ABSTRACT

Title of Document: TEMPORAL TRENDS OF AND INFLUENCE OF STORAGE METHODS ON CONCENTRATIONS OF PERFLUOROALKYL SUBSTANCES IN LIMED MUNICIPAL WASTEWATER BIOSOLIDS

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Perfluoroalkyl substances (PFASs) are a classification of anthropogenic chemicals used in a variety of consumer and industrial products. Compounds from two PFAS subgroups, perflurocarboxylic acids (PFCAs) and perfluorosulfonic acids (PFSAs) are known to be persistent and have been detected in environmental and biotic samples worldwide. While long-chain PFCAs and PFSAs have been in a phase-out process within the United States and some have been regulated in Europe, these compounds have continued to be produced in developing countries. The sustained use of PFCA and PFSA compounds in consumer products, as well as the ability of some PFASs to degrade into these compounds, has led to their presence in the wastewater treatment (WWT) process. This study analyzes archived limed biosolids from a municipal WWT plant for temporal trends of 8 PFCAs and 4 PFSAs over an eight year period. This study also compares storage methods to determine influence on PFCA concentrations.

TEMPORAL TRENDS AND INFLUENCE OF STORAGE METHODS ON CONCENTRATIONS OF PERFLUOROALKYL SUBSTANCES IN LIMED MUNICIPAL WASTEWATER BIOSOLIDS

by

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CHAPTER 1: BACKGROUND INFORMATION AND RESEARCH JUSTIFICATION

1.1 WASTEWATER SOLIDS

Modern advanced wastewater treatment (WWT) processes are in place in the United States (USA) as a means of reducing concentrations of pathogens, suspended particles, nutrients, and organics prior to effluent release into natural water bodies. During this process, both organic and inorganic solids are removed from the wastewater and the resulting sludge requires both treatment and disposal. In the USA, these sludges are typically treated and then land-applied for nutrient recovery/soil reclamation or sent to a landfill or incinerated for disposal (Laturnus et al. 2007; NEBRA 2007; USEPA 2009c).

1.1.1 BIOSOLIDS AS AGRICULTURAL AMENDMENTS

The use and disposal of wastewater sludge for land application in the USA is regulated under 40 CFR Part 503 as a way to manage any potential health or environmental risks associated with the material. Current regulations include limits on heavy metal concentrations as well as application restrictions based on human pathogen concentrations. (Itanpour et al. 2004; USEPA 1993; USEPA 2009a) These pathogen requirements set forth by the United States Environmental Protection Agency (USEPA) ensure that the wastewater sludges are treated prior to land application. Common treatment processes include anaerobic digestion, stabilization with lime, composting, and heat treatment (H. Wang et al. 2008). The treated sewage sludge, or biosolids, is then classified based on pathogen counts within the material. Class A biosolids are defined as those with fecal coliform densities of less than 1000 most probable number (MPN) per gram (g) of dry weight solids or densities of *Salmonella* sp. of less than 3MPN per 4g of

dry weight solids. Class B biosolids, despite treatment, have detectable pathogen counts and, therefore, restrictions on use, including limitations to: application frequency, harvesting, animal grazing, and public contact. All land-applied biosolids must meet concentration limits for heavy metals. (USEPA 1993)

The land-application of biosolids has been shown to be a sustainable and cost-effective way to improve upon soil properties, through the addition of organic matter, as well as a route to introduce micro- and macronutrients into soils (M. Guo et al. 2012; Robinson et al. 2012; Goss et al. 2013; Wuest & Gollany 2013). The addition of organic matter to soils is desirable in that it can improve upon the physical and chemical properties of soil. This can include an enhancement of the cation exchange capacity, soil structure, porosity, water holding capacity, and hydraulic conductivity as well as decrease the bulk density of the soil (H. Wang et al. 2008; Goss et al. 2013). Sludge amendments to soil have also been shown to promote plant growth in agricultural settings (Antolín et al. 2010) as well as in urban landscapes (Scharenbroch et al. 2013), further supporting the positive impact the product can have.

In 2004, an estimated 55% of biosolids produced in the USA were utilized for land application purposes, 79% of which were applied for agricultural use (NEBRA 2007). Current approximations of biosolid use in the USA show that 60% of the estimated 7 million tons produced yearly are utilized for land application (Egan 2013). However, the use of biosolids as a sustainable fertilizer is not without concern. There is evidence of varying public attitudes within the public regarding the land-application of biosolids. More specifically, despite public acknowledgement of the beneficial uses of biosolids, there are perceived health and safety concerns from land applications (Itanpour

et al. 2004; Robinson et al. 2012; USEPA 1993; Lowman et al. 2013; USEPA 2009a). Concerns regarding the health effects of the land-application of biosolids typically arise more commonly from residents who live within close proximity to application areas (Robinson et al. 2012) and are often associated with emitted odors, the potential for pathogen presence, and reported acute health complaints from the application process itself, including eye, nose, and throat irritation and gastrointestinal tract and skin infections from the aerosolization of biosolid dust particles (Lowman et al. 2013; Robinson et al. 2012). Additionally, there is growing scientific evidence that numerous organic contaminants, as a result of society's reliance on chemical products, are prevalent within municipal biosolids (Chari & Halden 2012; Clarke & Smith 2011), leading to concern that land-applied biosolids may act as an environmental source of these pollutants or their degradation products (Gorgy et al. 2011; Barron et al. 2010; Lozano et al. 2012; Sabourin et al. 2009; Sepulvado et al. 2011), many of which are considered endocrine disruptors with a potentially high ecological risk.

1.1.2 Persistent Organic Chemicals in Biosolids

Modern society utilizes and relies on an extensive variety of chemical products. This rampant use, be it industrial, medical, or household, often results in the chemicals within these products being washed down the drain and, thus, swept into the WWT process. While some of these contaminants are broken down during the treatment of wastewater, such as via biological or photolytic processes, many are not and, and quite often they become associated with wastewater solids.

To date, the USEPA does not regulate concentrations of organic chemicals in biosolids. However, nation-wide surveys of biosolids are performed regularly as a means

to evaluate metal and organic chemicals concentrations and determine any potential concerns regarding environmental and human health. A survey of 40 cities across the USA was conducted in 1982 and National Sewage Sludge Surveys (NSSSs) have been carried out in 1988-89, 2001, 2006-07. (H. Wang et al. 2008; USEPA 2009c) Organic chemicals, such as dioxins, pharmaceuticals, plasticizers, and flame retardants, have been analyzed in NSSSs due to their frequent presence in biosolids and/or environmental and health concern (USEPA 2009c). Through these surveys and risk assessments, the USEPA has concluded that, while chemicals are present, the concentrations of these organic contaminants in biosolids are not high enough to draw concerns regarding environmental or human health risk (USEPA 1993; Smith 2009). Furthermore, any monitoring requirement would be very costly and hard to implement. The USEPA continues to monitor and evaluate the potential health and environmental impacts of organic chemicals detected in biosolids (USEPA 2009a).

In similar fashion to the USEPA, the European Union (EU) issued a directive (86/278/EEC) in 1986 as a means to prevent potential environmental and human health impacts that may arise as a result of the land-application of biosolids. This directive set limits for some metal concentrations in agriculturally applied biosolids as well set forth acceptable treatment guidelines for pathogen reduction. (M. Guo et al. 2012; EuropeanParliament 1986; Robinson et al. 2012; Laturnus et al. 2007; Goss et al. 2013; Smith 2009; Wuest & Gollany 2013; Itanpour et al. 2004). More recently, the EU has also proposed limits on select organic chemicals in agriculturally-applied biosolids, including proposed restrictions on bis(diethylhexyl)phthalate (DEHP), linear alkylbenzene sulfonates (LASs), nonylphenols (NPs), nonylphenol ethoxylates (NPEs),

polyaromatic hydrocarbons (PAHs), and polychlorinated bisphenyls (PCBs). However, some EU member states have instituted even stricter limits than those proposed on the concentrations of these chemicals allowed in agriculturally applied biosolids. (H. Wang et al. 2008; Laturnus et al. 2007; Goss et al. 2013; Egan 2013).

Concern regarding persistent organic pollutants in biosolids centers around the fact that research has shown numerous anthropogenic organic chemicals to be not only present in biosolids, but in agricultural soils after biosolids applications as well (Andrade et al. 2010; Lozano et al. 2010; Lozano et al. 2012; Sepulvado et al. 2011; Barron et al. 2010). This, in turn, has increased concern regarding the potential for the transport of the chemicals further into the environment, magnification of contaminants in food webs, uptake by plants, potential build-up in agricultural soils, etc. Numerous bodies of work have shown pharmaceutical and personal care products (PPCPs); such as antibiotics, antidepressants, antibacterials, steroids, hormones, and contraceptives, as well as surfactants, plasticizers, and flame retardants to be present in biosolids samples worldwide (Scharenbroch et al. 2013; USEPA 2009c; Clarke & Smith 2011; Chari & Halden 2012; Hyland et al. 2012). Research has indicated that many of these compounds can be persistent within soils for many years, due to the properties that cause them to become associated with wastewater solids in the first place (Andrade et al. 2010; Barron et al. 2010; Clarke & Smith 2011). On the other hand, once in the soils, some compounds may naturally degrade, with the risk of this degradation leading to more toxic and/or persistent compounds (Laturnus et al. 2007; Lozano et al. 2012). Other chemicals introduced into agricultural soils via biosolids applications may leach further into the soil core (Barron et al. 2010) and/or make their way into groundwater or tile water (Gottschall

et al. 2013). There is also concern that compounds may be taken up or become associated with vegetation later consumed by humans or animals (Laturnus et al. 2007) or introduced into the food chain by organisms that reside in the soil and are exposed to the chemical contaminants introduced by biosolids amendments (Kinney et al. 2008).

While research studies have demonstrated organic contaminants to be present in the environment due to biosolids applications, it is not entirely clear if these detected concentrations are high enough or extensive enough to have an impact on environmental and/or human health. Factors such as chemical toxicity properties, doses, and duration of exposure to the chemical influence the impact on human and biotic health (Bars et al. 2012) and in some cases, low pollutant concentrations can still induce negative effects in the surrounding biota (Schultz et al. 2013). However, while no immediate risk from compounds in biosolids is clear, it is evident that more information is needed regarding the relationship between chemical pollutants, biosolids, human health and the environment, particularly since chemical usage often changes due to factors including the increase in consumption, introduction of new chemical formulations into products, and governmental regulation of compounds. As the regulation and phase-out of some chemicals of concern increases, the analysis of biosolids for these compounds can be used as an indicator to the effectiveness of the regulatory and phase-out efforts.

1.2 PERFLUOROALKYL SUBSTANCES

Per- and polyfluoroalkyl substances (PFASs) are a classification of thousands of fluorinated surfactants that have been used extensively in both consumer and industrial applications since the 1950s. It is the unique and highly desirable chemical and physical properties of PFASs, including hydrophobicity, oleophobicity, and surfactancy, which

have led to their use in a wide-range of products. (Lindstrom, Strynar & Libelo 2011a; Buck et al. 2012) Common applications of PFASs have included use in fluoropolymer synthesis and as additives in aqueous fire-fighting foams (AFFFs), pesticides, surface coatings for textiles and papers, electrical insulators, lubricants, metal cleaners, and varnishes (Lehmler 2005; Prevedouros et al. 2006). Such widespread use of these compounds over time has led to their release and worldwide detection in environmental, biotic, and human samples and, in turn, drawn the scrutiny of numerous regulatory agencies. Furthermore, this extensive use of PFASs has resulted in their presence throughout the wastewater treatment process (Kim et al. 2012; Kunacheva et al. 2011; Loganathan et al. 2007; Campo et al. 2014).

The general structure of most PFASs is characterized by a partially or fully fluorinated hydrophobic moiety and a hydrophilic functional group. The hydrophobic segment of the PFAS can be comprised of various structures, including alkyl chains, polyethers, and aromatic groups, and various elements, such as nitrogen (N), chlorine (Cl), silicone (Si), and/or sulfur (S). Examples of various PFAS structure types are provided in Figure 1-1. The fluorinated moiety is what gives PFASs their unique characteristics. The fluorine within this structure allows for both hydrophobicity and oleophobicity, as well as chemical and thermal stability. This includes stability during exposure to acids, bases, and reducing and oxidizing agents. The degree of these characteristics depends on the extent of fluorination of the hydrophobic tail and location of fluorination. The hydrophilic moieties of PFASs are also quite diverse and are typically classified based on their ionicity (anionic, cationic, nonionic, or amphoteric). Some common hydrophilic structures include sulfonates, carboxylates, phosphates, and

quarternary ammonium. The various structures and elemental compositions of both the hydrophobic and hydrophilic moieties are what allow for the creation of such a vast number of PFASs. (Kissa 2001; Buck et al. 2012)

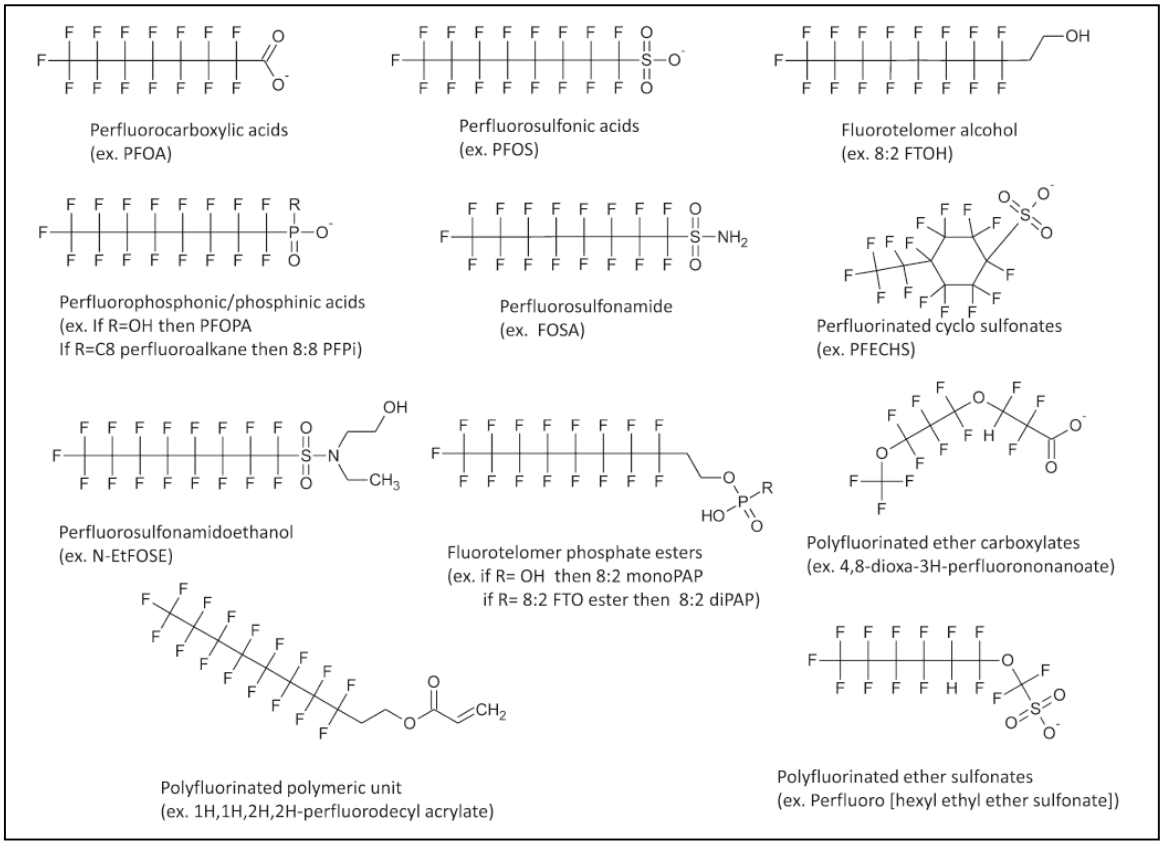


Figure 1-1: Examples of Various PFASs Structures (Lindstrom et al., 2011)

Prior to its phase-out by the primary world manufacturer (The 3M Company) in 2002, electrochemical fluorination (ECF) was the main process for producing PFASs. The ECF method forms linear and branched compounds through a process that involves the rearrangement and breakage of the carbon chains within linear hydrocarbon compounds when reacted with hydrogen fluoride (HF) and electricity. For instance, ECF typically produces a ratio of 70-80% linear C-F chains to 20-30% branched chains for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) – two

persistent PFASs. This process commonly results in the creation of PFAS compounds with 6-, 8-, and 10-carbon chains. (De Voogt & Sáez 2006; Buck et al. 2011; Lindstrom, Strynar & Libelo 2011a) An example of the use of ECF for the production of perfluorooctanesulfonyl fluoride (POSF), a building block for many PFASs, including the persistent PFOS, and its derivatives is presented in Figure 1-2.

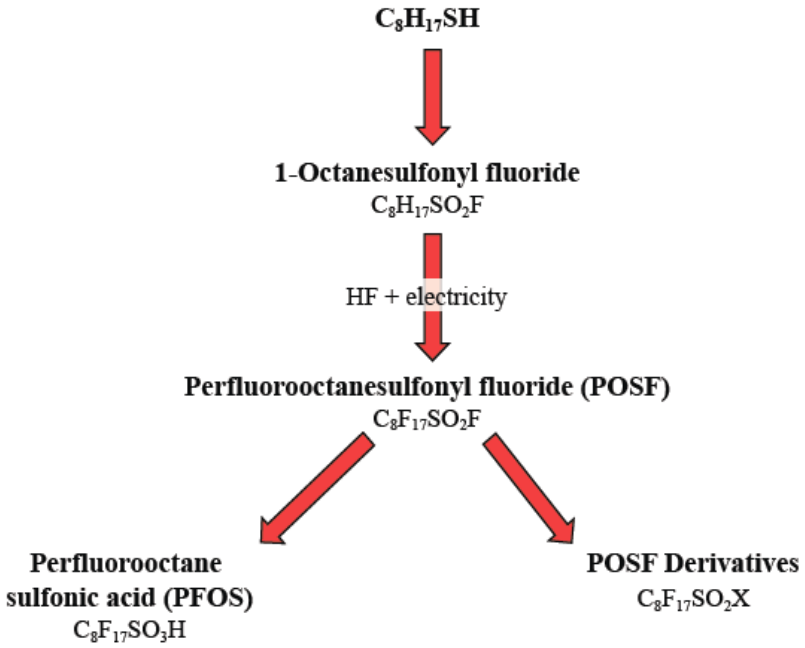


Figure 1-2: Production of POSF via Electrochemical Fluorination (Buck et al., 2011)

Due to the phase-out of the production of PFASs via ECF by The 3M Company, telomerization (TM) has become the more commonly used process for PFAS manufacturing. Using this method, perfluorinated iodides are produced by reacting perfluoroethylene with perfluoroethyl iodide. These perfluorinated iodides are then used to create a variety of predominantly straight-chained PFASs, including perfluorocarboxylic acids (PFCAs), fluorotelomer alcohols (FTOHs), and fluorotelomer

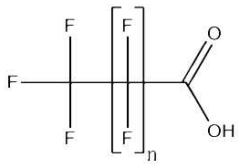
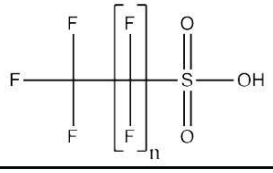
sulfonates (FTSs). (De Voogt & Sáez 2006; Lindstrom, Strynar & Libelo 2011a; Buck et al. 2011; Kissa 2001)

1.2.1 Perfluorocarboxylic Acids and Perfluorosulfonic Acids

Due to the vast number of compounds within the PFAS grouping, this research will center on compounds within the perfluorocarboxylic acid (PFCA) and perfluorosulfonic acid (PFSA) classifications. These two classifications were chosen because compounds found within these groups have been shown to be very persistent and prevalent within the environment and are of concern to regulatory agencies worldwide.

