated alkyl substances. For example, WWTPs 1, 3, 5, and 9 all incorporate primary gravitational settling, trickling filters, and activated sludge in their treatment processes (Table 4.2), and if the treatment processes dictate the fluorinated alkyl substance's behavior, the effluents should exhibit similar class outcomes. On the contrary, four different distributions of classes

were observed at each of the 4 plants (Figure 4.2). In WWTP 1, all classes were significantly reduced in the effluent, except for FOSA, which showed an enhancement. In WWTP 3, the perfluoroalkyl sulfonates were the only class to exhibit significant removals in the effluent. The perfluoroalkyl sulfonates and fluorotelomer sulfonates were removed, and the perfluoroalkyl carboxylates and FOSA increased at WWTP 5. All classes demonstrated significant increases in the effluent at WWTP 9. Four different outcomes at four different WWTPS that utilize the same treatment technologies indicate that the overall trend differences in the fluorinated alkyl substances removal (or enhancement) rates are not solely influenced by the treatment processes. This observation is further verified by examining WWTPs 7 and 8. These are the only two plants that employ mixed media filters in the plants' treatment. WWTP 7 was the only plant where there were no observed total class increases in the final effluent; however, WWTP 8 had effluent increases with the perfluoroalkyl carboxylates. With a limited data set (i.e. single sampling of influents and effluents at each plant on a single day), observed trends merit postulations of sources, but definitive conclusions cannot be drawn with the data currently available and are subject to further investigations.

This present study demonstrated a high-volume-injection method for the analysis of fluorinated alkyl substances in municipal wastewater, illustrated that these compounds are present at varying degrees at every plant sampled, and are point sources of fluorinated alkyl substances. Each WWTP had a unique distribution of analytes, despite similar treatment. This study gave

insight to removal and enhancement of fluorinated alkyl substances' levels throughout the wastewater process, and it also raised additional areas of work. This data set included analyzing 24 h composites of wastewater influents and effluents from one day. Further investigations involving repetitive sampling at plants, hourly grab samples over a defined period of time, sampling after each treatment stage, and seasonal sampling are only a few of the research areas needed to address whether the observed trends described here are representative of the fate of fluorinated alkyl substances throughout the wastewater treatment process.

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5. BEHAVIOR OF FLUOROCHEMICALS DURING WASTEWATER TREATMENT

Melissa M. Schultz, Christopher P. Higgins, Richard G. Luthy,
Douglas F. Barofsky, and Jennifer A. Field

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Abstract

Fluorochemicals have widespread applications and as a result of extensive consumer use, fluorochemicals may be released to municipal wastewater treatment plants via domestic wastewater. A field study was conducted at a full-scale municipal wastewater treatment plant to determine the mass flows of selected perfluoroalkyl sulfonates, perfluoroalkyl carboxylates, fluorotelomer sulfonates, and perfluoroalkyl sulfonamides in wastewater and sludge. Samples of wastewater (raw influent, primary effluent, trickling filter effluent, secondary effluent, and final effluent) and sludge (primary, thickened, activated, anaerobically digested, and storage lagoon) were collected over a duration of 10 days and were analyzed byliquid chromatography (LC), electrospray ionization (ESI) tandem mass spectrometry (MS/MS). Both removals and increases of fluorochemical concentrations in wastewater treatment plants were observed. Perfluoroalkyl sulfonates were found to increase significantly (~200%) in the plant mass balance (30 days). Fluoroalkyl sulfonamide acetic acids were also found to increase approximately 500% throughout the sludge treatment process with a residence time of a year. In this study, perfluoroalkyl carboxylates were overall removed by the wastewater treatment plant. When the assumption is made that the monitored plant is representative of wastewater treatment plants nationwide, fluorochemicals are discharged in wastewater effluents at a rate of 3180 kg/year and are introduced to terrestrial environments via biosolids at a rate of 7080 kg/year. If this assumption is valid, wastewater treatment plants are

point sources of fluorochemicals and cannot be overlooked when determining origins and fate of fluorochemicals in the environment.

Introduction

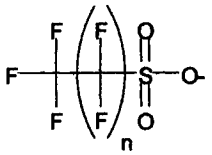
Fluorochemicals have ignited widespread interest due to their ubiquitous, worldwide presence in the environment, including air (1-3), surface waters (4-12), groundwater (13-15), biota (16-21), and sediment (22), and their occurrence in serum of nonoccupationally-exposed humans(20,23-28). The fluorination of organic compounds imparts unique physical and chemical properties, including significant thermal and chemical stability, permitting applications where conventional hydrocarbon chemistries would decompose (29). The fluoroalkyl tails are both hydrophobic and oleophobic (i.e. oil-repelling). The distinct physical and chemical properties of fluorochemicals make them valuable constituents in a wide range of industrial and commercial applications, including adhesives, cleaners, coatings, shampoos, electroplating, fire-fighting foams, herbicides and insecticides, polishes, wetting agents, repellants for furniture, carpets, and clothing (29).

As fluorochemicals are widely used in household and consumer-based products and have many industrial applications, a primary route for this chemical class into aquatic and terrestrial environments is by municipal wastewater treatment disposal. The untreated fluorochemicals may enter the environment via wastewater effluent, septic discharge or land application of biosolids. Previous studies have indicated the presence of fluorochemicals in

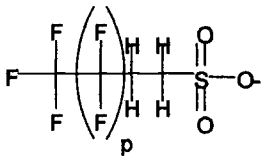
treated wastewater. In a multi-city study (6 cities), conducted by the 3M Company, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) were observed in all sampled wastewater effluents and PFOS was present in the majority of biosolids sampled (30). Alzaga and Bayona also analyzed wastewater effluents and quantitated PFOA and perfluorodecanoate (PFDA) in two urban wastewater treatment plant (WWTP) effluents (31). Fluorochemicals were observed in all 10 WWTP influents and effluents sampled by *Schultz et al.* and each plant demonstrated different distributions of fluorochemicals despite similar treatment processes (32). In 9 out of the 10 plants sampled, at least one class exhibited increased levels in the effluent as compared to the influent concentrations. In a parallel study, fluorochemicals were analyzed in domestic sludge (22). Total perfluoroalkyl sulfonate concentrations were substantitally more abundant than the total perfluoroalkyl carboxylate levels. Fluoroalkyl sulfonamides, 2-(*N*-methylperfluorooctanesulfonamido) acetic acid (*N*-MeFOSAA) and 2-(*N*-ethylperfluorooctanesulfonamido) acetic acid (*N*-EtFOSAA), intermediates found to degrade to PFOS (33), were present in sludge often exceeding the concentration of PFOS.

The focus of this study was to determine mass flows of the most abundant perfluoroalkyl sulfonates, perfluoroalkyl carboxylates, fluorotelomer sulfonates, and fluoroalkyl sulfonamides (Figure 5.1) in a municipal wastewater treatment plant using previously reported analytical methods for fluorochemicals in wastewater (32) and sludge (22). The scale of removal from the waste stream, transfer between aqueous and particulate phases, or

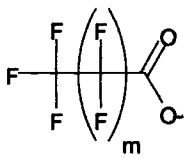
transformation of products will indicate the respective importance of each stage of wastewater treatment on the fate of fluorochemicals and on their release into the environment. In this manner, this study provides insight on the role wastewater treatment plays in the release of fluorochemicals to the environment.



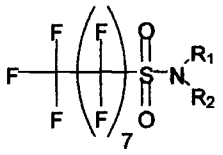
n = 5 Perfluorohexanesulfonate (PFHxS)
n = 7 Perfluorooctanesulfonate (PFOS)
n = 9 Perfluorodecanesulfonate (PFDS)



p = 5 1H, 1H, 2H, 2H - Perfluorooctanesulfonate (6:2 FtS)



m = 4 Perfluorohexanoate (PFHxA)
m = 6 Perfluorooctanoate (PFOA)
m = 7 Perfluorononoate (PFNA)
m = 8 Perfluorodecanoate (PFDA)
m = 9 Perfluoroundecanoate (PFUnA)
m = 10 Perfluorododecanoate (PFDoDA)
m = 12 Perfluorotetradecanoate (PFTA)



R_1 = H, R_2 = H Perfluorooctanesulfonamide (FOSA)

R_1 = H, R_2= CH_2COO^- Perfluorooctanesulfonamideacetate (FOSAA)

R_1 = CH_3, R_2= CH_2COO^- 2-(*N*-methylperfluorooctanesulfonamido) acetate (*N*-MeFOSAA)

R_1 = CH_2CH_3, R_2= CH_2COO^- 2-(*N*-ethylperfluorooctanesulfonamido)acetate (*N*-EtFOSAA)

Figure 5.1. Fluorochemicals detected in wastewater and sludge.

Experimental Section

Study site. The municipal wastewater treatment plant selected for this mass flow study is located in the Pacific Northwest, United States, and serves a population of approximately 50,000 people. Raw sewage entering the treatment plant is first passed through a screen to remove larger solids (Figure 5.2). The wastewater then flows to the primary clarifier, where additional solids settle to the bottom and are removed as primary sludge. The primary sludge and the waste activated sludge (from the activated sludge system) are mixed in a 3:1 (v/v) ratio (primary sludge : waste activated sludge) and are thickened for one day before the supernatant is decanted and fed back into the raw influent. The thickened sludge is passed to the anaerobic digestor where it is digested 30 days. After digestion, the sludge is further stabilized in a storage lagoon for one year, and then is land-applied. For the aqueous stream, the primary effluent leaves the clarifier and undergoes aerobic treatment. The aerobic treatment involves two stages: trickling filters followed by activated sludge treatment. After the activated sludge treatment, the wastewater undergoes further settling in the secondary clarifier. The secondary effluent is then chlorinated and dechlorinated before discharging into a river. The overall residence time for the aqueous stream through the WWTP is approximately 8 – 10 hours.