Compounds within the PFCA and PFSA groupings are characterized by a fully fluorinated alky chain with either a carboxylic acid functional group (for PFCAs) or a sulfonic acid functional group (for PFSAs). The general structures for both groups and specific PFCA and PFSA compounds analyzed in this study are presented in Table 1-1.

Table 1-1: PFCA and PFSA Structures and Compounds Studied

Perfluorocarboxilic Acids			
Compound Name	**Acronym**	**n**	
Perfluorobutanoic acid	PFBA	2	
Perfluoropentanoic acid	PFPeA	3	
Perfluorohexanoic acid	PFHxA	4	
Perfluoroheptanoic acid	PFHpA	5	
Perfluorooctanoic acid	PFOA	6	
Perfluorononanoic acid	PFNA	7	
Perfluorodecanoic acid	PFDA	8	
Perfluoroundecanoic acid	PFUnA	9	
Perfluorosulfonic Acids			
Compound Name	**Acronym**	**n**	
Perfluorobutane sulfonic acid	PFBS	3	
Perfluorohexane sulfonic acid	PFHxS	5	
Perfluorooctane sulfonic acid	PFOS	7	
Perfluorodecane sulfonic acid	PFDS	9	

1.2.1.1 Influence of PFCAs and PFSAs on Environmental and Human Health

PFCA and PFSA compounds have been detected in environmental and biota samples worldwide, including human samples. These compounds can be/have been introduced into the environment be either direct sources, such as industrial discharge, or indirect sources, such as long-range atmospheric transport of precursor compounds that then degrade into PFCAs and PFSAs (Prevedouros et al. 2006). Additionally, the inability of PFCA and PFSA compounds to be biodegraded both aerobically and anaerobically leads to their persistence within the environment. (Frömel & Knepper 2010) Various PFASs were detected by Zushi et al (2010) in sediment cores from Tokyo Bay, Japan. The collection and sampling of sediment cores showed not only the presence of PFASs in the matrix, but allowed for the determination of temporal trends of these compounds as well, indicating increases of some compounds in sediment over time, such as PFOA and PFNA, and decreases of other, such as PFOS. (Zushi et al. 2010) A study conducted in Tierra del Fuego and Antarctica showed the presence of PFCAs and PFSAs in various biotic and soil samples. Of note were detections of high concentraions of: PFHxA in algae, PFHxA and PFOS in penguin dung, PFHxA in guano, and PFHxA in fish. (Llorca et al. 2012) On the other side of the world, Cai et al (2012) analyzed surface water, sea ice, and snow samples for PFASs in the North Pacific Ocean, Arctic Ocean, and Bering Sea. PFCA compounds were found to be the primary PFASs detected in surface water samples. PFASs were also present in the ice and snow samples analyzed and it was theorized that their presence was due to atmospheric deposition. (Cai et al. 2012) Additionally, PFCA and PFSA compounds have been detected in seabird eggs collected in the Canadian Arctic, with total PFCA concentrations increasing between

1975 and 2011. (Braune & Letcher 2013) The presence of PFASs in biota has led to concern over the potential bioaccumulation of these compounds within the food chain. A study conducted in China demonstrated the ability of some compounds to accumulate within aquatic organisms. In this study, a variety of invertebrate and fish species were collected from Bohai Bay in northern China, with PFASs being detected in most samples. Higher trophic level animals, such as fish and squid, accumulated more PFASs than those located at lower levels. It was determined that the consumption of particles was a primary pathway of PFASs into benthic invertebrates and, therefore, into the food chain. (Yang et al. 2012)

Additionally, PFCA and PFSA compounds have been detected extensively in a variety of human samples, including blood, serum, breast milk and tissue. In 2004, Kannan et al. published a study of PFOS, PFHxS, PFOA, and perfluorooctanesulfonamide (PFOSA) in human blood samples collected worldwide. Whole blood, serum, and plasma samples from various countries in North America, South America, Europe, and Asia were analyzed from 473 donors located in assorted locales, including suburban, urban, and industrial. It was found that at least two of the four compounds analyzed were detectable in all countries. PFOS was the most predominant compound (concentrations greater than 30ng/mL), with PFOA being detected the second most frequently. (Kannan et al. 2004) A study PFASs in the plasma of 600 American Red Cross blood donors from six locations within the United States analyzed for 11 compounds from samples collected in 2010 and compared them to studies performed in 2000-01 and 2006. Results showed many of these compounds to be present in all years, with long-chain compounds being the most prevalent. While the

study indicated that concentrations of some compounds have decreased since 2000-01 (PFOS in particular), they also indicated that others were still steadily detected over time. (Olsen et al. 2012) An Italian study published in 2013 showed the presence of these compounds in human milk. The study conducted by Barbarossa et al. analyzed for PFOA and PFOS in 37 milk donors. It was demonstrated that while concentrations are lower in breast milk than in blood, PFOA and PFOS are transferred from the blood to the milk during breastfeeding. Additionally, the study determined that concentrations were higher in those donors who were nursing their first child. The study draws concern over breast milk as a source of these compounds to newborns. (Barbarossa et al. 2013) PFASs have also been shown to accumulate in human tissue. A Spanish study conducted on various human tissues collected during human autopsies examined the accumulation of various PFCAs and PFSAs in different tissue sample types. It was discovered that PFBA was both detected the most frequently and at the highest concentrations in the lungs and kidneys while PFHxA occurred in the highest concentrations within liver and brain tissues. PFOA had highest concentration in bone samples with both it and PFOS being detected the most frequently in the tissue type. In general, it was determined that the lungs were the region of the body with the highest PFAS concentrations. (Pérez et al. 2013) It is estimated that the half-life for PFCA and PFSA compounds in the human body are 0.53 years (for 5*m*-PFOA – an isomer of linear PFOA – in young females) to 90 years (for PFHxS in older females/all males). (Zhang et al. 2013)

The presence of PFCAs and PFSAs in human and environmental samples has led to concerns regarding their impact on health. Numerous animal studies, as well as human health surveys, have been conducted in an attempt to determine what and to what extent

the health effects of these compounds are. A study published by Ding et al. in 2011 on the toxicity of seven PFASs on lettuce and green algae showed the potential for these compounds to influence the root elongation abilities of lettuce after a 5-day exposure as well as the acute toxicity these compounds may have to algae after a 4.5-hour exposure. It was also observed, in both cases, that toxicity increased with fluorinated chain length. (G. Ding et al. 2011) Conversely, 48-hour acute immobilization test performed with seven different PFASs on water fleas indicated that, while the tested compounds still seemed to demonstrate toxicological effects, the toxicity increased with decreasing fluorinated carbon chain length. (G.-H. Ding et al. 2012) Partial life-cycle assays and acute toxicity studies on freshwater mussels have also demonstrated the toxicological potential of these compounds. A study by Hazelton et al. on the influence of PFOS on mussel heath showed that during the glochidia larval stage, both the duration of viability and probability of metamorphosis were decreased after exposure. (Hazelton et al. 2012) Another toxicity study conducted using PFOS, this time studying the effects on zebrafish, further demonstrates toxicity potentials. Chen et al. exposed zebrafish embryos to PFOS 48 to 96 hours (post fertilization). Results indicated that this exposure to PFOS during early development resulted in uninflated swim bladders, a less developed gut, and a curved spike 120 hours post fertilization. Additionally, it was observed that numerous transcripts were misexpressed and genes were changed due to PFOS exposure. (Chen et al. 2014) In mammalian studies, Takahashi et al. performed repeated dose toxicity studies on rats to evaluate the reproductive and developmental toxicity of PFUnA. They found that exposure to PFUnA could inhibit weight gain of adults and body weight of pups, increase blood urea nitrogen, and increase liver weight, among other observations.

(Takahashi et al. 2014) While potential influence of PFASs on health is evident, it is important to note that oftentimes, laboratory studies utilize concentrations of these compounds not typically found in the environment. Additionally, toxicological effects can be species-dependent.

Numerous human health survey studies, conducted in hopes of determining the influence of PFASs on human health, can be found within the literature. One such study, conducted between 2008 and 2011, health surveys were conducted on residents and workers from an Ohio community that was exposed to PFOA for over 50 years after an industrial release. Of the 32,254 citizens that participated, it was found that 3,633 of them had reported functional thyroid disease. It was determined that an increase in PFOA exposure led to higher incident of functional thyroid disease. A primary observation of this survey was the association of high PFOA exposure and hyperthyroidism or hypothyroidism among women. (Winquist & Steenland 2014) Shankar et al (2011) compared serum concentrations of PFASs from the United States National Health and Nutritional Examination Survey 1999-2000 and 2003-2008 cycles to incidences of chronic kidney disease (CKD). A positive correlation between PFAS concentrations in serum and CKD was found to occur. (Shankar et al. 2011) Another health study, this time performed on children, from birth through seven years old, looked into the association between PFAS concentrations in blood serum and antibody concentrations. It was found that children's antibody responses to immunizations were lowered with higher serum PFAS levels, a trend that was the most apparent when the patients were five years of age. (Grandjean et al. 2012) There is also evidence that PFASs may exhibit toxicity to human cells. In a study conducted by Gorrochatategui et

al (2014) on the human placental chloriocarcinoma cell line JEG-3, it was observed that long-chain PFASs displayed cytotoxic effects. It was noted that this was, in part, due to the propensity of the cells to uptake the long-chain compounds. It was also observed that the presence of PFASs could interfere with membrane lipids. (Gorrochategui et al. 2014)

1.2.2 Regulatory Action

The extensive detection of PFCA and PFSA compounds within the environment and human samples, as well as the tendency of long-chained compounds to persist, has drawn the scrutiny of numerous regulatory agencies. In particular, much of the concern from regulatory agencies has been focused on PFOA and PFOS due to their widespread past use and/or formation during manufacturing processes. In 2000, The 3M Company, in a voluntary agreement with the USEPA, set forth to phase-out the use of PFOS and its related compounds by 2003 (USEPA 2000; Zushi et al. 2011). Further action was taken in the USA when The 3M Company, along with seven other major PFOA manufacturers, entered a voluntary stewardship program with the USEPA to reduce the emission of PFOA, PFOA precursors, and long-chain PFCAs (homologues of PFOA). Reduction targets, based on emissions from 2000, were 95% reduction by 2010 and 100% by 2015. (USEPA 2006; Zushi et al. 2011) A 2012 progress report on the stewardship program shows that the US operations of all but one company have met the 2010 emission reduction targets and several have met the 2015 targets. However, non-US operations of several of the stewardship companies have not yet met the 2010 emission goals. (USEPA 2013) The European Union issued a directive (2006/122/ECOF) in 2006 limiting the use of PFOS (EuropeanParliament 2006). In 2009, The Stockholm Convention on Persistent Organic Pollutants (POPs), an international treaty that aims to limit or abolish POPs,

listed PFOS, PFOS salts, and POSF as part of Annex B of the Convention. This requires that the 179 parties of the treaty must restrict the application of these compounds to specific uses (as outlined by the Convention). (StockholmConvention 2011; Zushi et al. 2011) The restriction on use and emissions of long-chain PFCA and PFSA compounds in developed nations has led to (1) the increase in use of short-chain PFCA and PFSA compounds (Ritter 2010; Zushi et al. 2011) and (2) an increase in production in developing countries, such as China (Zushi et al. 2011; Xie et al. 2013).

1.2.3 Scope of Work and Objectives

The use of PFASs in a variety of consumer products has led to their detection within the WWT process. PFCA and PFSA compounds have been detected in both solids and aqueous samples throughout various treatment stages in wastewater treatment plants (WWTPs) worldwide (Sinclair & Kannan 2006; Loganathan et al. 2007; R. Guo et al. 2010; Kunacheva et al. 2011; Navarro et al. 2011; Gómez-Canela et al. 2012). Mass flow studies of these compounds in WWTPs have indicated that they are not removed by traditional wastewater treatment and there is a potential for the increase in concentration of some of these compounds during the treatment process (Sinclair & Kannan 2006; Kunacheva et al. 2011), likely a result of their formation from the biotransformation of other PFAS compounds that act as precursor compounds (Parsons et al. 2008; Rhoads et al. 2008; Frömel & Knepper 2010; N. Wang et al. 2011). This inability of the WWT process to remove PFCA and PFSA compounds, as well as their potential to increase in concentration during the treatment process, indicates that WWTP effluent and sludge can become a secondary source of these compounds into the environment. The sampling of various WWTPs has indeed shown that PFCA and PFSA compounds can be present in

both wastewater effluent and final solids (Campo et al. 2014; Kunacheva et al. 2011; Sinclair & Kannan 2006). The structure of PFCAs and PFSAs, a hydrophilic head group attached to an oleophobic/proteinophilic perfluorinated alkyl group, gives them their unique surfactant abilities and makes determining partitioning coefficients difficult. However, it has been demonstrated that these compounds will bind to organic matter, with those containing a longer carbon chain having a greater propensity for sorption. (Rayne & Forest 2009) Through numerous studies performed on wastewater sludges and biosolids, it has been determined that various PFCA and PFSA compounds are present in wastewater solids worldwide (Gómez-Canela et al. 2012; Navarro et al. 2011; Venkatesan & Halden 2013; Kunacheva et al. 2011). Few studies exist on the fate of PFCA and PFSA after land-application of biosolids, but there is an indication that these compounds have the potential to: leach into the soil horizon over time (Sepulvado et al. 2011), spread to surface and/or groundwater (Lindstrom, Strynar, Delinsky, et al. 2011b), be taken up by plants (Yoo et al. 2011; Wen et al. 2014; Zhao et al. 2014), and accumulate in earthworms (Yoo et al. 2011; Wen et al. 2014; Zhao et al. 2014). Additionally, while there are many studies on PFAS concentrations in wastewater sludges and biosolids, these studies are limited in that they often represent a single or short time period and can be constrained by the number or samples analyzed. To the best of the author's knowledge, this is the first investigation into the study of long-term trends of PFCAs and PFSAs in limed biosolids from a single WWTP.

This study focuses on the temporal trends of the PFCA and PFSA compounds (listed in Table 1-1) in limed biosolids from a municipal WWTP over an eight-year period. The results from this study aim to expand upon the understanding of how (1)

total PFCA and PFSA concentrations have changed and (2) individual compound compositions have changed. Additionally, this study explores the influence that storage methods may have on PFCA concentrations in historical samples.

CHAPTER 2: TEMPORAL TRENDS OF PERFLUOROALKYL SUBSTANCES IN LIMED BIOSOLIDS FROM A LARGE MUNICIPAL WASTEWATER TREATMENT PLANT

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2.1 ABSTRACT

Perfluoroalkyl substances (PFASs) are a classification of anthropogenic chemicals used in a variety of consumer and industrial products. Compounds from two PFAS subgroups, perflurocarboxylic acids (PFCAs) and perfluorosulfonic acids (PFSAs) are known to be persistent and have been detected in environmental and biotic samples worldwide. While long-chain PFCAs and PFSAs have been in a phase-out process within the United States and some have been regulated in Europe, these compounds have continued to be produced in developing countries. The sustained use of PFCA and PFSA compounds in consumer products, as well as the ability of some PFASs to degrade into these compounds, has led to their presence in wastewater treatment plants (WWTPs). This study analyzes archived limed biosolids from a municipal WWTP for temporal trends of 8 PFCAs and 4 PFSAs over an eight-year period. Results indicated no

significant changes in PFCAs between 2006 and 2013 while detectable PFSA concentrations decreased during this time period. This study also compared storage methods and determined that use of glass containers with polytetrafluoroethylene (PTFE)-lined lids had no apparent influence on PFCA concentrations.

2.2 INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are a class of anthropogenic compounds that have been utilized in various consumer and industrial products since the 1950s. The distinctive characteristics that result from the C-F bonds within the perfluoroalkyl moiety of theses compounds include: oleophobicity, hydrophobicity, as well as chemical and thermal stability (De Voogt & Sáez 2006; Buck et al. 2011). The perfluoroalkyl moiety, in conjunction with various chemical structures and hydrophilic functional groups has allowed for the formation of thousands of different PFASs that have been incorporated in and used to create a wide range of products. The extensive use of PFASs, due to their desirable physical and chemical properties, has resulted in their eventual release into the environment and subsequent detection in various environmental and biotic samples worldwide, including: air (Müller et al. 2012), freshwater (Clara et al. 2009; Kovarova et al. 2011), seawater (Benskin et al. 2012), sediment (Clara et al. 2009), arctic snow (Young et al. 2007), biota (Houde et al. 2011; Kovarova et al. 2011), bird eggs (Gebbink et al. 2011; Braune & Letcher 2013), as well as human blood samples (Kannan et al. 2004; D'eon & Mabury 2011).