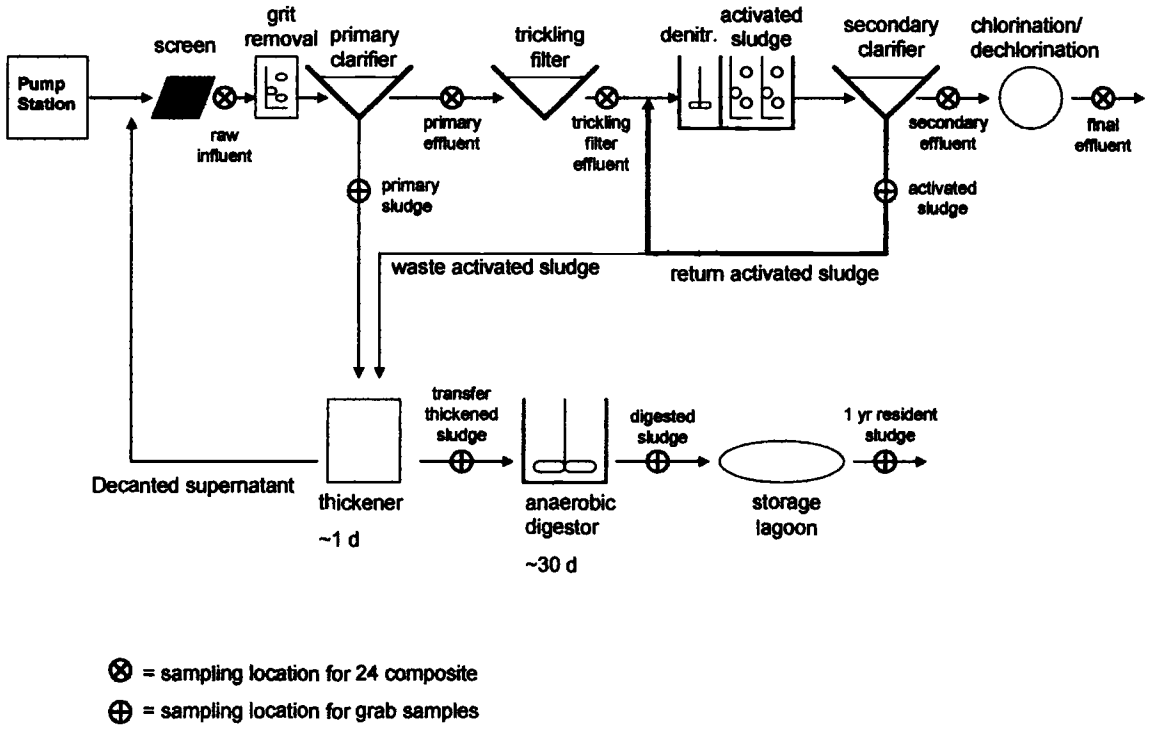


Figure 5.2. Schematic of the wastewater treatment plant

Sample collection and preparation. Flow-dependent 24 h composite samples of raw influent, primary effluent, trickling filter effluent, secondary effluent, and final effluent were collected over a ten d period in July of 2004. To better understand the diurnal variation of the fluorochemical mass flows, grab samples of raw influent and final effluent were collected each hour for 27 continuous hours for one day beginning on the fourth and concluding on the fifth day of sampling. On five days during the ten day sampling period, (days 1, 3, 5, 7, and 9) grab samples of primary, activated, thickened, and anaerobically-digested sludge were sampled. On days 1 and 3, the different types of sludge were collected four times during the day, and on days 5, 7 and

9, the sludge was sampled once daily. Lagoon sludge, sludge that has a residence time of one year, was sampled once on day 5 during the ten day study. All wastewater and sludge samples were collected and stored in high density polyethylene bottles. Wastewater samples that were analyzed within 48 h upon arrival to the laboratory were kept refrigerated at 4°C until analysis. Wastewater samples not analyzed within 48 h were stored at -20°C and thawed to room temperature prior to analysis. Sludge samples were frozen within hours of collection (-15°C) and remained frozen until extraction. Particulate matter (suspended solids present in the wastewater samples) was separated from the raw influent and primary effluent by centrifugation and then air dried. During the 10d sampling period, the outside temperature ranged from 12°C (night) to 41°C (day). There was no precipitation during the sampling period.

Analytical methods. Liquid chromatography with electrospray ionization tandem mass spectrometry (LC ESI-MS/MS) was used to analyze the wastewater samples and the sludge and particulate matter extracts. The method used for analyzing the wastewater samples by high-volume-injection LC ESI-MS/MS is described in *Schultz et al.* (32). The precision of the wastewater method was defined by the analysis of variance (ANOVA) for the fluorochemicals in the raw influents and final effluents, which were relative standard of errors of ±2.1% and ±2.0%, respectively. The accuracy of the method was established by determining the propagation of uncertainty (s_x) for the calibration curve and performing standard addition experiments. All s_x

were ≤ 8.1%, and the standard addition recoveries ranged from 77% - 95.1% (± 2.4%) and 85.3% - 95.5% (± 2.3%) in the raw influents and final effluents, respectively. The limit of quantitation (LOQ) for the fluorochemicals was analyte dependent and ranged from 0.14 ng/L to 3 ng/L.

Sludge and particulate matter samples were extracted using liquid solvent extraction followed by a solid-phase-extraction clean-up step and analyzed by LC ESI-MS/MS as described by *Higgins et al.* (22). The method precision indicated by relative standard deviations was <20% for 81% of quantifiable sludge samples. Recoveries of the fluorochemicals onto digested and primary sludge was >70% for most analytes. The method detection limits ranged from 0.7 to 2.2 ng/g (dry wt.) for all analytes.

Results and Discussion

Occurrence of fluorochemicals in wastewater and sludge. The wastewater treatment plant where this mass balance was undertaken was subject to previous studies by *Schultz et al.* and *Higgins et al.* (22,32). 24 h flow dependent composites of raw influent and final effluent were sampled for one day in April 2004 as part of a national wastewater sampling campaign to validate the LC ESI-MS/MS method for the analysis of fluorochemicals. Digested sludge was also sampled from the plant in a parallel study determining the concentrations of fluorochemicals in sludge and sediment. When comparing the two wastewater studies, the quantitifiable fluorochemical concentrations displayed a similar range, 1 ng/L to 30.5 ng/L (32) in the April

collection compared to 1.1 ng/L to 32.5 ng/L (Table 5.1) in the July sampling. This suggests that there might not be a dramatic seasonal variation in fluorochemical concentrations at this plant. At this plant's location, April falls at the latter end of a 6-month rainy season, whereas July falls in the middle of fairly consistent period of rare precipitation events. Although the fluorochemical concentrations seemed similar between the two studies, the observed analytes differed. Perfluorobutane sulfonate (PFBS) and perfluoroheptanoate (PFHpA) were not detected during the 10 d mass flow study; however both compounds were previously observed at this wastewater treatment plant and at the majority of plants sampled nationally. Perfluorodeacne sulfonate (PFDS) and PFDA were nondetectable in the April sampling, but were consistently seen in this study. Since the specific source of fluorochemicals in wastewater is widely speculative and unknown it is hard to surmise why certain fluorochemicals are present one day and not the next.

The concentration range observed in the digested sludge from this mass flow study was in good agreement with the digested sludge samples collected in April 2004. The concentration of the quantifiable fluorochemicals in the digested sludge ranged from 4.81 ng/g (dry wt.) to 167 ng/g (dry wt.) in April 2004 (22), and ranged from 1.8 ng/g (dry wt.) to 160 ng/g (dry wt.) in the present study (Table 5.2). The observed analytes in both sludge studies remained constant.

Table 5.1. Fluorochemical concentrations (ng/L) in raw influent, primary, trickling filter, secondary, and final effluents.[a,b]

Date	Hydraulic flow MGD	Raw Influent								
		PFHxS	PFOS	PFDS	6:2 FtS	PFHxA	PFOA	PFNA	PFDA	FOSA
19-Jul	6.735	9.9	32.5	7.5	9.1	24.5	16.1	nd	5.5	nd
20-Jul	7.0619	15.3	17.1	2.9	10.6	19.8	9.9	nd	4.1	nd
21-Jul	7.3009	5.3	6.9	4.8	5.5	17.6	18.8	nd	5.4	nd
22-Jul	7.4719	9.9	17.1	3.2	12.9	20.9	21.6	nd	6.3	nd
23-Jul	7.745	5.8	11	3.9	10.3	16.6	10.3	nd	4.4	nd
24-Jul	6.7679	6.7	9.6	3	6.4	19.1	10.1	nd	2.7	nd
25-Jul	6.943	6.9	15	7.1	4.9	12	9	nd	8.6	nd
26-Jul	6.7579	7.8	8	0	9	17.6	13	nd	5.2	nd
27-Jul	6.8999	5.5	13.5	6.5	6.4	17.1	23.8	nd	10.1	nd
28-Jul	6.95	3.8	15.9	1.1	5.1	29.1	16.9	nd	4.1	nd
Average ± SE[c]	**7.08**	**7.7 ± 1**	**14.7 ± 2.3**	**4.0 ± 0.8**	**8.0 ± 0.9**	**19.4 ± 1.5**	**15.0 ± 1.7**		**5.6 ± 0.7**	

[a]Each daily concentration reported is the average of duplicate analyses.
[b]The error associated with each measured concentration is ± 2.3% relative standard error.
[c]The standard errors of the reported 10 d average.

Table 5.1. (Continued)

Primary Effluent								
PFHxS	**PFOS**	**PFDS**	**6:2 FtS**	**PFHxA**	**PFOA**	**PFNA**	**PFDA**	**FOSA**
6.9	19.3	6.3	6.3	15.6	14	nd	7.7	nd
5.5	8.7	5.5	6.4	19.7	14.4	nd	16.7	nd
8.3	16	1.5	0	14.1	13.7	nd	3.8	nd
4.9	17.9	5.8	6.2	8.9	11.1	nd	8	nd
2.8	15.5	5.7	9.8	9.7	4.8	nd	6.6	nd
4.8	22.1	6.7	6.9	9.3	8.8	nd	4.5	nd
4.6	26.1	8.2	4.2	11.3	10.6	nd	6.5	nd
6.1	22.5	2.9	8.9	7.5	9.9	nd	5.8	nd
4.4	17.3	4.8	0	12.4	7.8	nd	1.1	nd
4.3	10.9	3.6	5.1	10.6	9.8	nd	1.4	nd
5.3 ± 0.5	**17.6 ± 1.7**	**5.1 ± 0.6**	**5.4 ± 1**	**11.9 ± 1.2**	**10.5± 1**		**6.2 ± 1.4**	

Table 5.1. (Continued)

Trickling Filter Effluent								
PFHxS	**PFOS**	**PFDS**	**6:2 FtS**	**PFHxA**	**PFOA**	**PFNA**	**PFDA**	**FOSA**
3.8	25.1	16.6	2	10.8	5.3	nd	3	nd
2	22.2	19.1	5.1	6.6	5.5	nd	2.7	nd
2.3	26.5	19.7	5.1	6.2	18.8	nd	5.4	nd
3	24.3	19.1	12.1	14.9	10.1	nd	4.6	nd
1.3	31.1	13.3	5	11.2	9.9	nd	4.1	nd
1.1	29.5	22.9	7.6	6.5	9.4	nd	3.1	nd
0.8	26.6	16.3	3.1	13	6.3	nd	1.4	nd
0.5	24.8	12.6	3.3	7.7	4.1	nd	2.6	nd
0.8	31	9.7	1.2	9.5	8.1	nd	2.1	nd
<LOQ	27	16.4	8.1	12.9	9.1	nd	3.5	nd
1.7 ± 0.4	**27.0 ± 1**	**16.6 ± 1.2**	**5.3 ± 1**	**9.9 ± 1**	**8.7 ± 1.3**		**3.3 ± 0.4**	

Table 5.1. (Continued)

			Secondary Effluent					
PFHxS	**PFOS**	**PFDS**	**6:2 FtS**	**PFHxA**	**PFOA**	**PFNA**	**PFDA**	**FOSA**
2.4	26.3	4.1	5.5	3.6	11.2	3.3	2	4.6
3.2	23.3	2.5	1.5	5.3	16.4	5.4	1.7	9.6
1.5	26.6	6.7	2.1	3.9	12.9	6.2	3.6	7.9
1.1	23.8	10.1	3.7	5.4	13.8	8.1	1.2	7
1.5	22.5	1.3	4.6	3.3	14.3	7.9	1.6	12
2	21.5	3.6	9.1	5.4	10	12.7	7.5	23.5
2.5	16.8	2.2	3.2	6.4	9.4	2.8	0.8	2.4
0	12	0.1	3.4	4.7	6.5	2.8	1.2	7.6
<LOQ	16.8	4.3	2.8	6.2	8.7	6.6	3.1	5.9
0	17	1.8	4.9	5.6	9.8	9.7	3.8	0
1.6 ± 0.4	**20.7 ± 1.5**	**3.7 ± 0.9**	**4.1 ± 0.7**	**5.0 ± 0.3**	**11.3 ± 0.9**	**6.6 ± 1**	**2.7 ± 0.6**	**8.1 ± 2**

Table 5.1. (Continued)

				Final Effluent				
PFHxS	**PFOS**	**PFDS**	**6:2 FtS**	**PFHxA**	**PFOA**	**PFNA**	**PFDA**	**FOSA**
0.6	22.8	15.1	7	5.6	11.5	3.7	0.6	2.6
3.2	27.1	7.4	8.7	5	10.1	3.8	5.1	3.3
1.1	22.3	8.7	7.3	7.4	15.4	1.5	2.2	4.3
1.8	22.1	7.9	14	6.4	13.1	2.8	3.5	4.6
3	31.3	11	19.8	5.7	14.6	3.5	3.9	4.5
0	33.6	12.9	8.6	8.3	8.2	3.1	2.9	17.1
0.6	16.2	4.8	8.5	7.3	11.5	2.5	1	1.7
0.6	28.9	6.2	11.6	7.4	9.9	5.9	1.1	3.8
0	14.7	2.2	6	4.6	8.8	3.2	0.9	0
0.9	21.5	5.5	6.1	6.1	11	4.3	2	4.1
1.2 ± 0.4	**24.1 ± 1.9**	**8.2 ± 1.2**	**9.8 ± 1.4**	**6.4 ± 0.4**	**11.4 ± 0.7**	**3.4 ± 0.4**	**2.3 ± 0.5**	**4.6 ± 1.5**

Table 5.2. Fluorochemical concentrations (ng/g, dry wt.) in primary, thickened, activated, and digested sludges, raw influent and primary effluent particulate matter.[a,b]

	Date	Hydraulic flow MG/day	Primary Sludge										
			PFHxS	PFOS	PFDS	FOSAA	N-MeFOSAA	N-EtFOSAA	PFOA	PFNA	PFDA	PFUnA	PFDoA
	19-Jul	0.108	nd	17.8± 6.7	22.2± 1.3	2.2± 0.4	5.5± 0.9	14.9± 1.9	2.3± 0.8	nd	1.6±. 0.2	4.2±1.6	1.6±0.1
	20-Jul												
	21-Jul	0.215	2.0± 1	40.5± 9.7	17.7± 2.9	2.2± 0.4	5.2± 2.2	21.0± 4.9	10.0± 4.3	4.4± 2.8	3.1± 1.4	2.1±0.4	1.2±0.4
	22-Jul												
	23-Jul	0.213	nd	83.8	22.9	<3	6.5	15.3	<6	nd	2.0	2.0	1.8
	24-Jul												
	25-Jul												
	26-Jul	0.107	11.7	50.4	20.6	3.4	8.9	23.8	12.2	6.4	3.9	2.5	1.8
	27-Jul												
	28-Jul	0.106	3.1	69.8	13.5	<3	5.6	25.8	11.2	10.3	3.4	2.3	1.3
Ave ± SE[c]		**0.150**	**3.4 ± 2.1**	**52.5 ± 11.4**	**19.4 ± 1.7**	**1.6 ± 0.7**	**6.3 ± 0.7**	**20.2 ± 2.2**	**7.1 ± 2.5**	**4.2 ± 2**	**2.8 ± 0.4**	**2.6±0.4**	**1.5±0.1**

[a] MG/day denotes million gallons per day, G/day denotes gallons per day.
[b] The standard errors of the reported 10 d average.

Table 5.2. (Continued)

Hydraulic flow G/day	Thickened Sludge										
	PFOS	PFDS	FOSAA	N-MeFOSAA	N-EtFOSAA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTA
29300	21.0± 2.6	70.9± 11.7	7.5± 0.7	51.5± 9.3	47.8± 6.9	<6	nd	5.3± 0.7	4.7±0.7	5.1±0.7	1.3± 0.2
30900	19.6± 1.7	59.0± 4.4	6.6± 0.2	39.4±3	48.8± 2.4	<6	nd	3.9± 0.4	4.4±0.7	4.2±0.4	0.9± 0.3
30800	51.3	58.7	6.6	34.8	43.0	<6	nd	3.7	3.9	4.1	1.2
29200	118	62.8	7.6	44.0	52.1	<6	nd	3.6	5.0	4.1	1.3
29800	20.5	57.4	6.2	35.8	47.8	<6	nd	3.4	4.0	4.1	1.2
30000	**42.4 ± 17.5**	**61.8 ± 2.5**	**6.9 ± 0.3**	**41.1 ± 3**	**47.9 ± 1.5**			**3.9 ± 0.4**	**4.4±0.2**	**4.3±0.2**	**1.2 ± 0.1**

Table 5.2. (Continued)

Hydraulic flow MGD	Activated Sludge (WAS + RAS)										
	PFOS	PFDS	FOSAA	N-MeFOSAA	N-EtFOSAA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTA
5.27	54.7±1.9	143±2	22.7±0.5	161±3	129±2	8.2±1.3	4.9±1.1	10.8±0.3	9.0±0.3	7.8±0	<3
5.27	50.7±1.9	140±4	21.2±1	148±3	136±4	7.9±05	4.1±0.2	9.7±0.2	10.5±1.4	7.6±0.3	<3
5.26	40.7	135	17.7	132	127	6.7	3.4	9.2	9.3	7.0	<3
5.27	39.0	132	18.3	136	126	4.9	3.1	8.6	9.5	7.2	<3
5.26	31.2	94	13.5	98.7	99.9	5.7	3.7	7.2	7.7	6.1	<3
5.27	**43.3±4.2**	**129±9**	**18.7±1.6**	**135±10**	**124±6**	**6.7±0.6**	**3.8±0.3**	**9.1±0.6**	**9.2±0.4**	**7.1±0.3**	**0**

Table 5.2. (Continued)

Hydraulic flow G/day	Digested Sludge										
	PFOS	PFDS	FOSAA	N-MeFOSAA	N-EtFOSAA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTA
29300	100±4	90.2± 4.1	9.4± 0.5	127±4	95.7± 3.3	1.8±0.3	10.3± 0.5	5.9± 0.3	6.1±0.3	3.8±0.2	<3
30900	160± 73	91.3± 2.7	9.6± 0.5	127±4	91.3± 4.1	<3	9.2±0.4	5.6± 0.2	5.9±0.4	3.8±0.2	<3
30800	91.6	89.9	12.4	139	101	<3	10.1	6.1	8.4	4.2	<3
29200	88.0	93.0	11.4	129	100	<3	10.2	6.4	6.4	3.6	nd
29800	81.1	92.6	10.2	127	101	nd	9.6	5.4	7.0	3.8	nd
30000	**104 ± 14**	**91.4 ± 0.6**	**10.6 ± 0.6**	**130 ± 2.3**	**97.8 ± 1.9**	**0.36 ± 0.36**	**9.9 ± 0.2**	**5.9 ± 0.2**	**6.8±0.4**	**3.8±0.1**	**0**

Table 5.2. (Continued)

Hydraulic flow G/day	PFOS	PFDS	FOSAA	Lagoon Sludge						
				N-MeFOSAA	*N*-EtFOSAA	PFOA	PFNA	PFDA	PFUnA	PFDoA
44,320	737± 26	223± 17	20.3± 0.5	333±8	302±9	11.1± 0.6	nd	9.8± 0.3	3.1±0	3.1±0.1
44,320	**737± 26**	**223± 17**	**20.3± 0.5**	**333±8**	**302±9**	**11.1± 0.6**	**nd**	**9.8± 0.3**	**3.1±0**	**3.1±0.1**

Table 5.2. (Continued)

				Raw Influent Particulate Matter				
PFHxS	PFOS	PFDS	FOSAA	*N*-MeFOSAA	*N*-EtFOSAA	PFOA	PFNA	PFDA
<2.78	8.73	15	<2.78	<5.56	7.43	<5.56	<2.78	<1.11
<2.63	4.79	11.68	3.35	<5.26	7.78	<5.26	<2.63	<1.05
<2.63	5.16	19.16	3.24	<5.26	7.6	<5.26	<2.63	<1.05
<2.38	2.53	8.49	3.37	<4.76	6.47	<4.76	5.31	<0.95
<2.94	5.05	14.24	3.65	<5.88	10.16	<5.88	<2.94	<1.18
	5.3 ± 1	13.7 ± 1.8	3.4 ± 0.1		7.9 ± 0.6		5.3	

Table 5.2. (Continued)

Primary Effluent Particulate Matter								
PFHxS	PFOS	PFDS	FOSAA	*N*-MeFOSAA	*N*-EtFOSAA	PFOA	PFNA	PFDA
<3.57	8.89	37.86	14.43	12.3	27.86	<7.14	<3.57	2.44
<2.08	2.67	25.08	2.67	4.55	11.83	<4.17	<2.08	1.12
<3.57	6.3	45.29	3.59	6.16	46.14	<7.14	<3.57	1.59
<4.17	13.3	88.17	9.53	19.83	53.33	<8.33	<4.17	4.13
<3.13	10.38	86.5	5.8	13.88	35.13	<6.25	<3.13	4.54
<2.50	9.61	42.6	5.18	13.4	38.2	<5	<2.5	2.59
<1.92	4.96	18.69	1.85	5.01	15.8	<3.85	<1.92	<0.77
	8.0 ± 1.4	49.2 ± 10.5	6.2 ± 1.7	10.7 ± 2.1	32.6 ± 5.7			2.7 ± 0.5

Solid-liquid partitioning of fluorochemicals. The solid-liquid partitioning of selected fluorochemicals was determined in grab samples of primary sludge, activated sludge, thickened sludge, anaerobically-digested sludge, raw influent and primary effluent (Figures 5.3a-c). The solid-water partitioning behavior of perfluorohexane sulfonate (PFHxS), PFOS, PFDS, PFOA, PFDA, perfluoroalkylsulfonamideacetic acid (FOSAA), and *N*-EtFOSAA was determined. The perfluoroalkyl sulfonate and carboxylate responses were detectable in both wastewater and sludge matrices. PFHxS responses in all solid matrices were detected at or below the quantitation limit; thus, the quantitation limit was used as the observed solid concentration to estimate its maximum solid partitioning behavior. PFOA was also detected at or below the quantitation limit in all solid matrices, except for the activated sludge; therefore its responses were treated in the same manner as PFHxS. FOSAA and *N*-EtFOSAA were not detected in the raw influent and primary effluent; thus, there are no reported values.