Long-chain compounds found within two PFAS subgroups, perfluoroalkyl carboxylic acid (PFCAs) and perfluoroalkane sulfonic acids (PFSAs), have drawn the attention of numerous regulatory agencies worldwide due to their persistence and

ubiquitous presence in the environment (USEPA 2006; USEPA 2000; EuropeanParliament 2006; StockholmConvention 2011). In particular, much of the concern from regulatory agencies has been focused on perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) due to their widespread past use and/or formation during manufacturing processes. In the United States (US) several manufacturers have entered a voluntary stewardship program with the US Environmental Protection Agency (USEPA) for the phase-out of PFOA and longer-chain PFCAs, as well as PFOS and PFOS-related compounds (USEPA 2000; USEPA 2006). A directive issued by the European Union (EU) in 2006 restricted the use of PFOS (EuropeanParliament 2006) and in 2009 PFOS was included in Annex B of the Stockholm Convention on Persistent Organic Pollutants (POPs) (StockholmConvention 2011; Zushi et al. 2011). This has led numerous companies to utilize short-chain PFASs within their product formulations as substitutes for the restricted compounds (Ritter 2010). However, as these chemicals are phased-out in developed countries, there is evidence that in developing countries, such as China, production has increased (Ritter 2010; Zushi et al. 2011; Xie et al. 2013).

Numerous studies have shown PFCA and PFSA compounds to be present within industrial, commercial, and domestic wastewater treatment processes. These compounds have been detected in both solids and aqueous samples throughout various treatment stages in wastewater treatment plants (WWTPs) worldwide (Sinclair & Kannan 2006; Loganathan et al. 2007; R. Guo et al. 2010; Kunacheva et al. 2011; Navarro et al. 2011; Gómez-Canela et al. 2012). Mass flow studies of PFCAs and PFSAs in WWTPs have indicated that they are not removed by traditional wastewater treatment and there is a

potential for the increase in concentration of some of these compounds during the treatment process (Sinclair & Kannan 2006; Kunacheva et al. 2011), likely a result of their formation from the biotransformation of other PFAS compounds that act as precursor compounds (Parsons et al. 2008; Rhoads et al. 2008; Frömel & Knepper 2010; N. Wang et al. 2011). This inability of WWTPs to remove PFCA and PFSA compounds, as well as their potential to increase in concentration during the treatment process, indicates that WWTP effluent and sludge can become a secondary source of these compounds into the environment.

This study focuses on the temporal trends of select PFCA and PFSA compounds in limed biosolids from a large municipal WWTP in the US prior to application to agricultural fields. The results help to broaden the understanding of how total PFCA and PFSA concentrations are changing in the US as well as determine the change in individual compound compositions in limed biosolids over time. This, in turn, allows for the potential influence that land-applied biosolids may have as a source for PFCA and PFSA compounds in the environment to be better understood. Finally, the study helps to show whether storage methods can impact PFCA concentrations in archived samples.

2.3 MATERIALS AND METHODS

2.3.1 SAMPLE COLLECTION/HANDLING

Samples were collected from a large municipal WWTP in the Mid-Atlantic region of the US. The plant serves a region of over 2 million people and has the capacity to treat 370 million gallons of raw wastewater per day. The WWTP consists of primary treatment, secondary treatment, nitrification-denitrification, filtration, and disinfection.

Solids from primary treatment as well as the secondary and nitrification treatment processes are thickened, combined, and dewatered through centrifugation. Lime is added to this sludge mixture on a dry weight basis of approximately 15% to neutralize pathogenic organisms, classifying the product as Class B biosolids. Biosolids from the WWTP are primarily land-applied to agricultural fields in the surrounding region according to USEPA guidelines. (Lozano et al. 2013)

Beginning in July 2005, limed biosolid samples were collected from the WWTP approximately every two to three months as part of previous studies on POPs in biosolids (Andrade et al. 2014; Bevacqua et al. 2011). Grab biosolids samples were collected from the WWTP directly after the liming process. In the previous studies, all samples were stored in wide-mouth glass jars as this is the preferred storage method for steroid hormones, triclosan (TCS), triclocarban (TCC), and polybrominated diphenyl ether (PBDE) congeners for the USEPA National Sewage Sludge Survey (NSSS) (USEPA 2009c). To remain consistent with storage practices in these previous studies, all samples were stored in glass jars with polytetrafluoroethylene (PTFE)-lined lids. However, in an effort to determine whether the PTFE-lined lids impacted PFCA concentrations in the historical samples, since PFCAs are used in the synthesis of PTFE (Ritter 2010), limed biosolids were also stored in high-density polyethylene (HDPE) containers beginning in June 2012. Samples from both storage methods were extracted and analyzed concurrently for comparison. Additionally, in cases where the limed biosolids stored in glass jars were in contact with the PTFE-lined lids, samples were extracted from the portions of the sample that were in contact with the lid as well as portions that were in contact with glass only. After collection, samples were placed on ice and transported to

the laboratory, where they were archived and stored at -20°C until analysis for PFCA and PFSA compounds.

2.3.2 STANDARDS AND REAGENTS

Standard solutions for perfluorobutanoic acid (PFBA) (98%), perfluropentanoic acid (PFPeA) (97%), pefluorohexanoic acid (PFHxA) (97%), perfluoroheptanoic acid (PFHpA) (99%), PFOA (96%), perfluorononanoic acid (PFNA) (97%), perfluorodecanoic acid (PFDA) (98%), perfluoroundecanoic acid (PFUnA) (95%), and perfluorobutanesulfonic acid (PFBS) (97%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Standard solutions for perfluorohexanesulfonic acid (PFHxS) (98%), PFOS (98%), and perfluorodecanesulfonic acid (PFDS) (98%) as well as isotope-labeled $^{13}C_5$-PFPeA, $^{13}C_5$-PFHxA, $^{13}C_8$-PFOA, $^{13}C_5$-PFNA, $^{13}C_3$-PFHxS, and $^{13}C_8$-PFOS were obtained from Wellington Laboratories (Guelph, ON, Canada). Organic solvents used were of high purity, HPLC grade (Burdick and Jackson; Fisher Scientific). Organic-free, UV-treated water (Hydro Service & Supplies, Inc.; Durham, NC, USA).

2.3.3 ANALYTICAL METHODS

Limed biosolids samples were extracted using a modified version of a previously published method (Navarro et al. 2011). All biosolids samples were spiked with 7.5 ng/mL of $^{13}C_5$-PFPeA, $^{13}C_8$-PFOA, and $^{13}C_8$-PFOS as surrogate standards prior to the extraction process. Fifteen milliliters (mL) of an acetonitrile:methanol (ACN:MeOH) solvent mixture (50:50 v/v) (Yoo et al. 2009) was added to 50mL polypropylene centrifuge tubes containing approximately 5 g of limed biosolid sample (wet weight). Samples were vortexed for 1 minute and sonicated in a heated water bath at a temperature

of 45°C for 25 minutes. Following sonication, samples were centrifuged for 5 minutes at 3200 x g. The supernatant liquid from each sample was decanted into new 50 mL centrifuge tubes. The extraction process was repeated two additional times, with the supernatant from each sample extraction combined with that of the previous extraction step, for a total of three extractions. All samples were extracted in triplicate. Extractions were performed in batches containing a maximum of 20 samples. Each extraction batch contained a blank consisting of laboratory-grade sand and a biosolids sample spiked with all 12 PFAS compounds.

Sample extracts were cleaned using Supelco Envi-Carb (500mg, 6mL) SPE cartridges (Sigma-Aldrich, St. Louis, MO, USA). Prior to sample loading, cartridges were conditioned with 10 mL of ACN:MeOH (50:50 v/v). Extracts were loaded and collected. Cartridges were washed with 3 mL of MeOH. This wash was also collected. Eluates were concentrated to 4 mL under a gentle stream of nitrogen, diluted in 175 mL of organic-free water, and acidified to a pH of approximately 4 using formic acid.

Diluted and acidified samples were loaded onto Oasis WAX (500mg, 6mL) SPE cartridges (Waters, Milford, MA, USA) at a rate of one drop per five seconds. Cartridges were previously conditioned with 10 mL of MeOH and 10 mL of organic-free water. After sample loading, cartridges were washed with 2.5 mL of 0.01% formic acid in MeOH. Compounds were then eluted and collected with two 10 mL washes of 0.5% NH_4OH in MeOH. The collected eluates were evaporated, reconstituted with 387.5µL of MeOH, and transferred to 1mL Thompson filter vials (0.45µm nylon filter) and spikes with 7.5ng/mL $^{13}C_5$-PFHxA, $^{13}C_5$-PFNA, and $^{13}C_3$-PFHxS as internal standards. The

final sample volume for high performance liquid chromatography, tandem mass spectrometry (HPLC-MS/MS) analysis was 0.5mL in MeOH..

2.3.4 INSTRUMENT ANALYSIS

Sample extracts were analyzed via HPLC-MS/MS to measure for PFAS compounds using a Waters 2690XE separations module (Waters Corporation, Milford, MA, USA) attached to a Quattro LC benchtop triple quadrupole mass spectrometer (Micromass Limited, Manchester, UK) with an electrospray interface. Chromatographic separation was obtained by injecting 10 μL of extract onto a Zorbax C8 (150 x 4.6 mm) reversed-phase liquid chromatography column in-line with a 4.6 x 12.5 mm guard column (Agilent, Santa Clara, CA, USA) at a temperature of 40°C (Powley et al. 2005). The mobile phase consisted of (A) 2 mM ammonium acetate in organic-free water and (B) MeOH and was run at a flow of 0.5 mL/min. Solvents were run at a gradient, with starting conditions of 90% solvent A and 10% solvent B and reduced to 20% A in 5 minutes and to 0% A in an additional 5 minutes. (García-Valcárcel et al. 2012) The gradient was held at 0% solvent A for 5 minutes. A MeOH:H_2O mixture (50:50 v/v) was run through the column for 3 minutes before being returning the gradient back to 90% solvent A, 10% solvent B.

The mass spectrometer source parameters were: capillary voltage 3.10kV in electrospray negative (ES-); cone voltage 105V; extractor voltage 1V; RF lens 0.1V; source temperature 140°C; desolvation temperature 400°C. Nitrogen was used as both the nebulizer (145L/hr) desolvation gas (450L/hr). Acquisition was done in the multiple-reaction monitoring mode (MRM). Standards were injected every ten samples in order to verify stability of the instrument during the analyses. Peak integration and quantitation

were performed automatically using MassLynx4.0 (Micromass Limited, Manchester, UK).

2.4 RESULTS AND DISCUSSION

For all samples in this study, only data for PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFOS, and PFDS are reported, as the remaining compounds were below MDL or not detectable.

2.4.1 COMPARISON OF STORAGE METHODS

Results from the analysis of the different storage methods from this study are presented in Figures 2-1a through 2-1e. While concentrations vary, no discernable pattern between samples stored at the bottom of a glass jar, sample stored in a glass jar but in contact with the PTFE-lined cap, or samples stored in a HDPE jar is evident. It is likely that the variability of the concentrations of PFCA compounds is due to the heterogeneity of the biosolids themselves, indicating, that for this matrix any impurities present from the PTFE likely have no significant impact one PFCA concentrations. Although it does not appear that storage methods have an impact of the concentration of PFCAs in biosolids, for more sensitive samples the manner of storage may have a significant effect on concentrations and this should be considered/investigated prior to sample collection.

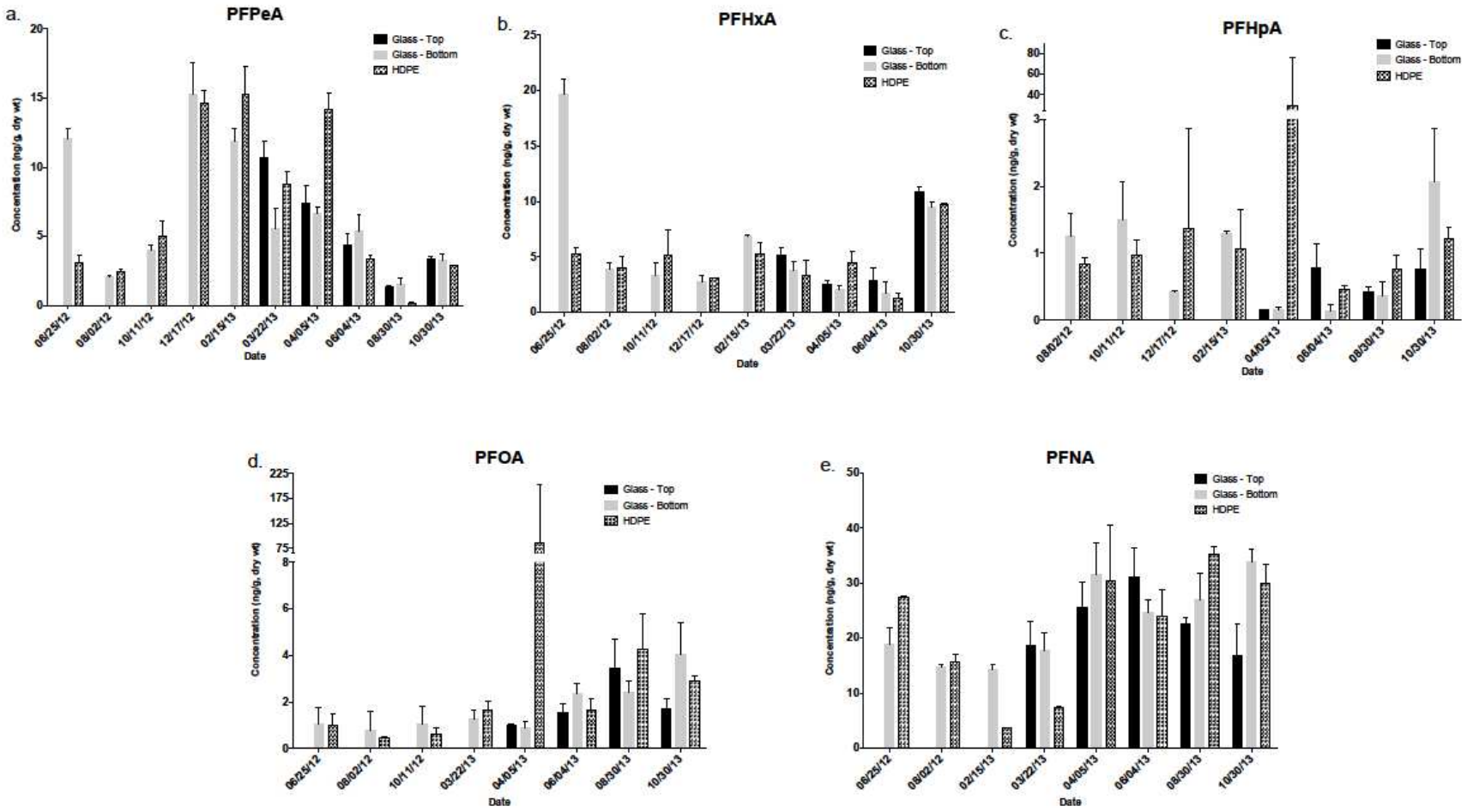

Figure 2-1: Concentrations Among Different Storage Methods for (a) PFPeA (b) PFHxA (c) PFHpA (d) PFOA and (e) PFNA. Bars represent the average concentration and standard deviation.

2.4.2 TEMPORAL TRENDS OF PFCAS AND PFSAS

In this study, concentration ranges of PFCAs over the 8-year study period were as follows: PFPeA, below detection limit (BDL) to 28.2 ng/g dry weight (dw); PFHxA, BDL to 27.5 ng/g dw; PFHpA, BDL to 121 ng/g dw; PFOA, BDL to 1098 ng/g dw; and PFNA, BDL to 387 ng/g dw. For the PFSA compounds, concentrations ranged from 0.892 to 75.6 ng/g dw for PFOS and 0.384 to 15.5 ng/g dw for PFDS. These ranges are generally within the varying range of those from previous studies on wastewater solids within the US (Venkatesan & Halden 2013) and Europe (Navarro et al. 2011; Gómez-Canela et al. 2012).

Trends of short-chain PFCAs for individual sample dates over the study tended to vary considerably, as shown in Figures 2-2 through 2-4. For PFPeA and PFHxA, average detected concentrations oscillated between 1 and 30 ng/g dw, which no discernable seasonal trends. PFHpA concentrations were consistently detected at trace levels below 4 ng/g dw, with the exception of seven sample dates where at least one replicate was detected at a much higher concentration. The moving average (MA) of the data set for each compound was calculated as a means of smoothing the short-term fluctuations. The MA for a sample was calculated by averaging the average concentration for that date with that of the previous sample date and the following sample date. A linear trend line was fit for the MA of each compound using Microsoft Excel and is shown in Figures 2-2 through 2-4. Information regarding the trend lines, including the equations and R^2 values are provided in Table 2-1. A linear regression on the moving average data was performed using GraphPad Prism as a means to determine whether a linear trend over time exists and the significance of the slope (data provided in Table 2-1).

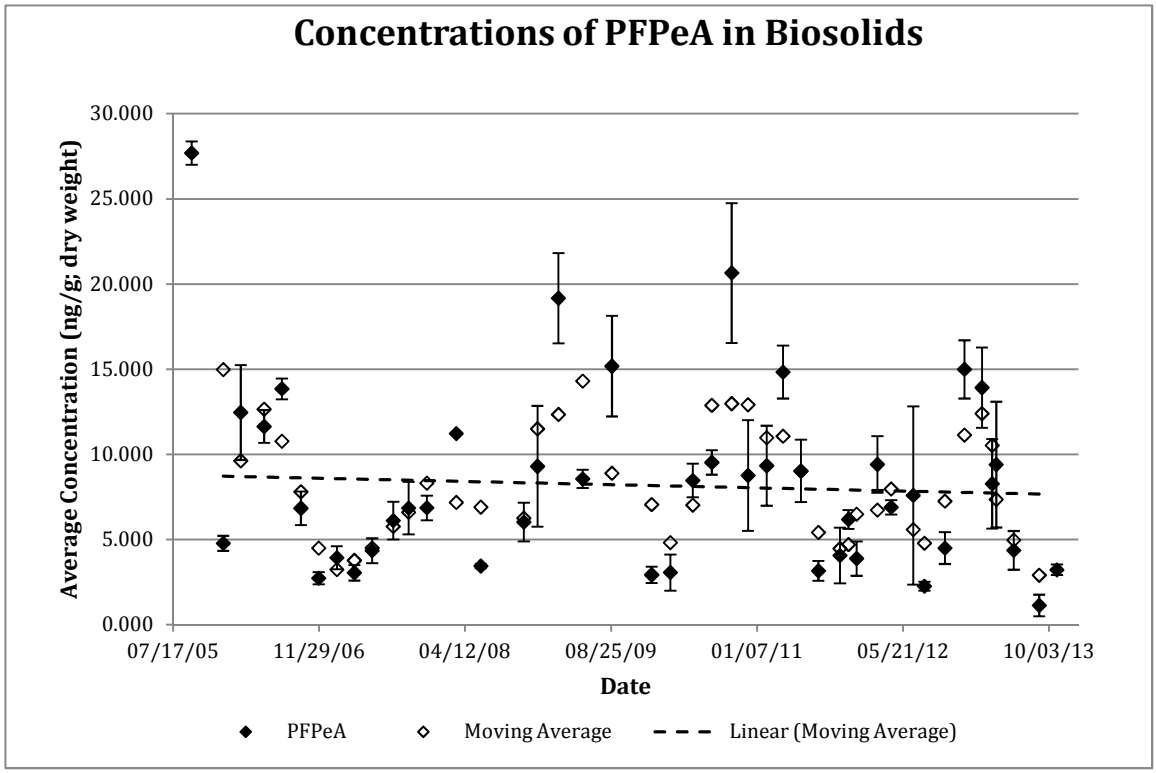

Figure 2-2: Trends of PFPeA Over Time. Points represent the average concentration and bars represent the standard deviation.