The solid-liquid partitioning of fluorochemicals is driven by the concentration of suspended solids (Figures 5.3a-c). At the low suspended solid concentrations (2.9 mg/L and 18.4 mg/L) present in the primary effluent and raw influent, the fraction of fluorochemicals sorbed onto suspended solids was less than 5%, except for PFDS which exhibited 30-40% sorption. The fraction of fluorochemicals on suspended solids increased as the concentration of suspended solids increased. In the thickened and digested sludges, each possessing suspended solid concentrations of 10,800 mg/L and

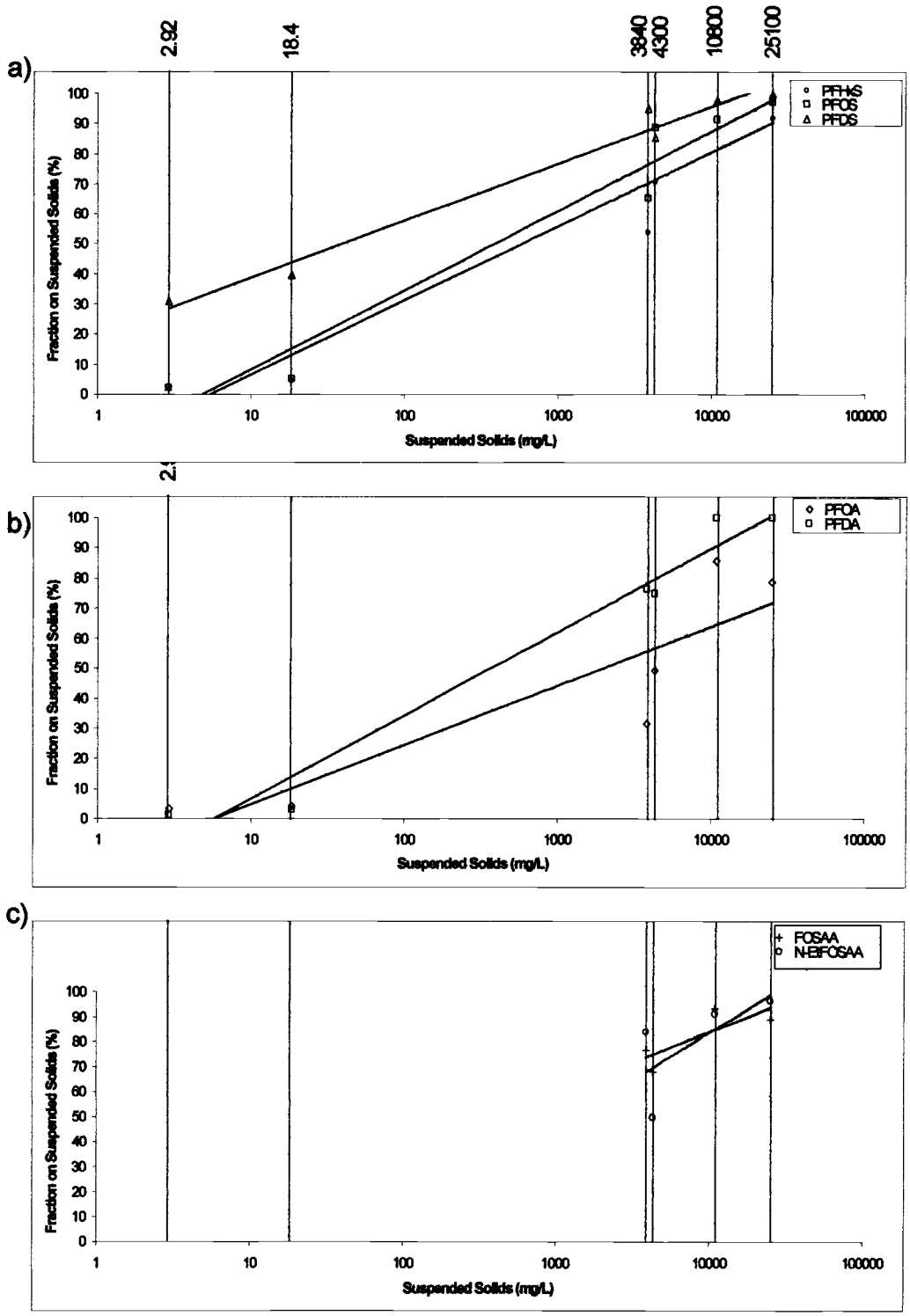


Figure 5.3.a-c. Partitioning of fluorochemicals to suspended solids in raw influent, primary effluent, primary sludge, activated sludge, thickened sludge, and digested sludge

25,100 mg/L, respectively, all fluorochemicals (> 78%) were mostly sorbed to suspended solids. The enhancement of the % sorbed fluorochemicals onto suspended solids in primary sludge and activated sludge as compared to primary effluent indicates that sorption onto sludge is an important removal process in the primary clarification and activated sludge treatment processes.

A solid-water partitioning pattern appeared within the perfluoroalkyl sulfonate and carboxylate classes. The affinity of the fluorochemicals to the solid phase increases with increasing carbon chain length (Figures 5.3a & b). This trend is consistent with previous observations from wastewater and sludge studies. In a wastewater study that analyzed influents and effluents, the shortest fluoroalkyl chain detected was PFBS, a fluoroalkyl sulfonate with four fluorocarbons and the longest fluoroalkyl chains observed contained 10 fluorocarbons, PFDS and PFDA, (32), whereas, *Higgins et al.* observed perfluorotetradecanoate (PFTA) in sludge, which has 14 fluorocarbons in fluoroalkyl chain (22). This observation is explained by the enhancement of hydrophobicity exhibited with increasing carbon chain lengths. Therefore, the longer the fluorocarbon chain, the more enriched the hydrophobic interaction of fluorocompounds with suspended solids.

The fluoroalkyl sulfonamide acetic acids, FOSAA and *N*-EtFOSAA, were not detected in the raw influent and primary effluent, and exhibited high fractions on suspended solids (50% - 96%), suggesting a high affinity for suspended solids. This observation is supported by previous studies where *Higgins et al.* detected FOSAA, *N*-EtFOSAA and *N*-MeFOSAA in sewage

sludge, often *N*-EtFOSAA and *N*-MeFOSAA were the most abundant fluorochemicals present in the sludge sample (22), and *Schultz et al.* did not find any evidence of *N*-EtFOSAA, *N*-MeFOSAA, or FOSAA in aqueous wastewater samples (32). Therefore, the presence of fluoroalkyl sulfonamide acetic acids in a wastewater treatment plant is most likely to be observed on suspended solids as opposed to in the wastewater since it has exhibited an affinity for partitioning onto the solid phase.

Daily and diurnal variations of fluorochemical mass flows. The daily variation of the mass flows for each fluorochemical class entering (raw influent) and exiting (final effluent) the plant are depicted in Figure 5.4. The total perfluoroalkyl sulfonates are comprised of PFHxS, PFOS, and PFDS. The total perfluoroalkyl carboxylates include PFHxA, PFOA, perfluorononoate (PFNA), and PFDA. 1H, 1H, 2H, 2H-perfluorooctane sulfonate (6:2 FtS) was the only fluorotelomer sulfonate observed during the sampling period. Likewise, perfluorooctanesulfonamide (FOSA) was the only fluoroalkyl sulfonamide detected in the wastewater matrix. The fluorochemicals raw influent mass flows have daily variation. For example, the mass flow entering the plant in the raw influent ranged from about 0.40 ± 0.04 g/day to 1.3 ± 0.05 g/day, 0.13 ± 0.03 g/day to 0.36 ± 0.02 g/day, and 0.82 ± 0.04 g/day to 1.4 ± 0.05 g/day for total perfluoroalkyl sulfonates, 6:2 FtS, and total perfluoroalkyl carboxylates, respectively (Figure 5.4). FOSA was not observed in the raw influent. The fluorochemical final effluent mass flows exhibited not only daily variation, but also different trends in their observed removals or increase in the

final effluent. For example, the total perfluoroalkyl sulfonates show significant increases, by at least a factor of 2, their final effluent for Wednesday (July 21), Friday (July 23), and Saturday (July 24), however, on Thursday (July 22) there is little change, and Sunday (July 25) exhibits some overall removal (~25% decrease) in the final effluent (Figure 5.4). 6:2 FtS showed slight (<0.1 g/day) increases or decreases for 7 out of the 10 days, a large effluent increase (0.30 g/day) on one day, and the influent and effluent mass flows remained constant for two days (Figure 5.4). The total perfluoroalkyl carboxylates and FOSA had consistent effluent trends. The total perfluoroalkyl carboxylates mass flows indicate significant removals in the effluent; the decreased concentrations ranged from 0.1 g/day – 0.88 g/day (Figure 5.4). FOSA showed significant increased mass flows in the effluent for 9 out of the 10 days sampled (Figure 5.4). No distinct patterns emerged between weekday fluorochemical usages as compared to the weekend.

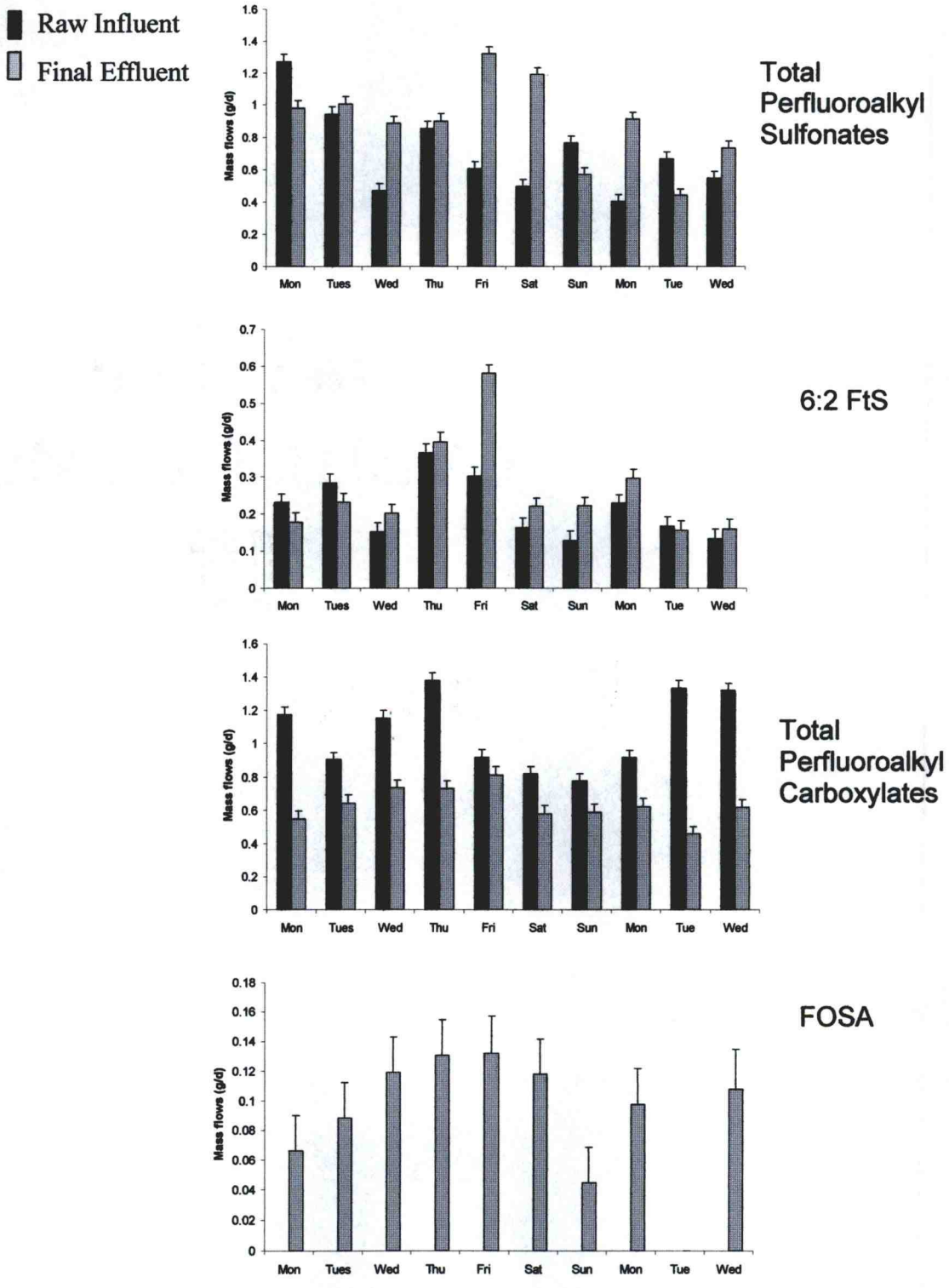


Figure 5.4. Daily variations of fluorochemical mass flows in the raw influent and final effluent

The diurnal variation of the predominant fluorochemicals in wastewater, PFHxS, PFOS, 6:2 FtS, PFHxA, PFOA, and FOSA, was determined during the mass flow study by collecting grab samples for 27 consecutive hours of the raw influent and final effluent beginning on day 4 at 8 AM and culminating on the morning of day 5 at 10 AM. There was good agreement between the average fluorochemicals concentration of the grab samples as compared to the concentrations of the flow-dependent 24 h composites collected during the same 24 h period (Table 5.3). The concentrations of PFHxS, PFOS, PFHxA, and PFOA showed heightened activity during the daytime hours of 8 AM through 6 PM (hour 20) (Figure 5.5). There was no significant variation of the concentration of 6:2 FtS in the raw influent. FOSA was not detected in the raw influent. The effluent levels remained almost constant for PFHxS, PFHxA, PFOA, and FOSA. The PFOA and FOSA effluent levels were higher than the lowest influent concentrations. The constant fluorochemical concentrations observed in the four analytes could be somewhat attributed to the activated sludge treatment. Activated sludge has a longer solids retention time (6 - 7 d) than wastewater and has concentrations at least 100x higher than the fluorochemical concentrations present in the wastewater. Since the partitioning of fluorochemicals is driven by the concentration of suspended solids, the activated sludge treatment can either remove fluorochemicals by sorption, or depending on their concentration in the wastewater, activated sludge could also act as a source, due to the high observed fluorochemicals concentrations.

PFOS and 6:2 FtS exhibit elevated effluent concentrations during the nighttime hours, approximately 9:00 PM (hour 21, Thursday) to 8 AM (Friday). As the residence time of the plant is approximately 8-10 h, the high effluent concentrations in the evening could be a result of heightened influent concentrations observed earlier in the day; however, the 6:2 FtS influent concentrations were constant. A possible explanation for the enhanced 6:2 FtS effluent concentrations could be a result of biodegradation of precursor molecules. It was proposed that 6:2 FtS is a degradation product of a parent compound, fluoroalkylthioamidosulfonates (15). This precursor compound or other similar compounds have the potential to degrade to 6:2 FtS during the biologically active trickling filters or activated sludge treatment. The abundant PFOS effluent concentrations could be a result of the residence time it takes for influent to make it through the plant, desorption of PFOS from activated sludge, or degradation of precursor molecules. Preliminary results have shown the formation of PFOS from *N*-ethyl-*N*-(2-hydroxyethyl)perfluorooctanesulfonamide (*N*-EtFOSE) by microbial activity present in municipal wastewater treatment sludge (33).

Table 5.3. Comparison of flow-dependent 24 h composite concentrations to the average concentration of combined 24 h grab samples collected during the same time period.[a] (ng/L)

Fluorochemical	24 h composite raw influent	24 h combined grab raw influent	24 h composite final effluent	24 h combined grab final effluent
PFHxS	5.8	7.7	3	3.8
PFOS	11	8.9	31.3	18
6:2 FtS	10.3	6.1	19.8	20.7
PFHxA	16.6	9.8	5.7	3.3
PFOA	10.3	11.9	14.6	16
FOSA	nd	nd	4.5	8.8

[a]nd = not detected

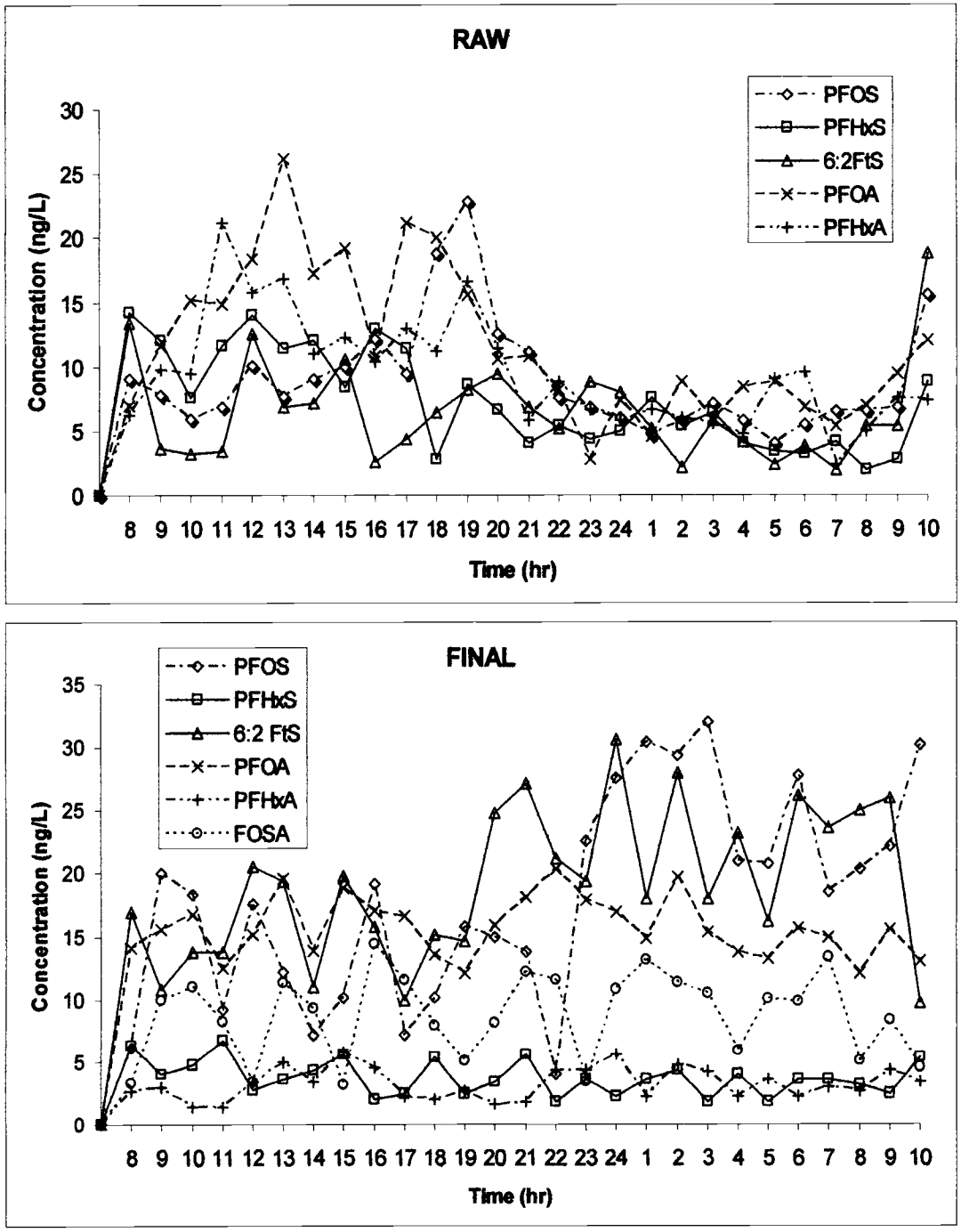


Figure 5.5. Diurnal variation of fluorochemical concentrations in raw influent and final effluent

Fluorochemicals observed in sludge treatment. The sludge analyzed in this study contained significant concentrations of fluorochemicals. Five types of sludge were analyzed, including primary, thickened, activated, anaerobically-digested, and storage lagoon sludge (Figure 5.2). Approximately 99% of the activated sludge that leaves the secondary clarifier is fed back to the aerobic basin as recycled activated sludge (RAS) (Figure 5.2). The remaining 1% of activated sludge is sent to the thickener as waste activated sludge (WAS). Primary sludge and WAS combine in the thickener, where dewatering occurs for approximately one day. With the exception of PFOS, the addition of the mass flows of primary sludge and WAS were in good agreement with the concentration of the fluorochemicals observed in the thickened sludge (Figure 5.6). The digested sludge has a residence time of 30 days. Increases of 38 ± 11%, 102 ± 6%, and 25 ± 3% for PFOS, *N*-MeFOSAA, and *N*-EtFOSAA, respectively, were observed in the digested sludge as compared to the levels in the thickened sludge (Figure 5.6). There was no significant change in the remaining fluorochemicals.