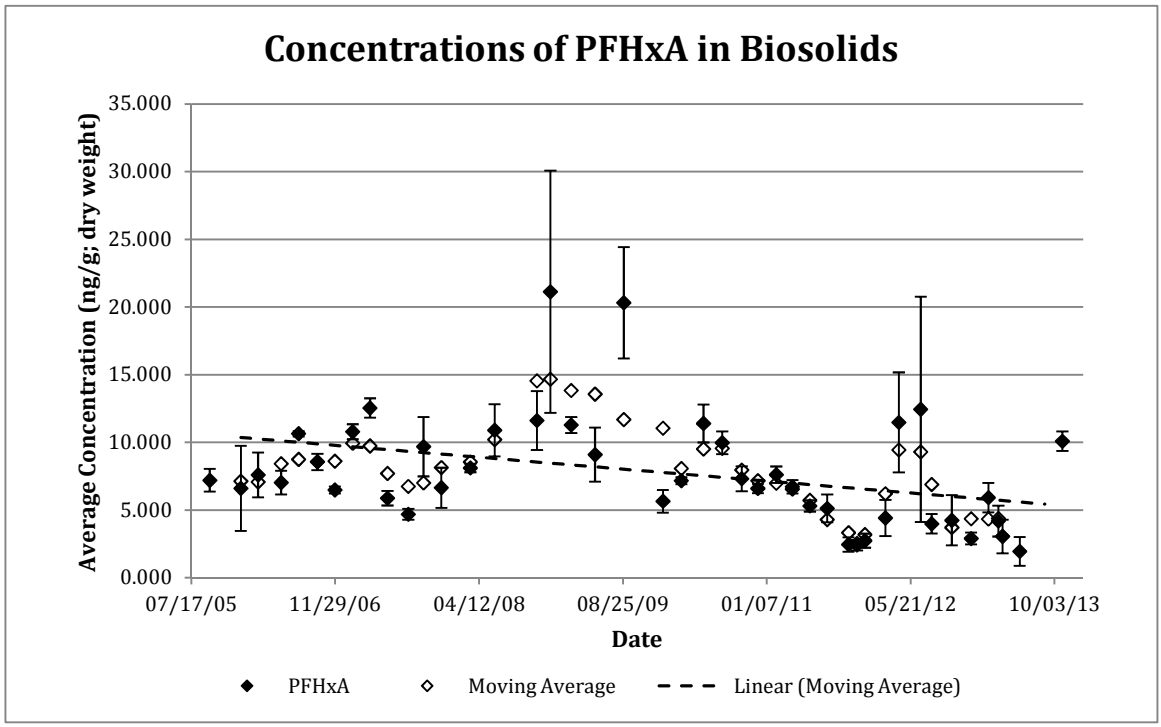

Figure 2-3: Trends of PFHxA Over Time. Points represent the average concentration and bars represent the standard deviation.

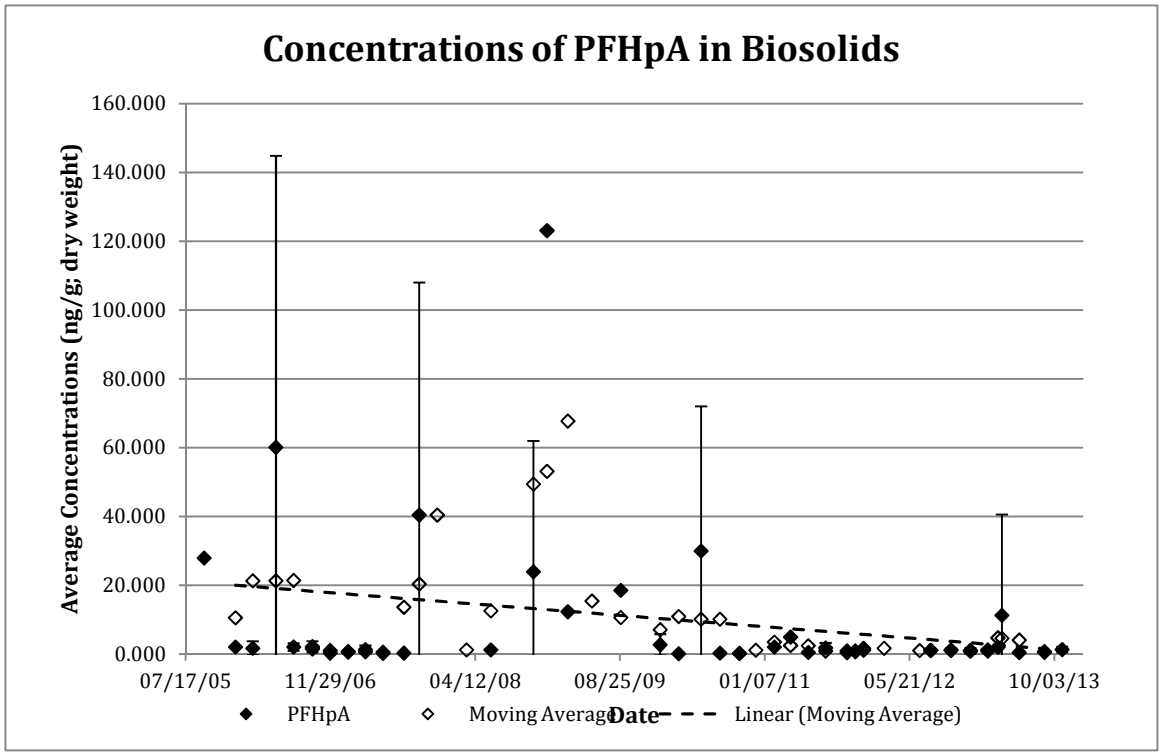

Figure 2-4: Trends of PFHpA Over Time. Points represent the average concentration and bars represent the standard deviation.

Given the trend line equation of Y = -0.0004*X + 8.776 (R^2 = 0.01) for PFPeA it can be determined that only 1% of the data variation can be characterized by a linear model. Additionally, the regression coefficient for the linear model (-0.0004) is not significantly different than zero (P=0.52) indicating that for the linear model there are no significant decreasing trends. For PFHxA, the linear model was a better, but still not ideal fit of the data. In this case, approximately 23% of the data variation can be characterized by the equation Y = -0.0018*X + 10.54. The linear regression analysis determined that the slope of this model was significantly different than zero (P=0.0017). Thirteen percent (13%) of the data variation of PFHpA was explained by the Y = -0.0066*X + 20.67 model. Similar to PFHxA, the slope of the linear PFHpA model was significantly different than zero (P=0.0173).

Table 2-1: Trend Line Equation and Linear Regression Analysis Results for Short-Chain PFCAs

	PFPeA	PFHxA	PFHpA
Linear Equation	Y = -0.0004*X + 8.776	Y = -0.0018*X + 10.54	Y = -0.0066*X + 20.67
R^2	0.01014	0.2266	0.1336
Regression Coefficient (RC)	-0.0004	-0.0018	-0.0066
Is RC Significantly Non-zero?	No	Yes	Yes
P	0.5205	0.0017	0.0173

Since temporal patterns in the results in Figures 2-2 through 2-4 are difficult to discern, the yearly averages for each compound are presented in Figure 2-5. While it would not be accurate to perform statistical analysis on the data as presented in this format, displaying the data in this layout shows some general increases in concentrations for PFPeA, PFHxA, and PFHpA during the 2008 and/or 2009 time period.

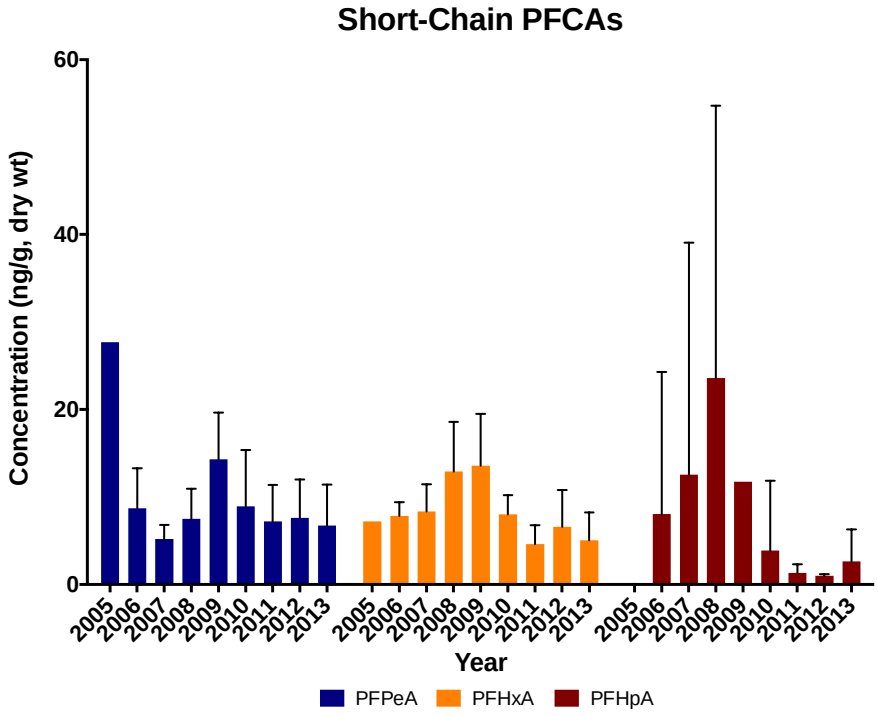

Figure 2-5: Average Yearly Concentrations for Short-Chain PFCAs. Bars represent the average concentration and standard deviation.

Long-chain PFCAs were analyzed in the same manner as short-chain PFCAs. Like their short-chained homologs, long-chain PFCA concentrations were also variable, but generally detected at higher concentrations than the short-chain PFCAs and are shown in Figures 2-6 and 2-7. Overall, PFOA concentrations were below 20 ng/g dry wt and PFNA concentrations were below 50 ng/g dry wt. Similar to the trend observed for PFHpA, there were a number of data points where PFOA was detected at concentrations much higher than the typically observed concentration. Many occurred simultaneously with increases in PFHpA concentrations. This is perhaps due to the degradation of a precursor compound into PFOA and PFHpA (Buck et al. 2011).

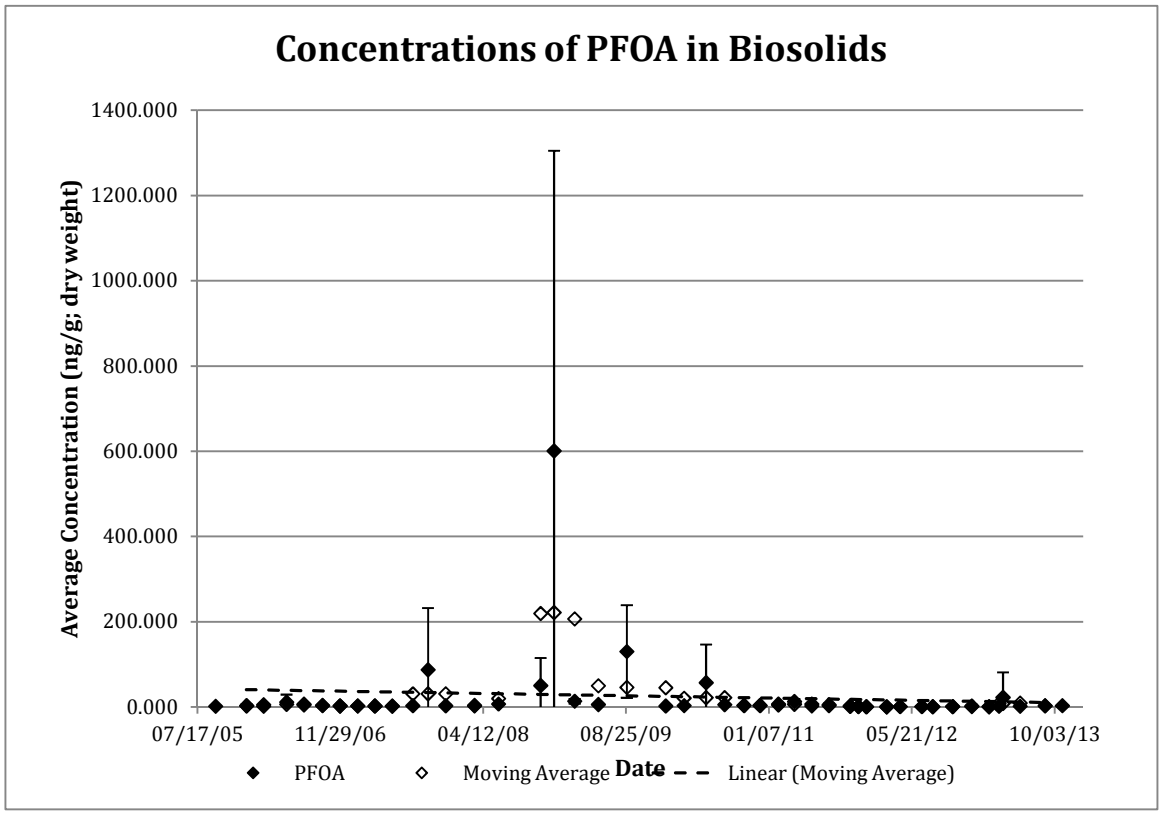

Figure 2-6: Trends of PFHpA Over Time. Points represent the average concentration and bars represent the standard deviation.

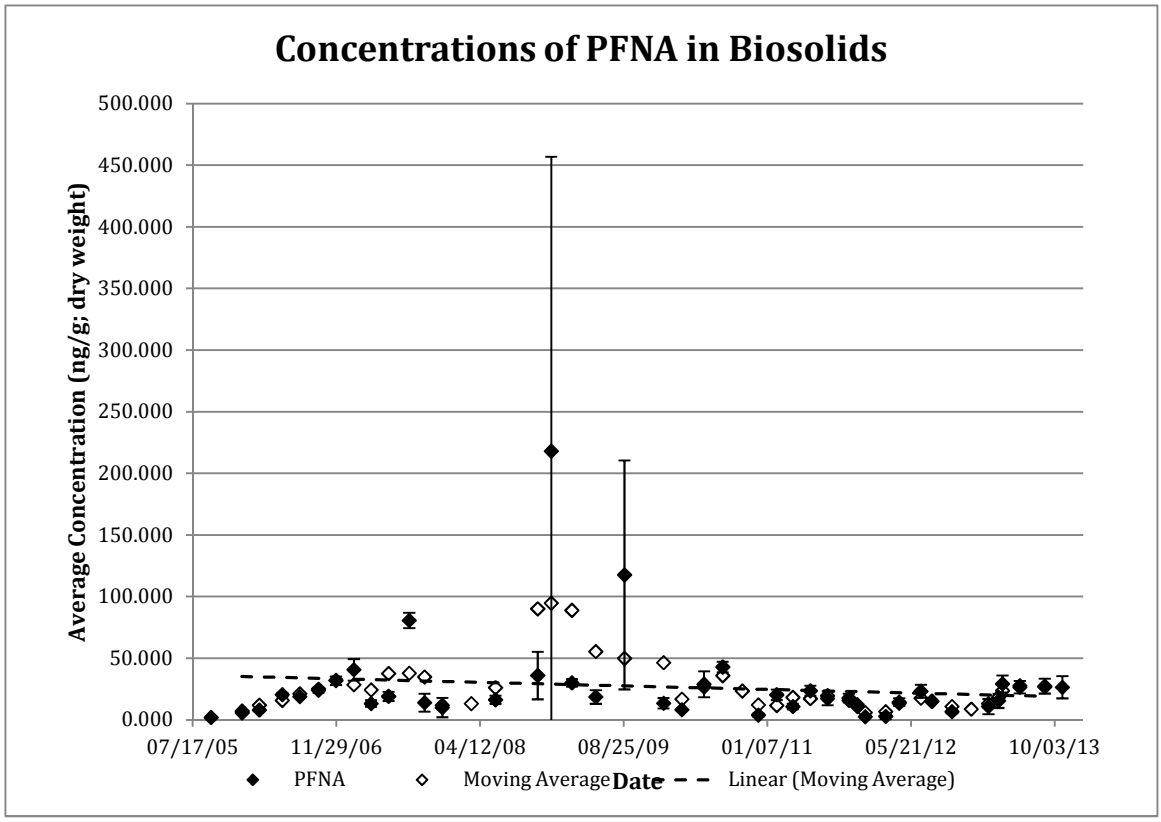

Figure 2-7: Trends of PFHpA Over Time. Points represent the average concentration and bars represent the standard deviation.

Information regarding the MAs of the linear trend lines for PFOA and PFNA, as well as the linear regression analyses performed on the MA data is provided in Table 2-2.

Table 2-2: Trend Line Equation and Linear Regression Analysis Results for Long-Chain PFCAs

	PFOA	PFNA
Linear Equation	Y = -0.0108*X + 42.13	Y = -0.0058*X + 35.88
R^2	0.02972	0.05572
Regression Coefficient (RC)	-0.0108	-0.0058
Is RC Significantly Non-zero?	No	No
P	0.269	0.1275

The linear model for PFOA characterizes approximately just 3% of the data variation between 2005 and 2013. Additionally, the regression coefficient for the linear

model (-0.0108) is not significantly different than zero (P=0.269) indicating that for this linear model there are no significant decreasing trends. Similarly for the PFNA data, the model accounts for a small percentage of the data variation (~6%) and the slope is not statistically significantly different from zero (P=0.13) indicating no significant decrease in concentrations over time.