The storage lagoon sludge offered a unique opportunity to analyze anaerobically-digested sludge that has a residence time of one year in an open-air basin. The % increases observed in the lagoon sludge as compared the digested sludge were 1400 ± 240%, 520 ± 50%, 620 ± 70%, 500 ± 50%, 380 ± 2% and 330 ± 1% for PFOS, *N*-MeFOSAA, *N*-EtFOSAA, PFDS, FOSAA, and PFDA, respectively (Figure 5.6). As the lagoon is an open-air lagoon and thus likely has a small oxic layer at the surface in addition

to significantly anaerobic layers below the surface, it is unclear whether the higherlevels observed reflect aerobic or anaerobic transformation of parent compounds such as *N*-EtFOSE.

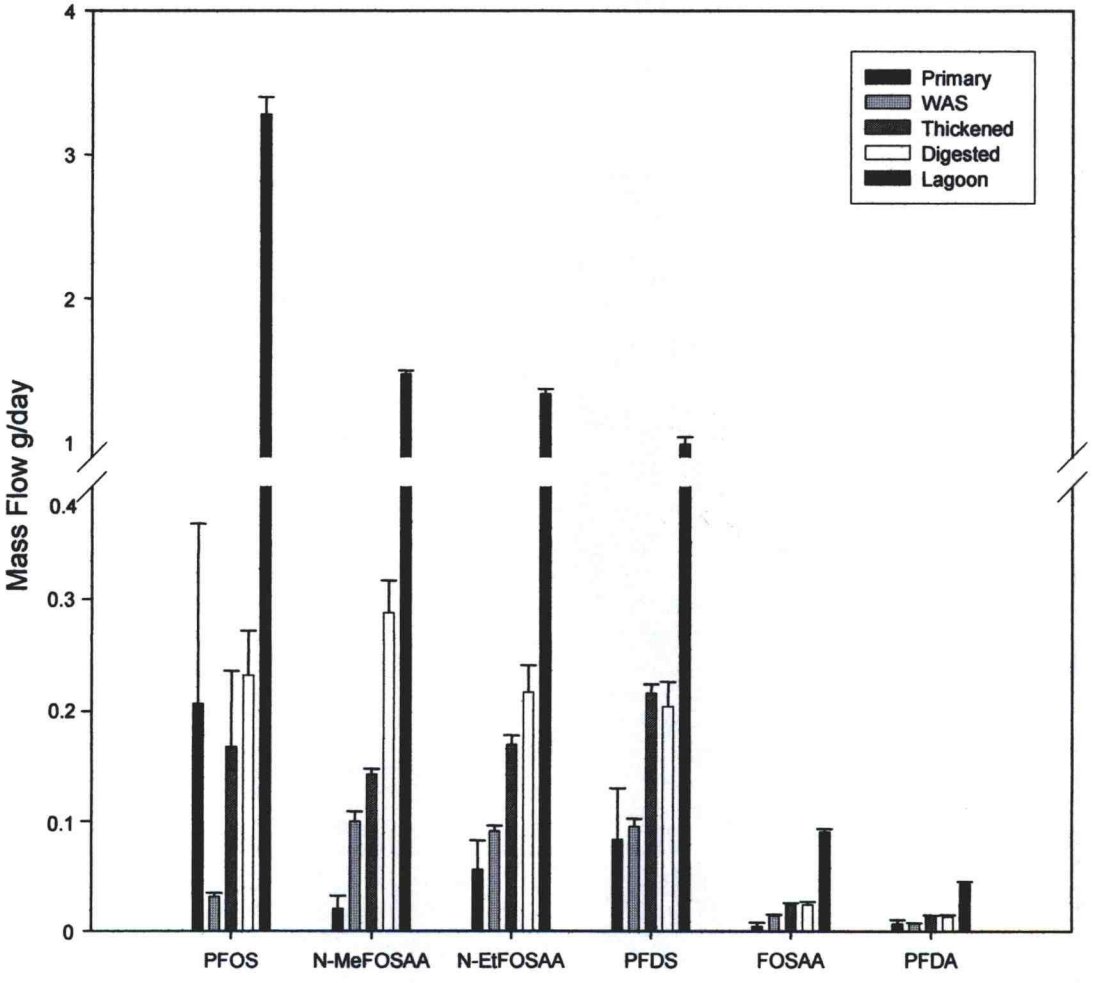


Figure 5.6. Fluorochemical mass flows in primary sludge, waste activated sludge (WAS), thickened sludge, digested, and storage lagoon sludge.

Mass flows of fluorochemicals during wastewater and sludge treatment. Mass balances of PFOS, PFDS, PFOA, and PFDA throughout a wastewater treatment plant were determined (Figure 5.7). These analytes were selected for this study because complete data sets were obtained for these analytes in both the wastewater and sludge matrices. The average mass flows were determined from the sum of analyte concentrations present in both wastewater raw influent and sorbed to the particulate matter present in the influent. The average mass flows of PFOA, PFDA, PFOS, and PFDS into the plant were 0.400, 0.151, 0.417, and 0.172 g/day respectively (Table 5.4).

Table 5.4. Average fluorochemical mass flows (g/day)[a]

	PFOS	PFDS	PFOA	PFDA
Raw Influent[b]	0.417	0.172	0.400	0.151
Primary Effleunt[b]	0.484	0.208	0.145	0.169
Trickling Filter Effluent	0.717	0.443	0.232	0.869
Secondary Effluent	0.552	0.0979	0.302	0.0709
Final Effluent	0.643	0.218	0.305	0.0620
Primary Sludge	0.206	0.0836	0.00273	0.00685
Thickened Sludge	0.167	0.215	<0.0186	0.0138
Digested Sludge	0.231	0.203	0.000998	0.0132
Waste Activated Sludge	0.0319	0.0953	0.00490	0.00673
Recycled Activated Sludge	3.36	10.0	0.515	0.708

[a]Daily mass flows were averaged over the duration of the study.
[b]Mass flow value determined from the sum of adsorbed and dissolved fluorochemicals.

Perfluoroalkyl Carboxylates. Only 76±9% and 56±12% of the initial mass flow for PFOA and PFDA were accounted for in the mass balance. PFOA exhibited 30% removal in the primary effluent and a 17% removal in the trickling filter effluent (Figure 5.7). With the exception of the 12% increase (which was not statistically different than the initial mass flow) in the primary effluent, PFDA was removed in each stage of wastewater treatment, exhibiting a 49% removal in the trickling filter effluent, a 19% reduction in the secondary effluent, and a 13% decrease in the final effluent. Aerobic biodegradation of perfluoroalkyl carboxylates is unlikely since earlier studies have not shown transformation of these compounds under those conditions (34). Furthermore, previous research has shown that perfluoroalkyl carboxylates are degradation end products of fluorotelomer alcohols under aerobic conditions (21,34-36). Previous work found that in 8 out of the 10 plants sampled, perfluoroalkyl carboxylate effluent concentrations were significantly higher than the influent levels, also suggesting the possibility of aerobic biodegradation of fluorotelomer alcohols or other precursor molecules to perfluoroalkyl carboxylates (32). However, the current plant, where the mass balance was conducted, was one of the two plants that showed significant decreases in the total perfluoroalkyl carboxylate concentrations. A possible explanation for the observed removals of perfluoroalkyl carboxylates during wastewater treatment may be explained by an affinity for the trickling filter media. This partitioning behavior was never determined and both PFOA and PFDA mass flows exhibited removals in the trickling filter effluents as compared to the primary

effluents. It is also possible that fluorotelomer alcohols or other potential precursor compounds are not present in the waste treated at this plant. In addition, the PFDA overall plant concentrations were low (raw influent = 5.6 ± 0.7 ng/L; final effluent = 2.3 ± 0.5 ng/L, Table 5.1), suggesting analytical variability at the lower limits of quantitation may impact the percent removals described here.

There was no evidence of anaerobic degradation of perfluoroalkyl carboxylates in the thickeners or the anaerobic digestor (Figure 5.7). For PFOA, 1.9±0.4% of the initial mass flow entered the thickener as combined primary sludge and WAS. Although there were detectable levels of PFOA in the thickened sludge, the responses were not quantifiable. After 30 days of anaerobic digestion, 0.25±0.01% of the corresponding influent levels of PFOA was observed in the anaerobically-digested sludge. Comparison of the mass flows of PFDA associated with primary sludge, WAS, thickened sludge, and anaerobically-digested sludge also yielded very good agreement despite the fact that the digested sludge sampled during this study does not correlate with the primary sludge due to the long residence time in the digestor (30 days). There was 9±3 % of the intitial flow entering the thickener, 9.1±4.8% of PFDA present in the thickened sludge, and 8.7±1.7% in anaerobically-digested sludge.