Yearly averages for long-chain PFCAs are presented in Figure 2-8. In a similar observation to the short-chain PFCA data, peaks in concentrations of PFOA and PFNA in 2008/2009 can be observed. Reasons for this trend are likely due to a few sample dates with increased average concentrations (as seen in Figures 2-6 and 2-7) skewing the average yearly concentrations.

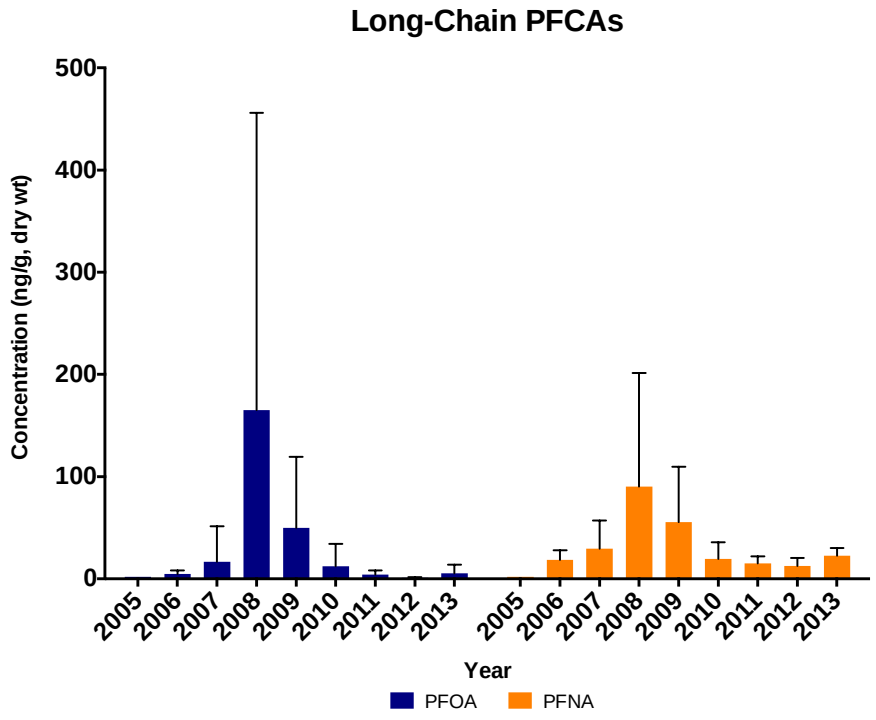

Figure 2-8: Average Yearly Concentrations for Long-Chain PFCAs. Bars represent the average concentration and standard deviation.

Analysis of archived limed biosolids samples collected between 2005 and 2013 for PFSAs indicates that PFDS concentrations have oscillated at concentrations less than 15 ng/g dw. PFOS showed a peak occurring in late 2006/early 2007 followed by a steady decline and then stabilizing at concentrations generally less than 30 ng/g dw by 2008. Results with the MAs and MA trend lines for PFOS and PFDS are presented in Figures 2-9 and 2-10, respectively. Moving average trend line equations, R^2 values, and linear regression statistical information is provided in Table 2-3.

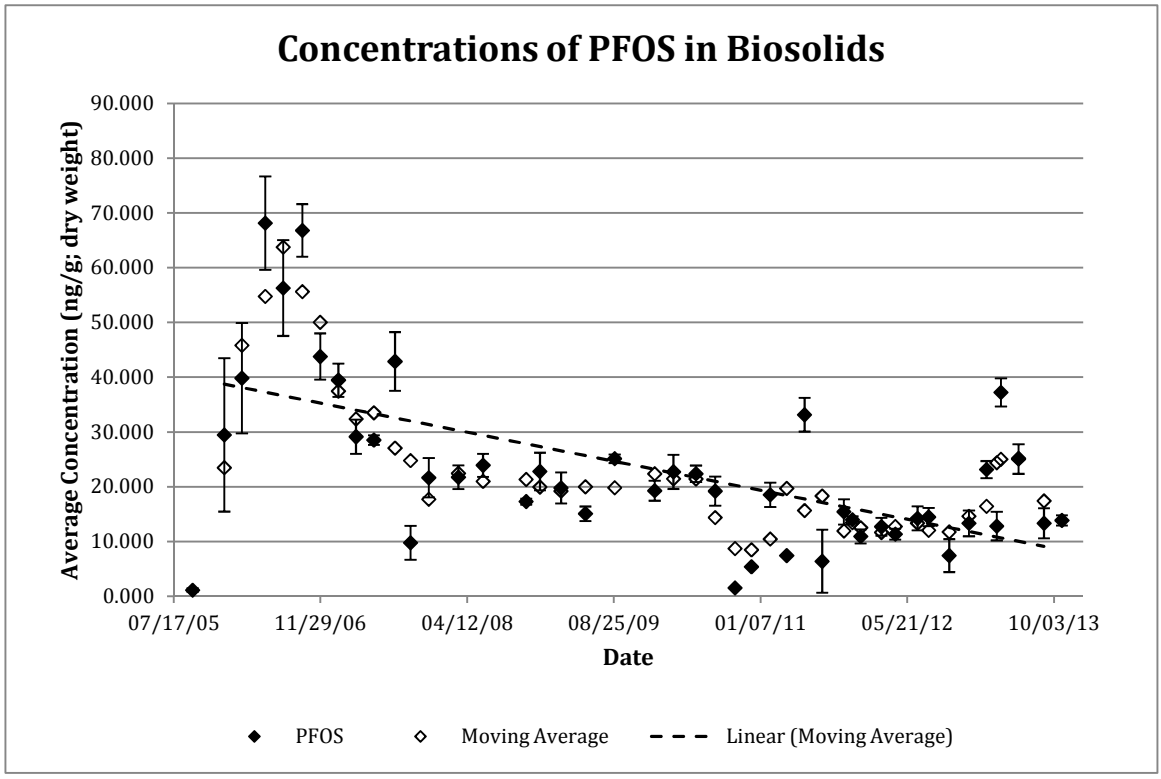

Figure 2-9: Trends of PFOS Over Time. Points represent the average concentration and bars represent the standard deviation.

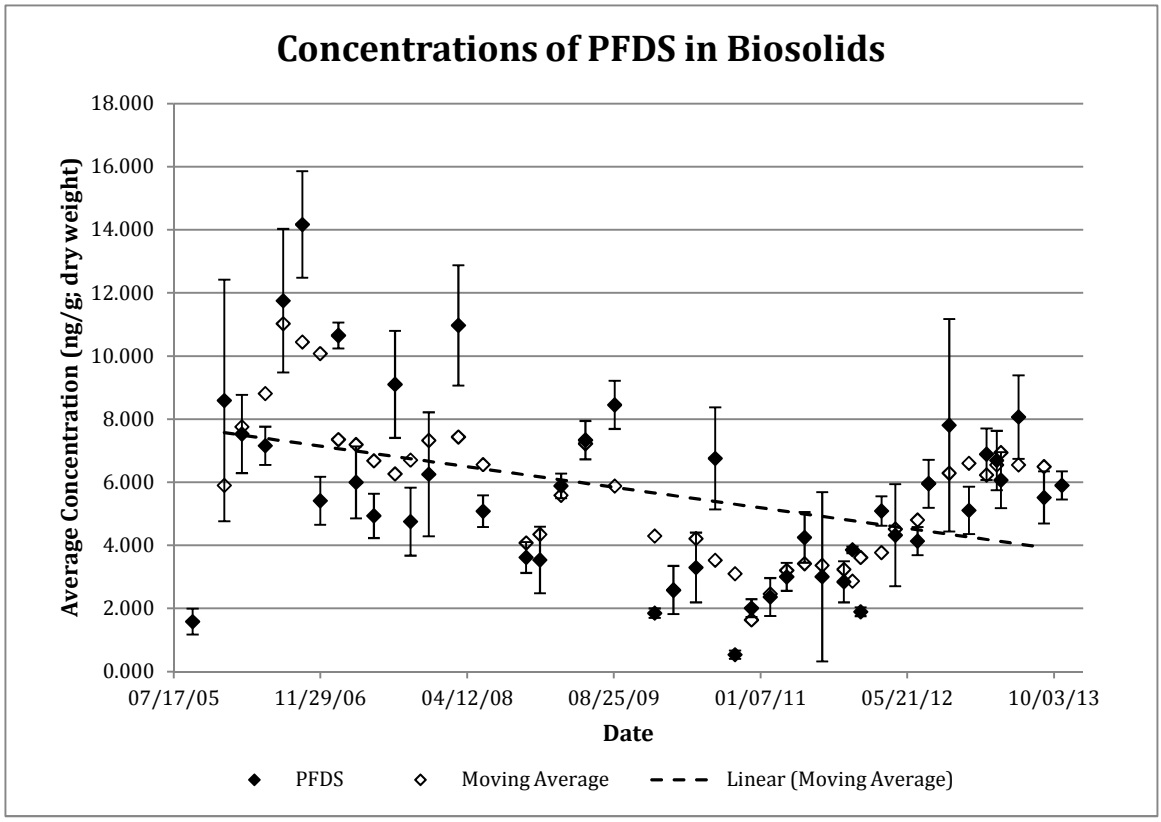

Figure 2-10: Trends of PFDS Over Time. Points represent the average concentration and bars represent the standard deviation.

Table 2-3: Trend Line Equation and Linear Regression Analysis Results for Long-Chain PFSAs

	PFOS	PFDS
Linear Equation	Y = -0.0106*X + 39.87	Y = -0.0013*X + 7.710
R^2	0.4884	0.2648
Regression Coefficient (RC)	-0.0106	-0.0013
Is RC Significantly Non-zero?	Yes	Yes
P	< 0.0001	0.0004

The linear trend line for the MA of the PFOS data accounts for approximately 49% of the data variation. The regression coefficient (-0.011) is statistically different ($P<0.0001$) than zero, indicating a slight downward trend overall. When linear regression analysis is performed on only the MA between 05/25/2006 and 10/02/2007 the slope ($Y = -0.07818*X + 80.98$; $R^2=0.89$) shows a sharper downward trend that is statistically different

(P=0.0001) than the overall trend, highlighting the degree of this decrease in PFOS during this time period. For PFDS, the linear model accounts for 26% of the data variation and linear regression analysis indicates that the regression coefficient (-0.0013) is significantly different (P=0.0004) than zero signifying a small decrease in concentrations between 2005 and 2013. Yearly averages for PFOS and PFDS are provided in Figure 2-11. The yearly average figures appear to be in agreement with the previous observations of decreases of both compounds (excluding the 2005 time point).

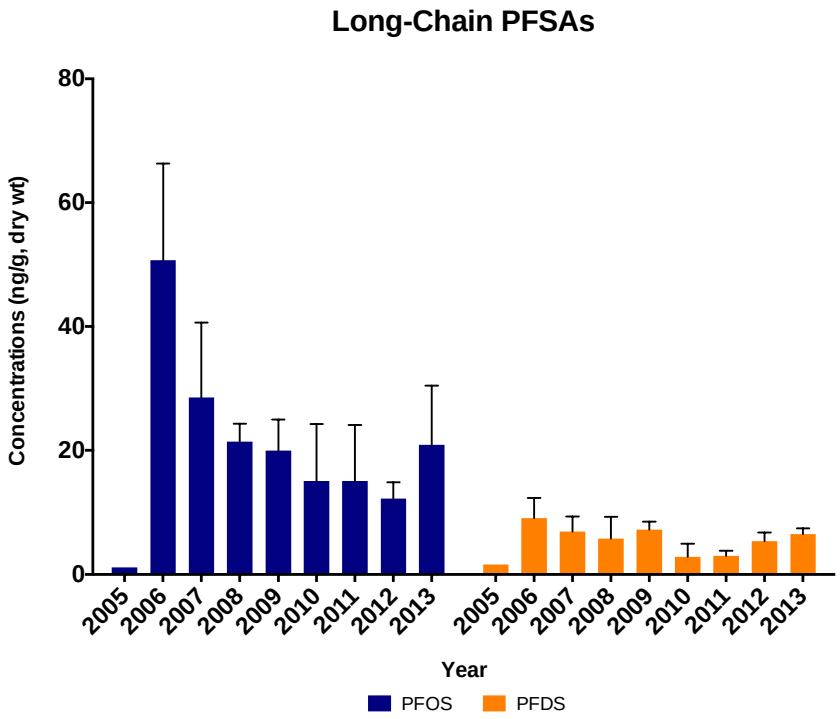

Figure 2-11: Average Yearly Concentrations for Long-Chain PFSAs. Bars represent the average concentration and standard deviation.

2.5 CONCLUSIONS

Long-term analysis of PFCAs and PFSAs in biosolids over time can help give insight into any changes in use of these compounds. PFHxA and PFHpA were the only PFCA compounds that showed a significant change in concentrations, when utilizing a linear model, during the study period. However, while PFHxA and PFHpA indicated a

significant decrease over time, the rate of change was quite low. Yearly averages of PFCA data showed an observable, but not statically analyzable, increase in concentrations of all compounds during 2008 and/or 2009. Both PFOS and PFDS showed significant decreases in concentrations between 2005 and 2013, with PFOS having the more noteworthy decrease. In particular, a major decrease in PFOS concentrations between May 2006 and October 2007 was observed. Yearly averages of these compounds supported these observations. Overall, PFOS was the only compound analyzed to show a key significant decrease in concentrations over the study period, all other compounds had no significant change or a very slight decrease.

While PTFE may contain PFCA compounds as impurities, a comparison in storage methods indicated that the material did not have a significant impact on PFCA concentrations in stored biosolids samples. While variability amongst the samples was evident, this was likely due to the heterogeneity of the biosolids themselves rather than any effects from differences in storage methods. It can be concluded that the use of storage containers containing PTFE-lined caps are suitable for biosolids samples to be analyzed for PFCAs.

CHAPTER 3: SUPPLEMENTAL SAMPLING INFORMATION AND METHODOLOGY DEVELOPMENT

3.1 WASTEWATER TREATMENT PLANT SPECIFICS

The District of Columbia Water and Sewage Authority (DC Water) serves the District of Columbia and its surrounding metro area, including counties in Maryland and Virginia. DC Water's Blue Plains Advanced Wastewater Treatment Plant (Blue Plains) serves a region of over 2 million people and has the capacity to treat 370 million gallons of raw wastewater per day.

The Blue Plains treatment process consists of (Figure 3-1):

- Preliminary treatment (grit & debris removal)
- Primary treatment
- Secondary treatment (activated sludge)
- Nitrification/Denitrification
- Filtration
- Disinfection

Biosolids from the Blue Plains plant consist of primary treatment sludge as well as secondary treatment and nitrification biological solids. Solids from primary treatment are settled and thickened in tanks via gravity and polymers. Gravity and polymer are also used to settle the solids from the secondary and nitrification treatment steps. Afterwards, these solids are thickened using dissolved air flotation (DAF) thickeners. Finally, both sets of solids are mixed, dewatered via centrifugation, and then stabilized using lime. Lime is added to this sludge mixture on a dry weight basis of approximately 15% to neutralize pathogenic organisms, classifying the product as Class B biosolids. The Blue

Plains facility produces over 1,200 wet tones of biosolids daily, the majority of which (>90%) are disposed of via land-application. (DCWater 2014; Lozano et al. 2013) Because the final biosolid product from Blue Plains has been dewatered, stabilized, and is not free flowing, it is considered a solid sludge product (USEPA 2009b).

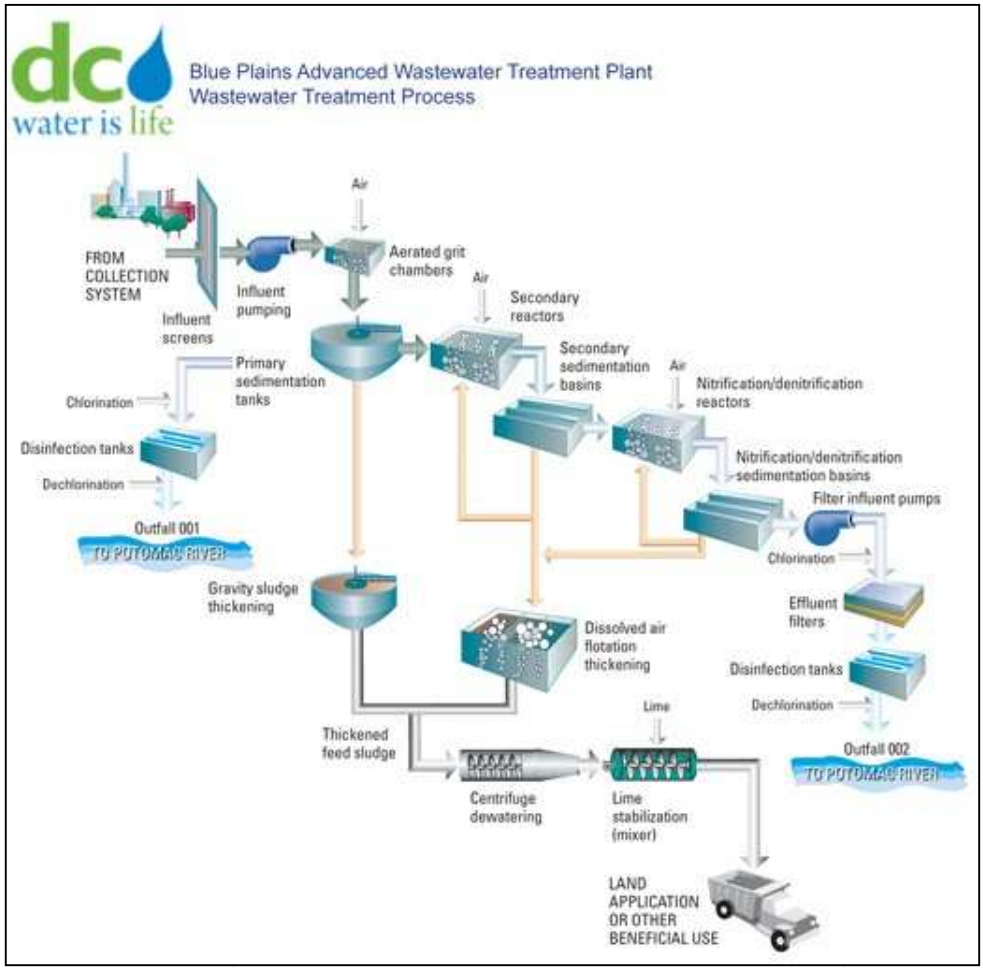

Figure 3-1: DC Water's Blue Plains Facility Wastewater Treatment Process (dcwater.com)

3.2 SAMPLE COLLECTION SPECIFICS

As outlined in Section 2.3.1, grab samples were collected approximately every two to three months, beginning in 2005, directly after the liming process. All samples were stored in glass jars with PTFE-lined caps as well as HDPE containers (beginning in

June 2012) for the determination of the influence of storage methods on PFCA concentrations in biosolids. Specific sample dates and location(s) within the different storage methods of samples analyzed are provided in Table 3-1.

Table 3-1: Collection Dates and Storage Locations of Samples Analyzed

Sample Date	Bottom of Glass Jar[1]	Top of Glass Jar[2]	HDPE Container	Sample Date	Bottom of Glass Jar[1]	Top of Glass Jar[2]	HDPE Container
09/19/05	X	--	--	08/05/10	X	--	--
01/05/06	X	--	--	10/12/10	X	--	--
03/06/06	X	--	--	12/07/10	X	--	--
05/25/06	X	--	--	02/09/11	X	--	--
07/25/06	X	--	--	04/06/11	X	--	--
09/28/06	X	--	--	06/06/11	X	--	--
11/28/06	X	--	--	08/05/11	X	--	--
01/29/07	X	--	--	10/18/11	X	--	--
03/30/07	X	--	--	11/16/11	X	--	--
05/30/07	X	--	--	12/14/11	X	--	--
08/10/07	X	--	--	02/23/12	X	--	--
10/02/07	X	--	--	04/10/12	X	--	--
12/03/07	X	--	--	06/25/12	X	--	X
03/13/08	X	--	--	08/02/12	X	--	X
06/05/08	X	--	--	10/11/12	X	--	X
10/30/08	X	--	--	12/17/12	X	--	X
12/16/08	X	--	--	02/15/13	X	--	X
02/26/09	X	--	--	03/22/13	X	X	X
05/20/09	X	--	--	04/05/13	X	X	X
08/28/09	X	--	--	06/04/13	X	X	X
01/11/10	X	--	--	08/30/13	X	X	X
03/16/10	X	--	--	10/30/13	X	X	X
06/01/10	X	--	--				

X = sample type extracted

-- = sample type not extracted

[1] Sample analyzed collected from bottom of glass sample jar with PTFE-lined cap.
[2] Sample analyzed collected from top of glass sample jar with PTFE-lined cap. Sample was in contact with cap.

3.3 METHOD DEVELOPMENT AND ANALYTICAL CHALLENGES

Surfactants, ions, and biosolids can present a number of challenges during the extraction and/or analysis process. The complexity of the biosolids material; including properties such as the large surface area of particles, charged particles, and interstitial spaces within particles, can influence chemical sorption to biosolids and make extraction of target compounds from the material difficult. Additionally, biosolids may contain large amounts of chemical compounds that are added through the WWT process, further complicating extraction of specific compounds from the material. (Chari & Halden 2012) As mentioned in Section 2.1, polymers and lime are added to wastewater solids at the Blue Plains facility (Lozano et al. 2013), these chemical additions, in conjunction with numerous other pollutants that are present in the biosolids material can further enhance the complexity of analysis of this material.

3.3.1 EXTRACTION CHALLENGES

The chemical properties of surfactants can make their extraction from environmental samples difficult. Hydrophobic and/or electrostatic interactions between sludges and surfactant compounds can make finding a suitable and efficient extraction method difficult (Petrović & Barceló 2004; Olkowska et al. 2011).

Methods were first tested using laboratory-grade sand spiked with PFCA and PFSA compounds (both straight standards and mass-labeled standards). The first method tested was that of Powley *et al.* (2005) where shaking and loose Supelco Envi-Carb added to microcentrifuge tubes were used for extraction and clean-up of 8 different PFCA compounds (carbon chain lengths of C = 6-12, 14) from liquid sewage sludge.

(Powley et al. 2005) This method was not found to be efficient for the determination of 12 PFCA and PFSA compounds in dewatered, limed biosolids, particularly for short-chained compounds as (1) Powley *et al.* did not analyze for compounds with carbon chain lengths less that 6 and (2) only had ^{13}C-PFOA to use as a surrogate standard, which is not a good indicator of recoveries for shorter-chained compounds. A method developed by Ahrens *et al.* (2009) for the detection of PFASs in sediments was then tested. This method again utilized shaking and, although this method was more efficient than that of Powley *et al.*, using Supelco Envi-Carb SPE tubes rather than loose Envi-Carb, and the recoveries from this method were an improvement, they still were not within the desired range. (Ahrens et al. 2009)

Trials with a sediment method published Higgins *et al.* (2006) were attempted. (Higgins & Luthy 2006) Communication with C. Higgins regarding more details of the method (since those published were vague in parts) led to the suggestion to try a recently published updated method by the Higgins group. Sepulvado *et al.* (2011) combined shaking with sonication and shaking for much improved recoveries of PFCA and PFSA compounds from spiked sand samples, with the exception of very long-chained PFCA compounds. (Sepulvado et al. 2011) Clean-up for this method was as presented by Powley *et al.* – loose Envi-Carb added to microcentrifuge tubes. However, this method was very time-consuming due to long shaking periods and, once attempted on limed biosolids, was not effective. The Envi-Carb alone was not sufficient for sample clean-up.

The literature was searched for papers using various types of SPE clean-up for PFAS analysis in solids samples. Yoo *et al.* (2009) presented comparison between various solvents, pretreatments, and clean-up methods for PFSAs extracted from industrial sludge

using sonication. The methods and trials presented in this paper were attempted and although Yoo *et al.* found better results with Oasis hydrophilic-lipophilic-balanced (HLB) SPE cartridges (Yoo et al. 2009), this study found Oasis WAX cartridges to yield better results. In a 2005 study comparing analytical methods for PFASs, Taniyasu *et al.* also found WAX cartridges to perform better than HLB cartridges for shorted-chain PFSA compounds (Taniyasu et al. 2005).

Variations on a method using shaking and sonication for extraction, coupled with Oasis WAX cartridges for clean-up were attempted. Eventually, a paper by Navarro *et al.* (2011) was discovered that combined many of the effective techniques from previous papers: (1) shaking and sonication, (2) initial clean-up using Envi-Carb SPE cartridges, and (3) further clean-up/analyte concentration using Oasis WAX cartridges (Navarro et al. 2011). The method was amended so that less time was required and less waste was produced. Additionally, the WAX cartridges were conditioned/rinsed using a method amended from that recommended by Waters Corporation for PFOA and PFOS isolation using their WAX cartridges. This led to the final method outlined in Section 2.3. However, despite the establishment of a method that seemed effective for all 12 compounds, inconsistencies with extraction efficiencies were encountered, leading to speculation of other problems within the analysis procedure.

3.3.2 MATRIX EFFECTS

Matrix effects can occur in the HPLC-MS/MS process when interfering substances coelute with the compounds of interest and influence the ionization efficiency. These interfering substances; which can originate from the sample extract itself, a previous sample (eluting from the HPLC column late), or column buildup, can increase

(ion enhancement) or decrease (ion suppression) the ion efficiency when the compounds of interest are being analyzed. This, in turn, can influence analyte detection concentrations (when compared with a pure standard), repeatability, and the limit of quantitation. There are several factors that may cause matrix effects including the chemical properties of the analytes of interest, pre-analysis clean-up procedures, the chromatography conditions, and the MS conditions. (Taylor 2005; Gosetti et al. 2010)

The amount of matrix effects on a chemical of interest can be influenced by the properties of the chemical itself. For example, very polar compounds are more likely to experience ion suppression (Taylor 2005). Additionally, when using reverse phase columns, more hydrophobic compounds are less likely to be influenced by matrix effects as they have a higher affinity for the column packing and elute later. Also, it has been observed that compounds with a large mass can suppress the ion signal of compounds that have a smaller mass. (Gosetti et al. 2010)

Sample extraction and clean-up procedures can have significant impacts on the degree of matrix effects encountered. Optimization of the extraction method can reduce the uptake of undesired compounds and use of proper clean-up steps can further decrease interfering compounds from the extract (Chambers et al. 2007). The difficulty in relying solely on optimization of sample preparation techniques to eliminate matrix effects arises when the techniques used to remove undesired compounds but retain the analytes of interest can, in fact, concentrate certain interfering compounds (Gosetti et al. 2010).

Chromatography conditions can also influence the degree of matrix effects on compound of interest. Salts, buffers, and acids added to the LC mobile phase to improve peak shape and separation, may, in fact, cause ion suppression of the compounds of

interest (Gosetti et al. 2010). Additionally, utilizing a fast LC gradient can also increase the degree of matrix effects. This is because with a quick gradient, the chromatographic separation between compounds, those of interest or otherwise, is reduced (Chambers et al. 2007). Modification of the mobile phase and/or stationary phase (utilizing a different column to better separate compounds of interest from interferences) can help reduce the degree of matrix effects (Gosetti et al. 2010).

Finally, conditions within the MS can influence the degree of matrix effects as well. Gosetti *et al.* (2010) recommend changing either the ionization mode of the MS method or using a different MS if matrix effects are a problem with the samples being analyzed.

3.3.2.1 Determining and Accounting for Matrix Effects

After various difficulties in determining an appropriate method for the extraction and analysis of the 12 PFCA and PFSA compounds, it was surmised that matrix effects may be occurring and impacting recovery of surrogate standards. The post extraction addition method (Taylor 2005) was used to determine whether matrix interferences were indeed occurring, as well as to what extent they were happening. Using the extraction method outlined in Section 2.3, 15 samples in total were extracted in three batches with various spiking techniques:

- Batch 1: 4 limed biosolids samples and 1 sand blank spiked with 10 ^{13}C-labeled PFAS compounds prior to extraction.
- Batch 2: 4 limed biosolids samples and 1 sand blank spiked with 10 ^{13}C-labeled PFAS compounds after extraction, prior to injection into instrumentation.

- Batch 3: 5 limed biosolids samples extracted and spiked with varying concentrations (5ng/mL, 10ng/mL, 25ng/mL, 50ng/mL, and 100ng/mL) of all 12 PFAS compounds to create a standard curve in the matrix for comparison to a standard curve in pure solvent.

The results of this experiment showed that matrix effects were indeed occurring and influencing perceived sample recoveries and compound concentrations. Figures 3-2 through 3-13 show "pure" standard curves (those created in solvent and used for instrument calibration) and matrix curves (those created by spiking biosolids samples, as outlined under the "Batch 3" bullet above) for PFCA and PFSA compounds analyzed. It is important to note that blanks for PFHxA, PFNA, and PFUnA showed significant concentrations for these compounds. While this was taken into account for curve creation, the matrix curves for these compounds were likely still affected.