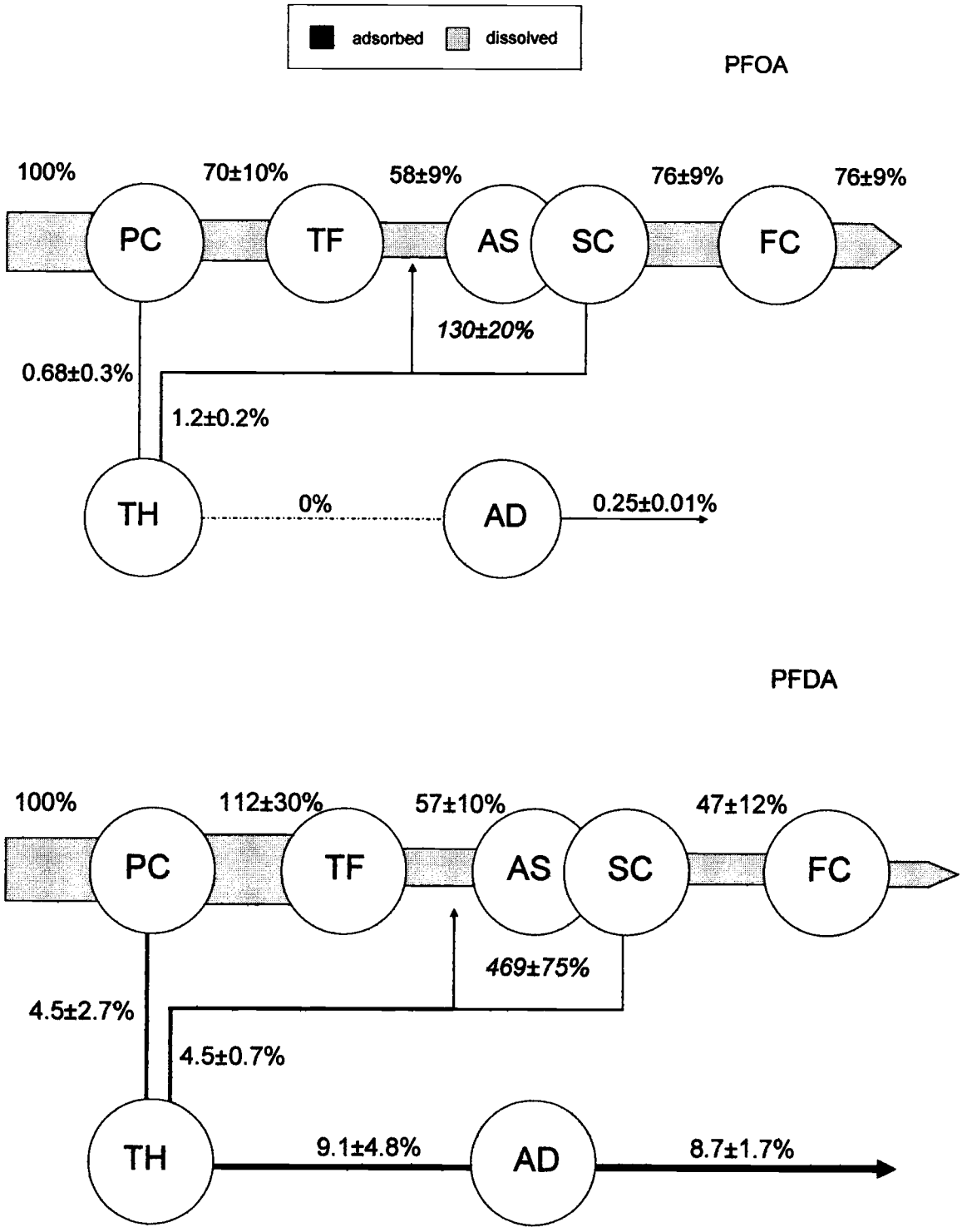


Figure 5.7. Mass flows of PFOS, PFDS, PFOA, and PFDA throughout the wastewater treatment plant.[a,b]

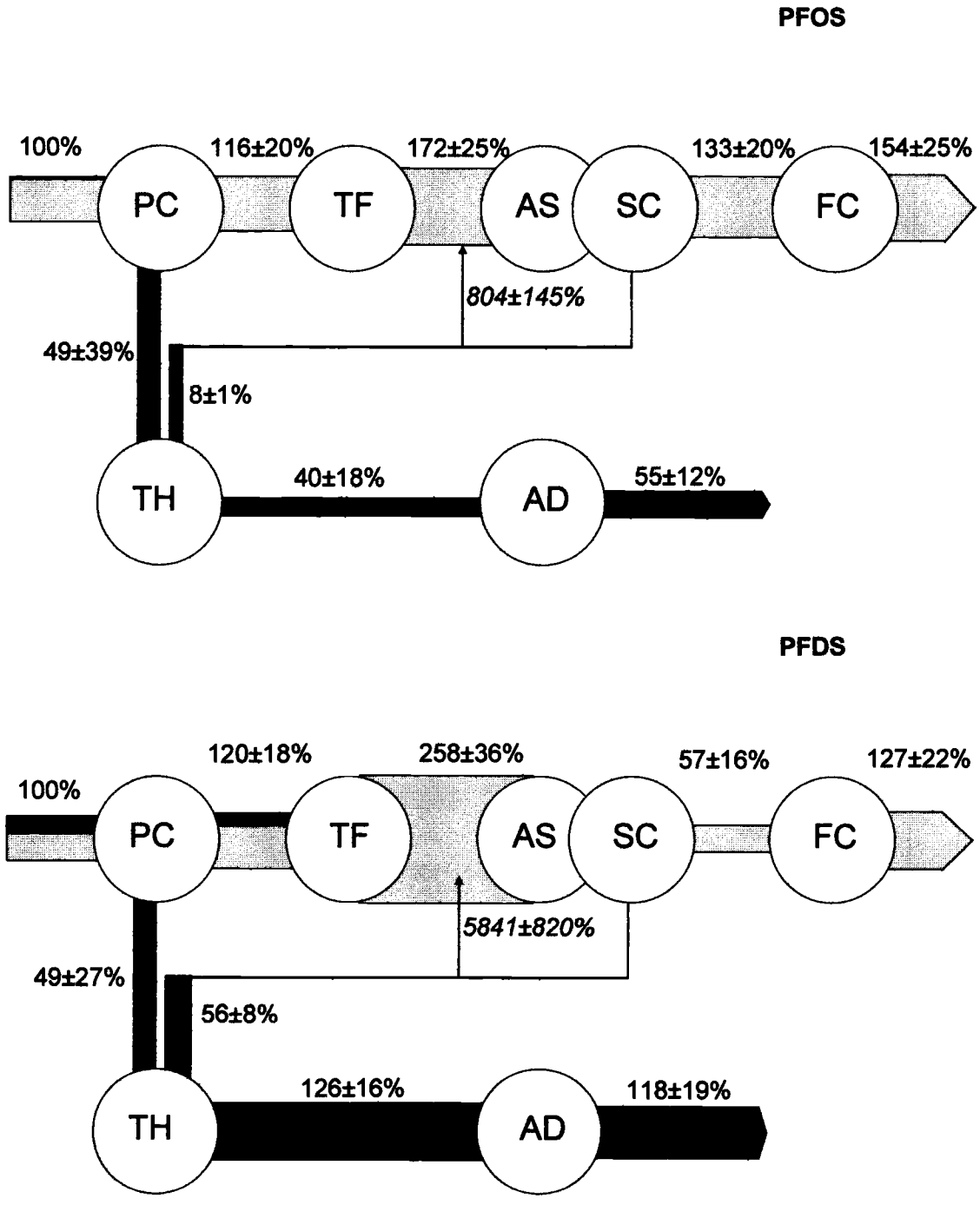


Figure 5.7. (Continued)

[a]PC denotes primary clarifier, TF denotes trickling filter, AS denotes activated sludge, SC denotes secondary clarifier, FC denotes final stage chlorination/dechlorination, TH denotes thickener, AD denotes anaerobic digestor.
[b]Recycled Activated Sludge (RAS) arrow not drawn to scale.

Perfluoroalkyl Sulfonates. After primary clarification, approximately 50% of PFOS and PFDS in the raw influent was removed upon settling as primary sludge (Figure 5.7); however, there was a 16% and 21% increase, respectively, in the mass flow of PFOS and PFDS present in the primary effluent, suggesting that biodegradation of precursor compounds may have occurred during the detention time (~1.2 h) in the primary clarifier. However the observed increases in the primary effluent for PFOS and PFDS are within the statistical variability, thus it is difficult to ascertain if the increases are valid. Increased concentrations were also observed for the *N*-MeFOSAA and *N*-EtFOSAA in the primary effluent particulate matter as compared to the raw influent particulate matter (Table 5.2). The fluoroalkyl sulfonamidoacetates are intermediates in the biodegradation pathway to the perfluoroalkyl sulfonates (37). There was an additional concentration increase of PFOS (48%) and PFDS (112%) during the biologically active trickling filter treatment as compared to the primary effluent levels. However, the activated sludge treatment (followed by secondary clarification) removed 23% and 78%, respectively, of the PFOS and PFDS present in the trickling filter effluent. This was in contrast to the chlorination and dechlorination processes, which resulted in increases of PFOS (16%) and PFDS (223%) relative to the secondary clarified effluent. The masses of PFOS and PFDS in the final effluent were 154±25% and 127±22% of the corresponding influent concentrations, indicating production of PFOS and PFDS during wastewater treatment.

There appears to be no significant production of PFOS and PFDS during the 30 day sludge treatment process (Figure 5.7). PFOS present in the combined WAS and primary sludge was 57±39% relative to the initial mass flow. There was 40±18% and 55±12% of PFOS present in the thickened and digested sludge, respectively, as compared to the initial mass flow. The variation observed in the different sludge mass flows for PFDS was also determined not to be statistically different. PFDS in the combined WAS and primary sludge that entered the thickener was 105±28% relative to the initial mass flow. PFDS was present in the thickened sludge and digested sludge contained 126±16% and 118±19%, respectively, as compared to the initial mass flow.

There is an overall 209±28% and 245±29% production of PFOS and PFDS observed when the mass flows of PFOS and PFDS exiting (in the final effluent and in the digested sludge) are combined and compared to the mass flow entering the plant (raw influent) (Figure 5.7). The production of PFOS and PFDS observed in the aqueous stream was also seen in the storage lagoon sludge (Figure 5.6) as was the production of the fluoroalkyl sulfonamide acetic acids, *N*-MeFOSAA and *N*-EtFOSAA. This evidence indicates that precursor compounds, such as *N*-EtFOSE, may be present in the wastewater treatment plant. Further research is needed to develop analytical methods for the determination of *N*-EtFOSE and other semi-volatile fluoroalkyl precursor compounds in wastewater and sludge to ascertain if their presence is contributing to the overall production of PFOS, PFDS,

N-MeFOSAA, and *N*-EtFOSAA observed in this study.

Discharge of fluorochemicals to aqueous and terrestrial environments. The mass flow data obtained from this study can be used to estimate the annual discharge of fluorochemicals to the environment via discharged wastewater effluent, land application of biosolids, or deposits to landfills. During the duration of the 10 d study, the total mass flow in the final effluent for all observed fluorochemicals was 1.9 g/day (Table 5.5). Assuming no seasonal variation, this corresponds to a total mass flow of 697 g/year of fluorochemicals introduced to the environment via wastewater final effluent from the observed plant. Using the data acquired from the storage lagoon sludge, which has a residence time of 1 year, the total fluorochemical discharge in generated biosolids is 7 g/day, which correlates to an annual biosolid disposal of 2670 g/year (assuming no seasonal variation) from the wastewater treatment plant (Table 5.5). At the monitored plant, there is 100% land application of generated biosolids.

Table 5.5. Estimated daily and annual fluorochemical mass flows of final effluent and biosolids discharged from wastewater treatment plants.[a]

[a]FE denotes final effluent.