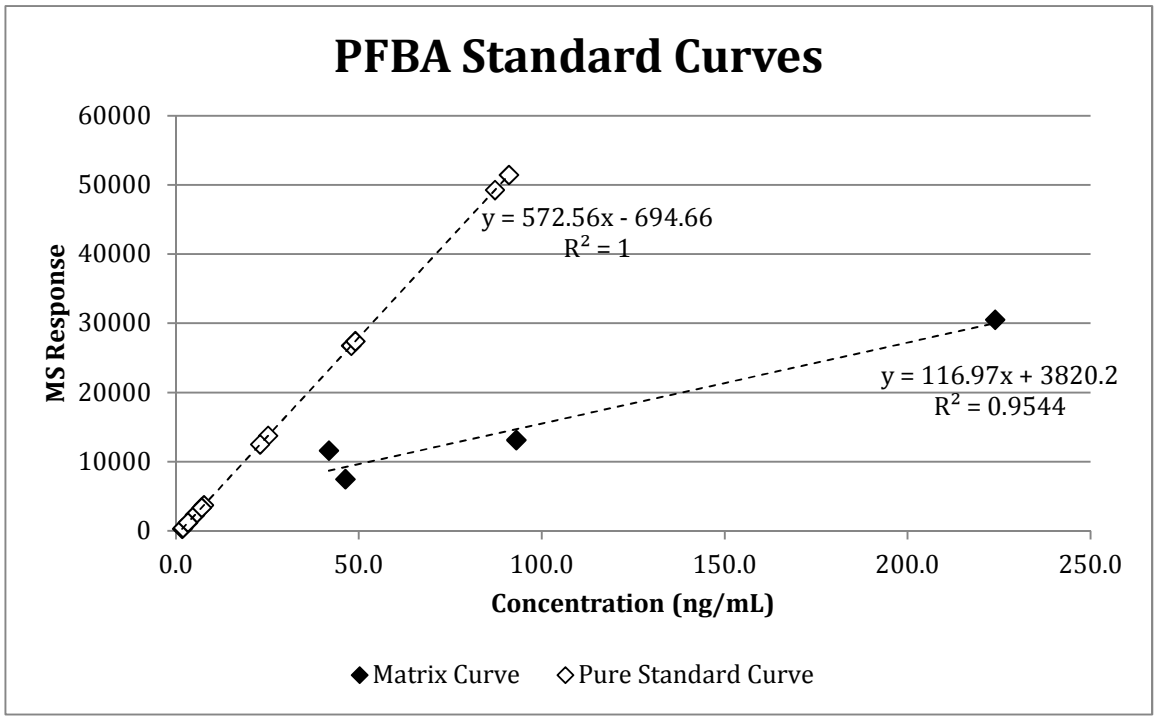

Figure 3-2: PFBA Matrix and Pure Standard Curves

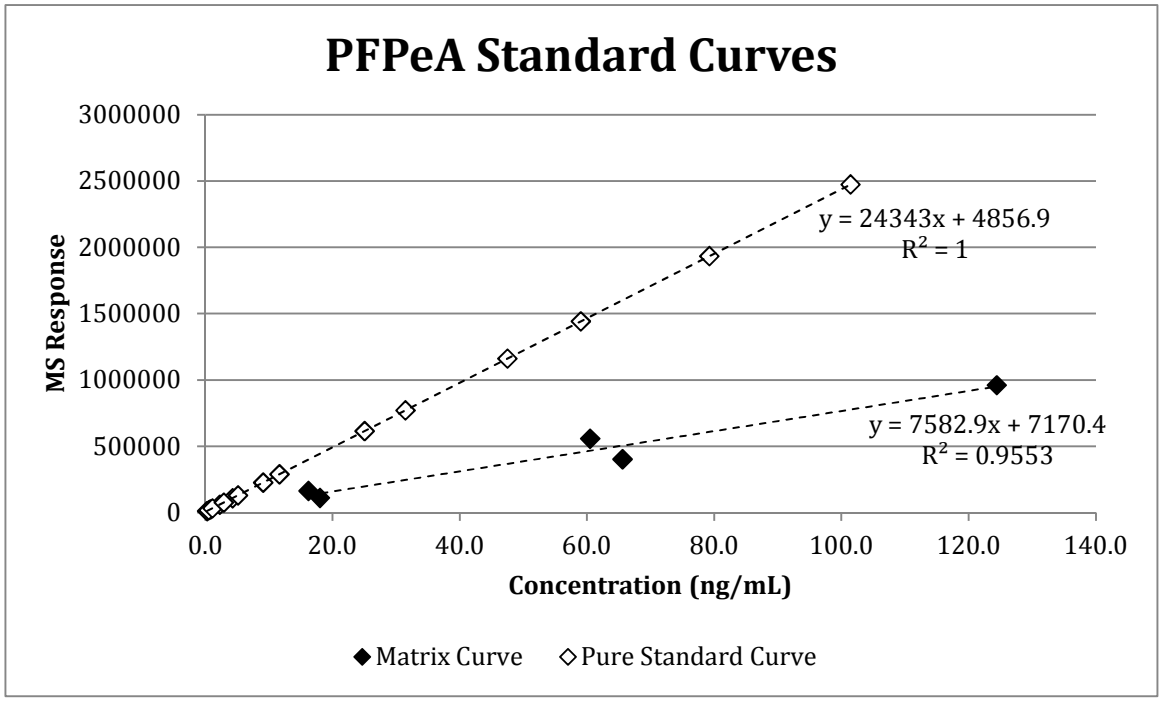

Figure 3-3: PFPeA Matrix and Pure Standard Curves

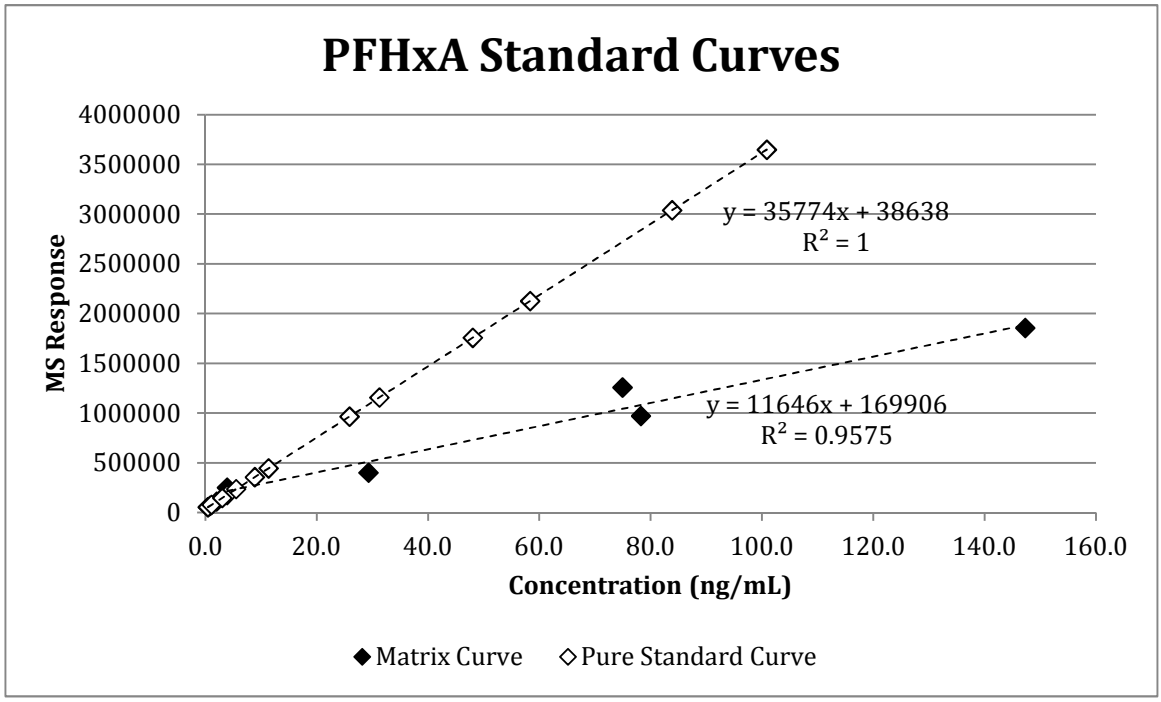

Figure 3-4: PFHxA Matrix and Pure Standard Curves

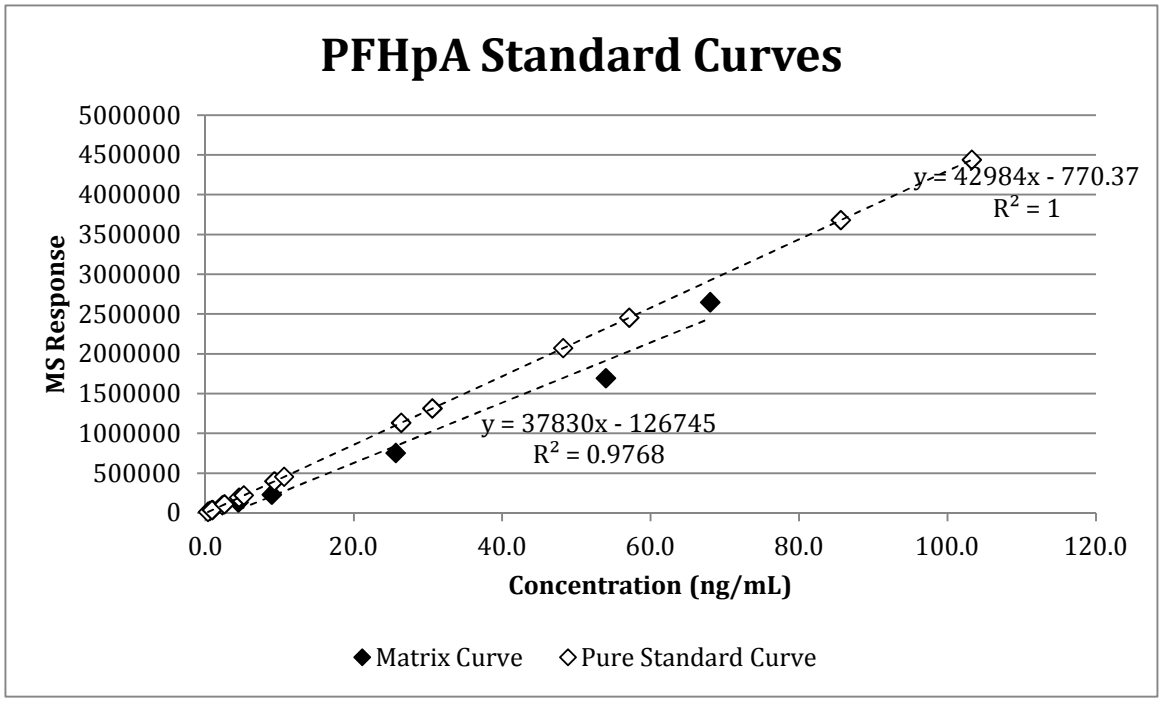

Figure 3-5: PFHpA Matrix and Pure Standard Curves

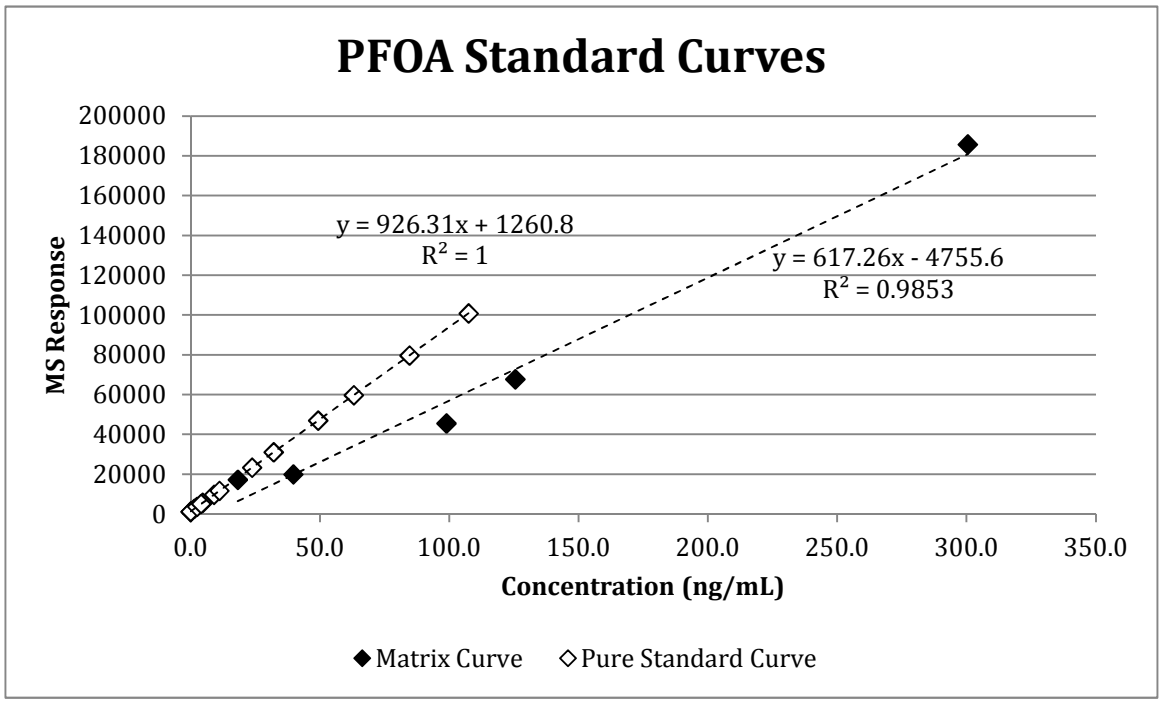

Figure 3-6: PFOA Matrix and Pure Standard Curves

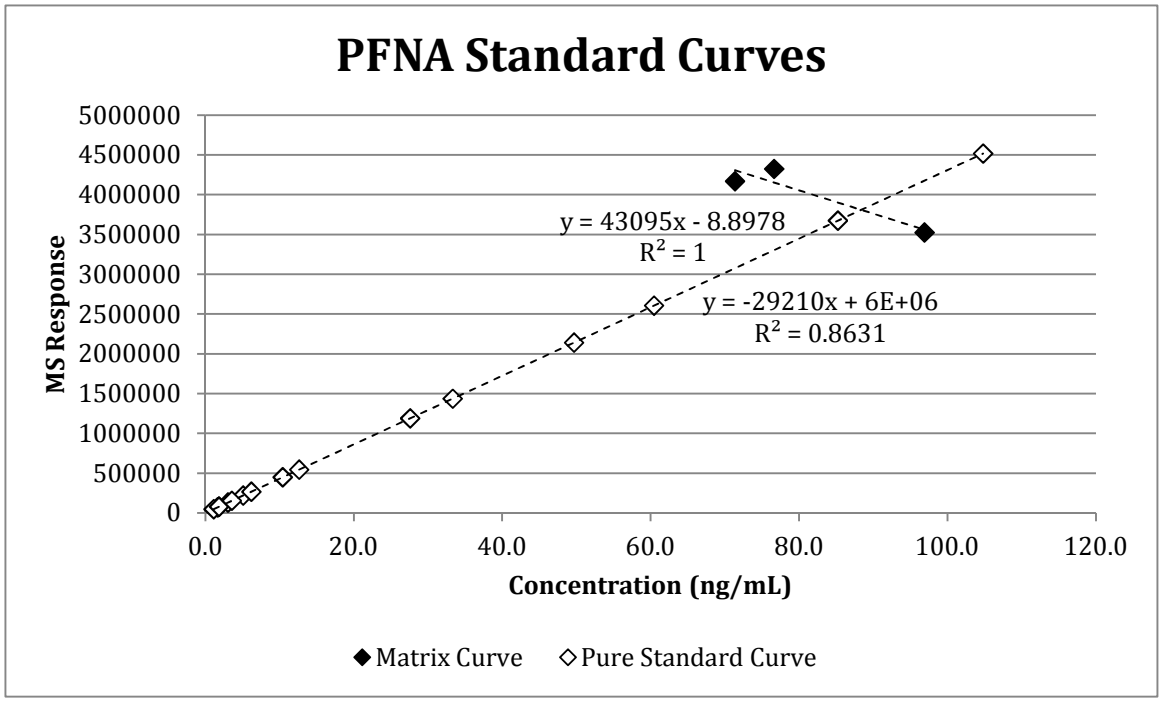

Figure 3-7: PFNA Matrix and Pure Standard Curves

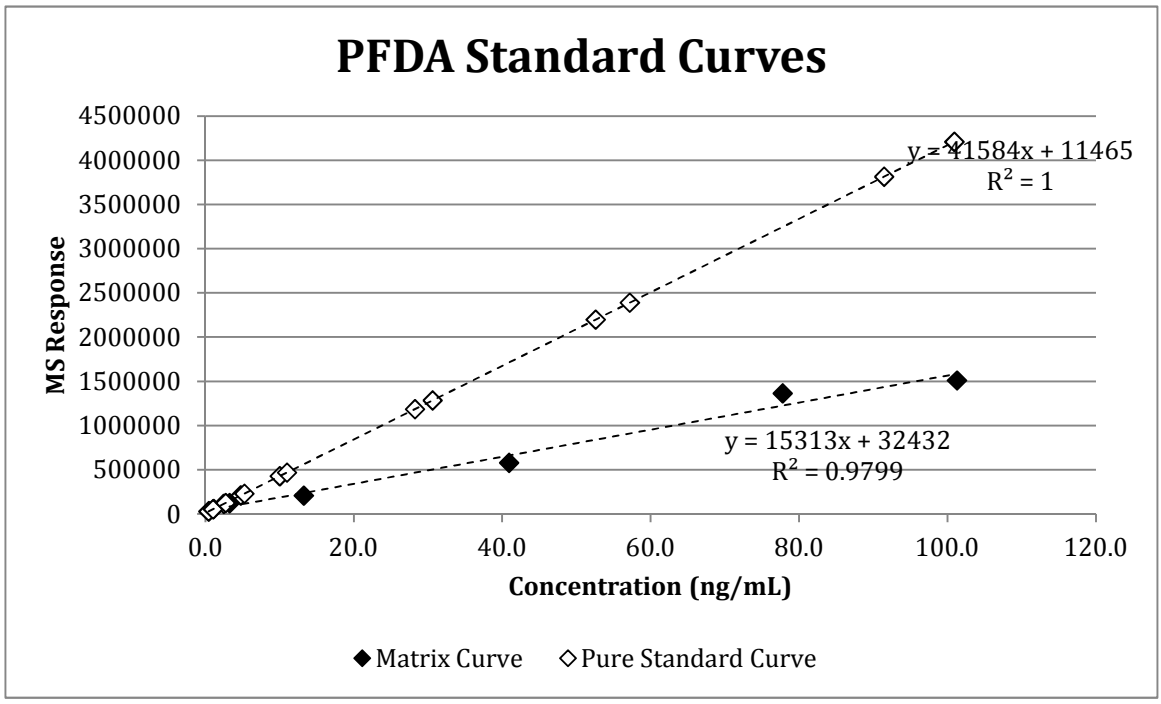

Figure 3-8: PFDA Matrix and Pure Standard Curves

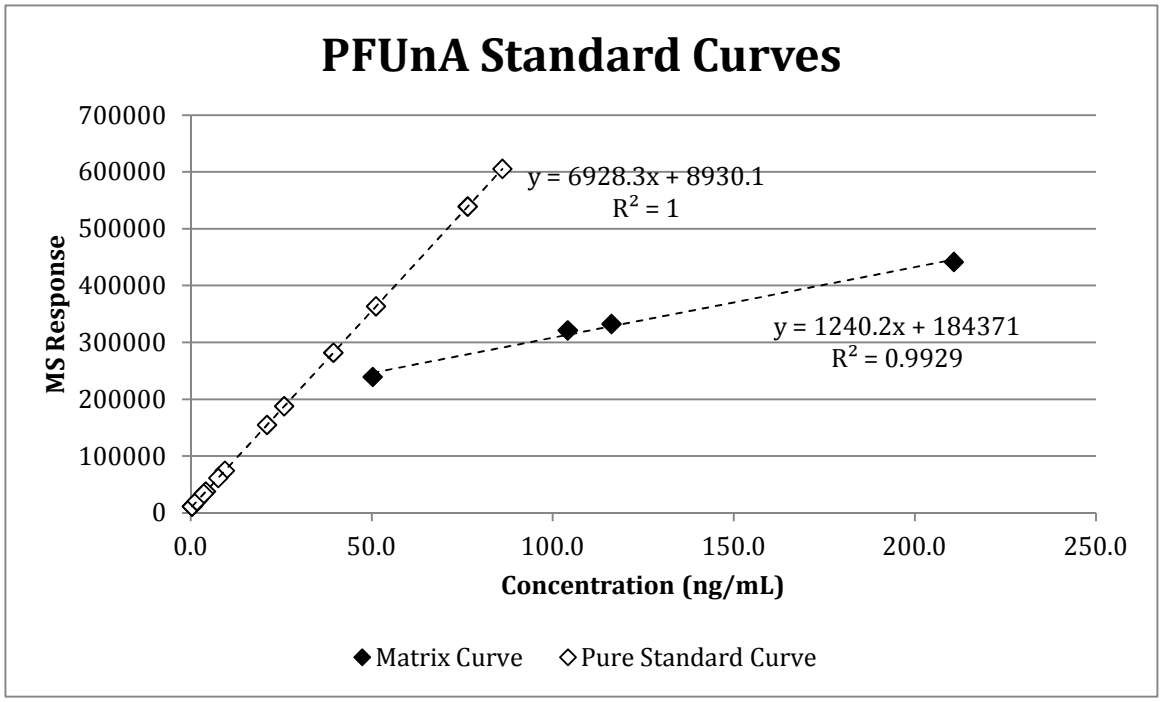

Figure 3-9: PFUnA Matrix and Pure Standard Curves

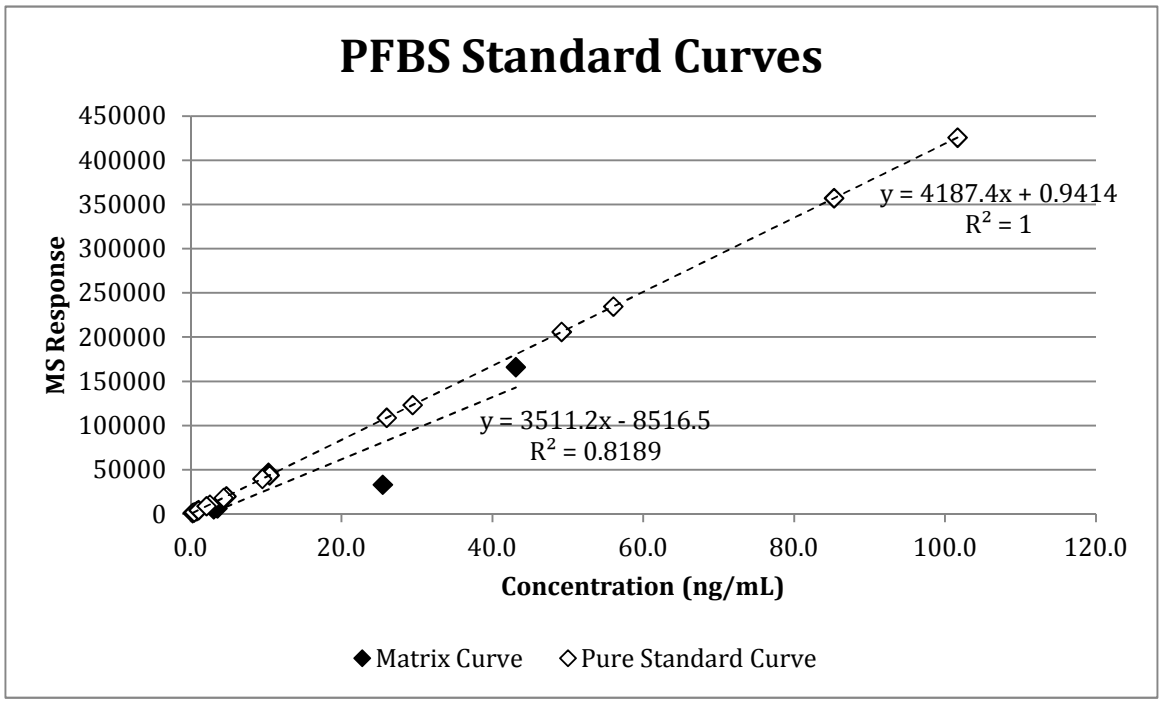

Figure 3-10: PFBS Matrix and Pure Standard Curves

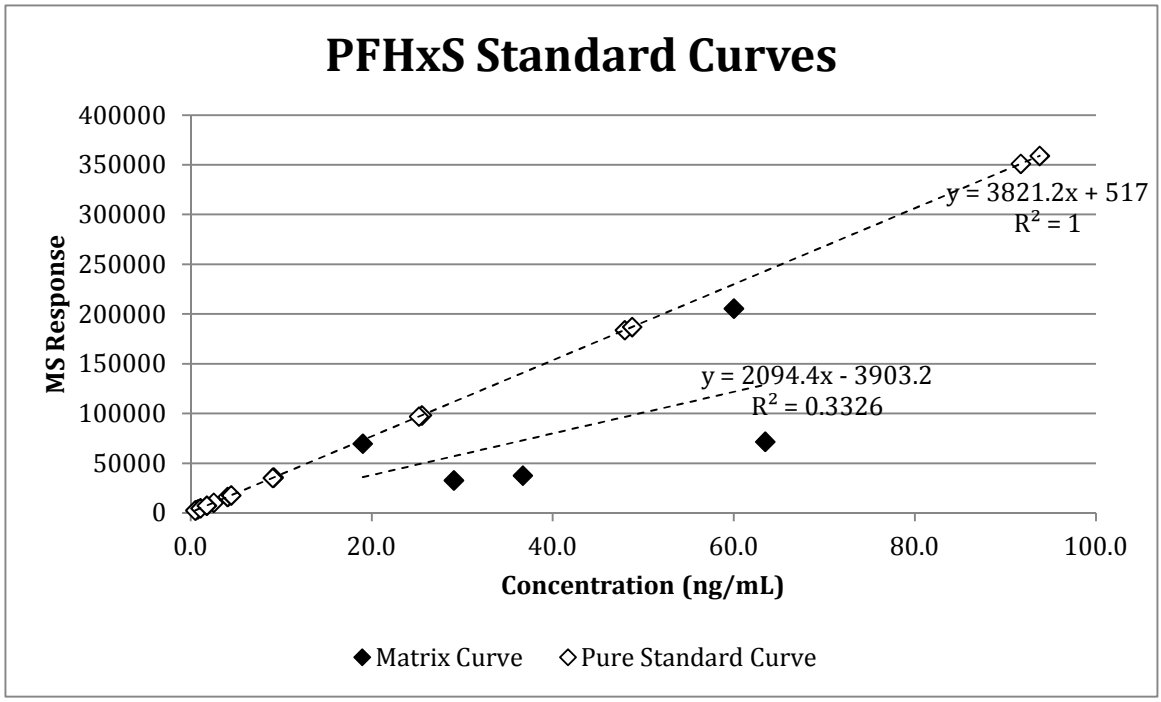

Figure 3-11: PFHxS Matrix and Pure Standard Curves

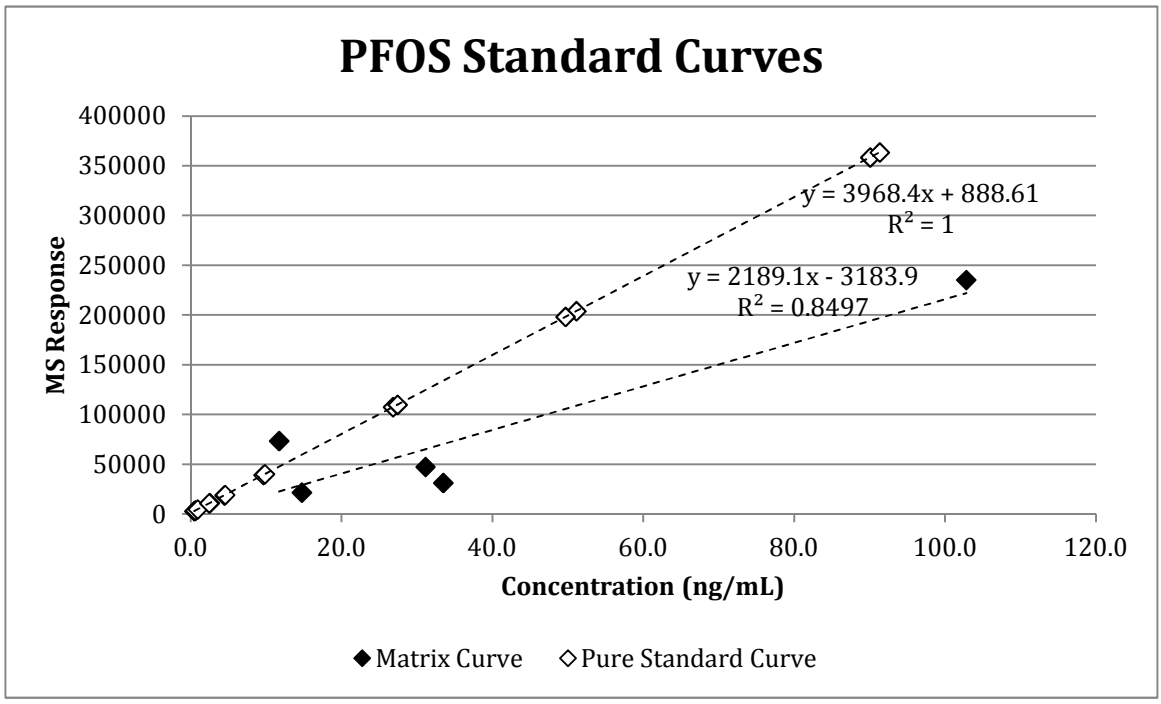

Figure 3-12: PFOS Matrix and Pure Standard Curves

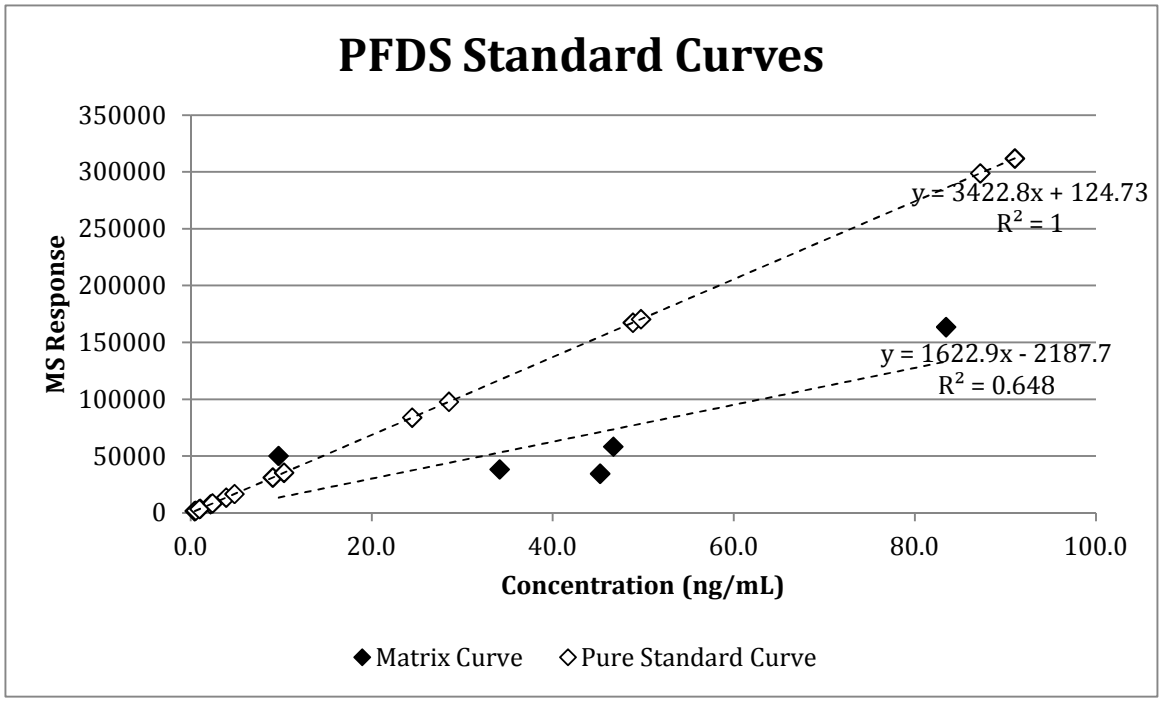

Figure 3-13: PFDS Matrix and Pure Standard Curves

Additionally, ^{13}C-labeled PFCA and PFSA compounds were spiked before (Batch #1 above) and after (Batch #2 above) extraction to compare loss of recovery from the entire extraction method to those encountered during the MS method. Results from the PFCA compounds are provided in Figure 3-14. Although not all loss of recovery was encountered during the MS method, it is apparent that the detection of PFCA compounds is strongly influenced during this step. The influence on PFUnA was greatest, with recoveries of ^{13}C-PFUnA from Batch #2 ranging from 19.1 to 23.2%.

Recoveries of PFSA compounds are presented in Figure 3-15. Only ^{13}C-PFHxS and ^{13}C-PFOS were analyzed as labeled compounds for PFBS and PFDS were not available. While Figure 3-15 implied little matrix effects occurred for these compounds, Figures 3-10 through 3-13 show that large amounts of matrix effects occurred for PFSAs. The is the reason for the large standard deviation shown in Figure 3-15 was due to matrix

effects causing not only suppression of the compounds analyzed, but, in some samples, enhancement as well. Use of a surrogate spike, as mentioned in Section 2.3.2, allowed for loss of analytes during the extraction process as well as instrument analysis to be taken into account.

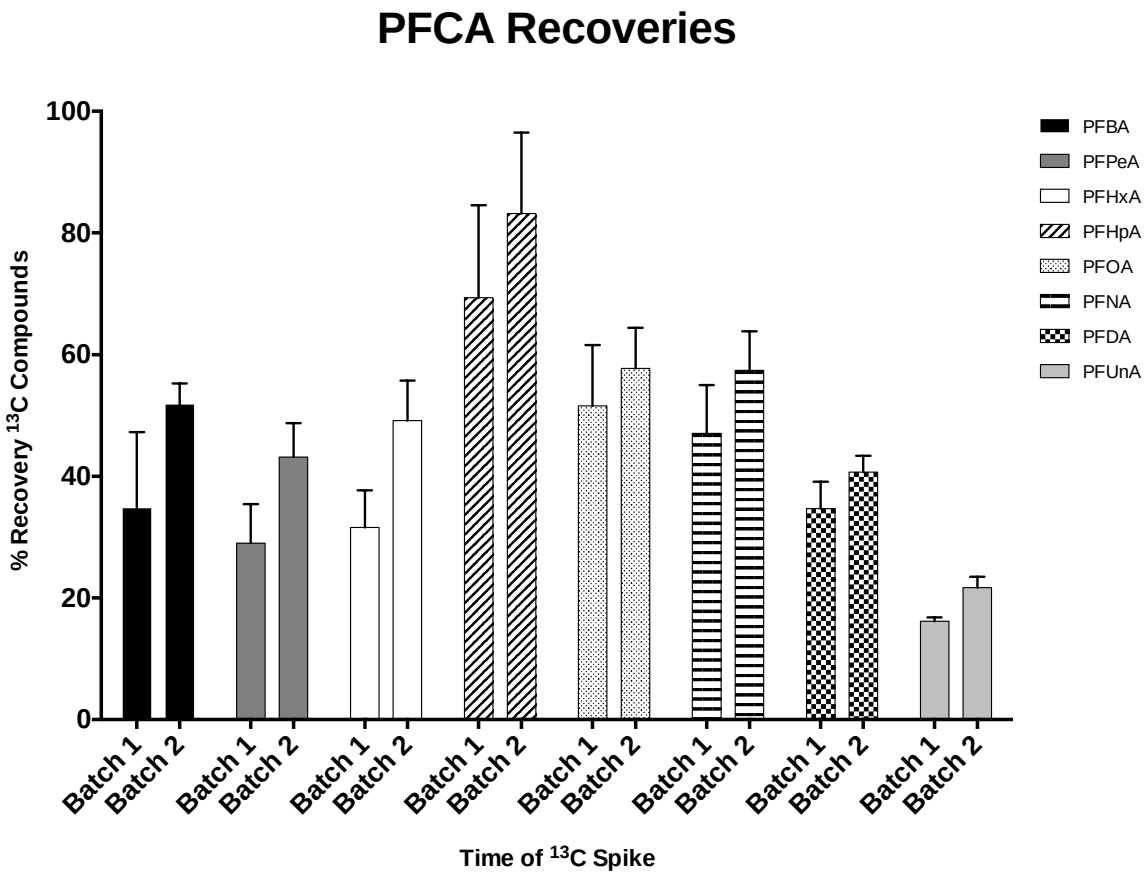

Figure 3-14: PFCA ^{13}C-Labeled Compound Recoveries. . Bars represent the average concentration and standard deviation.

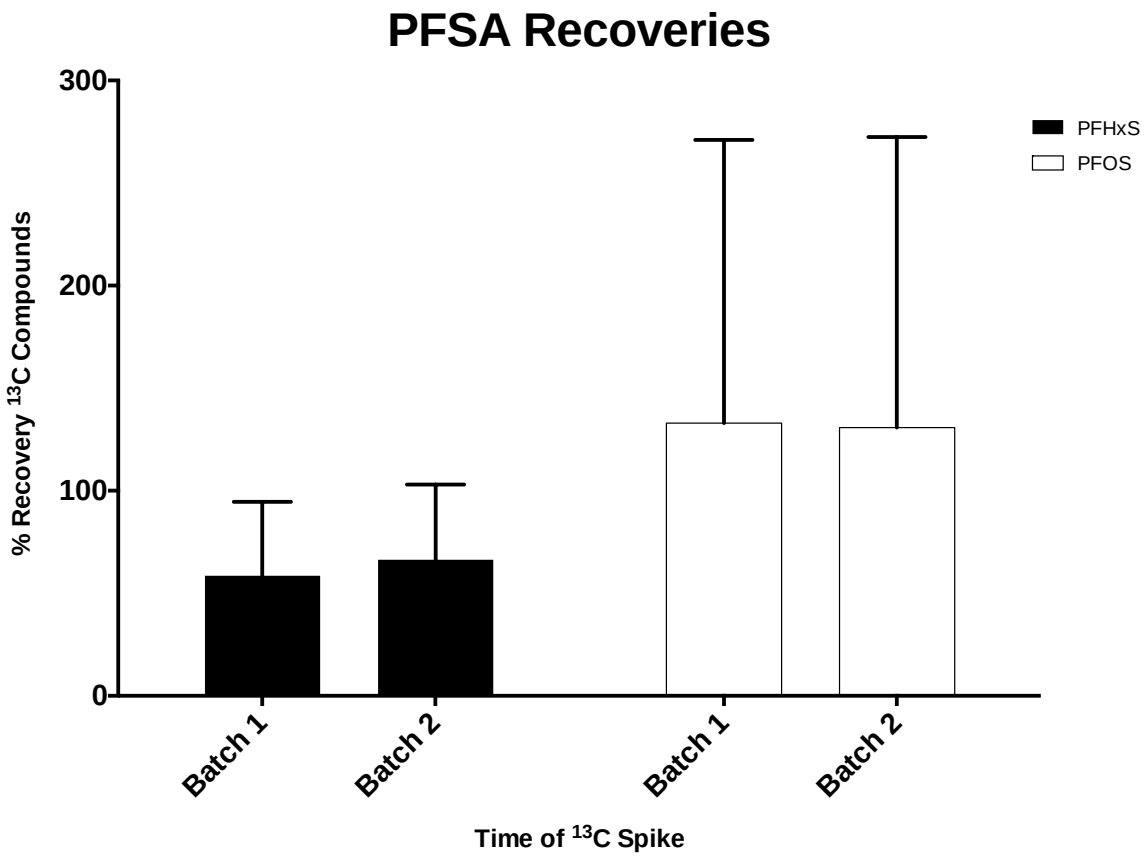

Figure 3-15: PFSA ^{13}C-Labeled Compound Recoveries. . Bars represent the average concentration and standard deviation.

3.4 HPLC-MS/MS PARAMETERS AND SETTINGS