[b]plant refers to the wastewater treatment where the mass balance was conducted.

	plant[b] FE avg conc. ng/L	plant[b] FE mass flow g/day	plant[b] FE mass flow g/year	national FE mass flow[c] g/day	national FE mass flow[c] g/year	plant[b] biosolids avg conc. ng/g (dry wt)	plant[b] biosolids mass flow g/day	plant[b] biosolids mass flow g/year
PFHxS	1.2	0.0	11.7	146	53400	<2.5	0	0
PFOS	24.1	0.6	235	2940	1070000	688	3.3	1200
PFDS	8.2	0.2	80.0	1000	365000	232	1.0	362
6:2 FtS	9.8	0.3	95.6	1195	436000	na	0	0
PFHxA	6.4	0.2	62.5	780	285000	na	0	0
PFOA	11.4	0.3	111	1390	507000	10	0.050	18.1
PFNA	3.4	0.1	33.2	415	151000	<2.5	0	0
PFDA	2.3	0.1	22.4	280	102000	9	0.044	16.0
PFUnA	na	0	0	0	0	3	0.014	5.1
PFDoA	na	0	0	0	0	3	0.014	5.1
PFTA	na	0	0	0	0	<2.5	0	0
FOSA	4.6	0.1	44.9	561	205000	na	0	0
FOSAA	nd	0	0	0	0	19	0.091	33.1
***N*-MeFOSAA**	nd	0	0	0	0	310	1.5	541
***N*-EtFOSAA**	nd	0	0	0	0	281	1.3	491
TOTAL	**71.4**	**1.9**	**697**	**8710**	**3180000**	**1560**	**7**	**2670**

Table 5.5. (Continued)

[c]Using a conservative estimate that 32,175 million of gallons of wastewater is treated daily in the United States{Environmental Protection Agency, 1996 #318}.

[d]Estimate that 7.6 million tons of biosolids will be generated during one year{Environmental Protection Agency, 1999 #319}

[e]"Beneficially used" denotes that the biosolids were either land applied, composted, or used as landfill cover and is estimated to be 66% of the total biosolids generated{Environmental Protection Agency, 1999 #319}

Table 5.5. (Continued)

national biosolids mass flow kg/day[d]	Biosolids beneficially used[e] kg/day[d]	national biosolids mass flow kg/year[d]	Biosolids beneficially used[e] kg/year[d]
0	0	0	0
13.0	8.6	4740	3130
4.4	2.9	1600	1060
0	0	0	0
0	0	0	0
0.189	0.125	68.9	45.5
0	0	0	0
0.170	0.112	62.0	40.9
0.057	0.037	20.7	13.6
0.057	0.037	20.7	13.6
0	0	0	0
0	0	0	0
0.359	0.237	131	86.4
5.9	3.9	2140	1410
5.3	3.5	1940	1280
29.4	**19.4**	**10700**	**7080**

The assumption is made that the wastewater treatment plant where the mass flow study was conducted is representative of wastewater treatment plants nationwide. With this assumption and using a conservative estimate that there is 32.175 billion gallons per day of wastewater currently being treated in the United States at publicly-owned treatment works (POWTs) (38), fluorochemicals are discharged in wastewater effluents to surface waters at a rate of 8710 g/day or 3180 kg/year in the United States (Table 5.5). Approximately 7.6 million tons of biosolids are generated annually at POWTs in the United States (39). When using this value, fluorochemical production of 29.4 kg/day and 10,700 kg/year in biosolids was estimated (39). About 66% of the generated biosolids are "beneficially used" (e.g. land applied, composted, or used as landfill cover) (39). Therefore, it can be estimated that fluorochemicals are introduced to terrestrial environments at a rate of 19.4 kg / day or 7080 kg /year. Assuming the previous assumptions are valid, wastewater treatment plants are point sources of fluorochemicals and their contributions cannot be overlooked when assessing the origins and fate of fluorochemicals in the environment.

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6. SUMMARY AND CONCLUSIONS

For the last half of the century, fluorinated alkyl substances have been widely used in an array of industrial and commercial applications, including cleaners, coatings, fire-fighting foams, and stain repellants for furniture, carpets, and clothing. As a result of the widespread use, evidence for its global distribution was found when it was identified in tissues of wildlife, including, fish, birds, and marine mammals, collected from urbanized areas in North America and Europe and in less urbanized locations such as the Arctic and Pacific Ocean, where there are no known sources. Fluorinated alkyl substances are also detected in blood serum at low parts-per-billion concentrations in nonoccupationally-exposed humans.

Little is known about the sources and behavior of fluorinated alkyl substances in the environment. Municipal wastewater treatment is one of the principal routes for disposing of aqueous-borne surfactant waste to the environment; therefore, treated wastewater is potential environmental point source for introducing fluorinated alkyl substances. It is particularly important to understand how the mass flow and composition of mixtures comprising of fluorinated alkyl substances change as they pass through wastewater treatment facilities and how different types of treatment affect their removal. In attempt to analyze for the fluorinated alkyl substances, known aqueous-film-forming-foam (AFFF)-contaminated groundwater sampled from military bases was used to develop the liquid chromatography, electrospray ionization, tandem mass spectrometer (LC ESI-MS/MS method). (AFFF contains

perfluoroalkyl carboxylates and sulfonates in its formulations). While working on the method development, fluorotelomer sulfonates, which were intended to be used as internal standards and surrogates, were discovered in the groundwater. These included 1H, 1H, 2H, 2H-perfluorooctane sulfonate (6:2 FtS) and 1H, 1H, 2H, 2H-perfluorodecane sulfonate (8:2 FtS). To our knowledge, this is the first discovery of fluorotelomer sulfonates in the environment.

Fluorotelomer sulfonates were observed and quantified at Tyndall AFB and Wurtsmith AFB. To better understand the origin of fluorotelomer sulfonates, negative fast atom bombardment mass spectrometry (FAB/MS) in low and high resolution mode was used to analyze a fluorotelomer-based AFFF product sold on contract to the military. A high molecular chemical class was observed in low resolution and then high mass accuracy measurements were used to identify the class as fluorotelomer-based alkylthiosulfonates. Precursor-ion detection and product-ion fragmentation patterns acquired by LC ESI MS/MS confirmed this identification. In addition, LC ESI MS/MS analyses of the AFFF product indicated trace levels of telomer sulfonates. LC ESI MS/MS analyses indicated that none of the parent fluorotelomer-based alkylthiosulfonates were present in groundwater at any of the military sites. It has yet to be determined if the fluorotelomer sulfonates are from the original AFFF applied or a degradation product of the fluorotelomer-based alkylthiosulfonates.

Early attempts to analyze municipal wastewaters by direct injection LC MS/MS, the method used for determination of fluorinated alkyl substances in groundwater, proved unsuccessful because the levels of fluoroalkyl substances were at or below the detection limits, which were around 0.3 ppb. High-volume-injection, instead of solid-phase-extraction (SPE), was chosen as a sample concentration step because high-volume-injection requires less time and resources for method development, and there is no loss of analytes in the concentration process. By using a large volume sample loop, the detection limits were increased by at least a factor of 50. Centrifugation was used as the sample clean-up step. This high-volume-injection LC ESI-MS/MS method was applied to 24 h flow dependent composite wastewater influents and effluents collected from 10 plants nationwide.

Fluorinated alkyl substances were observed in wastewater at all treatment plants sampled. Each plant displayed a unique footprint of fluorinated alkyl substances, despite similar treatment processes, suggesting that the complexity of the source is a significant contributor to the fluorinated alkyl substance distribution observed in the effluent. In 9 out of the 10 plants sampled, at least one class of fluorinated alkyl substances, perfluoroalkyl sulfonates, fluorotelomer sulfonates, perfluoroalkyl carboxylates, and/or fluoroalkyl sulfonamides, exhibited significant increased concentrations in the effluent as compared to the influent concentrations. Detection of these analytes in final effluent at the ng/L level indicates that wastewater treatment plants are point sources of fluorinated alkyl substances.

More detailed studies were required to ascertain whether the trends observed with the "snapshot" wastewater data were representative of the impact wastewater treatment had on fluorinated alkyl substances. In addition, further information was desired to better understand the importance of each stage of wastewater treatment on the fate of fluorochemicals, whether it was transfer between aqueous and particulate phases, production or transformation of production. Therefore, a 10 d mass balance field study was conducted collecting both wastewater and sludge samples in the attempt to perform a mass balance of fluorinated alkyl substances through the wastewater treatment plant.

Perfluoroalkyl sulfonates, perfluoroalkyl carboxylates, fluorotelomer sulfonates, and perfluorooctane sulfonamide were all observed in the aqueous stream. The predominant species in the sludge was the fluoroalkyl acetic acids and perfluoroaklyl sulfonates. Daily variation was observed for both the raw influents and final effluents. No distinct patterns emerged between weekday fluorochemical usages as compared to the weekend.

Mass balances were performed on PFOS, perfluorodecane sulfonate (PFDS), perfluorooctanoate (PFOA), and perfluorodecanoate (PFDA). These analytes were chosen for this study because complete data sets were obtained for these analytes in both the wastewater and sludge matrices. Only 76±9% and 56±12% of PFOA and PFDS, respectively, were accounted for in the final effluent. Aerobic degradation of perfluoroalkyl carboxylates is unlikely since previous studies have not shown transformation of these compounds

under those conditions. There was also no evidence of anaerobic digestion of perfluoroaklyl carboxylates in the thickeners or the anaerobic digestor (total residence time ~30 days). However, a 331±2% of PFDA was observed in storage lagoon sludge, which has a residence time of one year. The lagoon is an open air lagoon, likely subject to a thin oxic layer with multiple layers of anaerobic layers below the surface. Therefore, it is unclear whether the higher levels reflect aerobic or anaerobic transformation.

There is an overall 209±28% and 245±29% production of PFOS and PFDS observed when the mass flows of PFOS and PFDS exiting (in the final effluent and in the digested sludge) are combined and compared to the mass flow entering the plant (raw influent). Little to no transformation of precursor compounds to PFOS and/or PFDS was found to occur in the 30 day digestor. Thus, the apparent concentration increase resulted from aerobic degradation of *N*-(ethylperfluorooctanesulfonamido) ethanol (*N*-EtFOSE) or other fluoroalkyl precursor compounds during the trickling filters and/or aeration basin. The production of PFOS and PFDS observed in the aqueous stream was also seen in the storage lagoon sludge (1400±240% and 500±50%, respectively) as was the production of the fluoroalkyl sulfonamide acetic acids (>500%) This evidence further suggests the presence of *N*-EtFOSE and other fluoroalkyl precursor compounds within the treatment plant. Further research is needed to develop analytical methods for the determination of *N*-EtFOSE and other semi-volatile fluoroalkyl precursor compounds in wastewater and

sludge to ascertain if their presence is contributing to the overall production of PFOS, PFDS and the fluoroalkyl acetic acids.

One of the questions this study addressed was what role wastewater treatment plays in the release of fluorochemicals to the environment. When the assumption is made that the monitored plant is representative of wastewater treatment plants nationwide, fluorochemicals are discharged in wastewater effluents at a rate of 3180 kg/year and are introduced to terrestrial environments via biosolids at a rate of 7080 kg/year. If this assumption is valid, wastewater treatment plants are point sources of fluorochemicals and cannot be overlooked when determining origins and fate of fluorochemicals in the environment.

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