Specifics regarding instrument settings as well as detection and quantification parameters for each individual compound, including collision energies and primary/secondary ions, are provided in Table 3-2.

Table 3-2: HPLC-MS/MS Parameters and Settings

Compound	Acronym	Primary Ion MW (g/mol)	Secondary Ion MW (g/mol)	Retention Time (min)	Cone (V)	Collision (eV)	Ion Mode
Perfluorocarboxylic Acids							
Perfluorobutanoic acid	PFBA	213.60	169.10	7.34	10	12	ES-
Perfluoropentanoic acid	PFPeA	263.20	219.60	8.27	10	12	ES-
$^{13}C_5$-Perfluoropentanoic acid	$^{13}C_5$-PFPeA	268.01	223.01	8.27	10	12	ES-
Perfluorohexanoic acid	PFHxA	313.10	269.60	8.57	15	12	ES-
$^{13}C_5$-Perfluorohexanoic acid	$^{13}C_5$-PFHxA	318.02	273.02	8.57	15	12	ES-
Perfluoroheptanoic acid	PFHpA	363.10	319.70	8.81	15	12	ES-
Perfluorooctanoic acid	PFOA	413.70	369.70	9.08	15	12	ES-
$^{13}C_8$-Perfluorooctanoic acid	$^{13}C_8$-PFOA	421.01	377.01	9.08	15	12	ES-
Perfluorononanoic acid	PFNA	463.10	419.70	9.31	15	12	ES-
$^{13}C_5$-Perfluorononanoic acid	$^{13}C_5$-PFNA	467.54	424.04	9.31	15	12	ES-
Perfluorodecanoic acid	PFDA	513.10	469.70	9.60	20	15	ES-
Perfluoroundecanoic acid	PFUA	563.60	519.20	9.86	20	15	ES-
Perfluorosulfonic Acids							
Perfluorobutane sulfonic acid	PFBS	299.10	80.60	8.27	50	40	ES-
Perfluorohexane sulfonic acid	PFHxS	399.10	80.60	8.81	80	45	ES-
$^{13}C_3$-Perfluorohexane sulfonic acid	$^{13}C_3$-PFHxS	402.07	80.60	8.81	80	45	ES-
Perfluorooctane sulfonic acid	PFOS	499.10	80.60	9.36	35	65	ES-
$^{13}C_8$-Perfluorooctane sulfonic acid	$^{13}C_8$-PFOS	507.05	80.60	9.36	35	65	ES-
Perfluorodecane sulfonic acid	PFDS	599.10	80.60	9.86	35	70	ES-

MW = molecular weight
ES- = elecrospray negative

CHAPTER 4: SUPPLEMENTARY DISCUSSION AND DATA

4.1 FURTHER DISCUSSION

4.1.1 RELATIONSHIP BETWEEN PFHPA AND PFOA CONCENTRATIONS

As mentioned in Section 4.2.4, several spikes in PFOA and PFHpA concentrations appeared to occur simultaneously. This may be due to the degradation of a fluorotelomer alcohol (FTOH) precursor compound into both PFOA and PFHpA. A 2009 study by Wang et al. showed the potential for 8:2 FTOH in soil to be degraded aerobically into various other fluorinated compounds, including the PFCAs PFOA and PFHxA (N. Wang et al. 2009). In their 2011 review of PFASs and their origins, Buck et al. adapted the biodegradation pathways of 8:2 FTOH outlined by Wang et al. (2009) based on research conducted after the 2009 study. The adapted pathway, Figure 4-1, shows that in addition to PFOA and PFHxA; PFPA and PFHpA can be formed during the aerobic biodegradation of 8:2 FTOH. (Buck et al. 2011) The similar spikes in PFOA and PFHpA concentrations may be due to a degradation of 8:2 FTOH during the WWT process, resulting in increased concentrations of these two compounds.

4.1.2 COMPARISONS WITH OTHER STUDIES

In 2013, Zennegg et al. presented a study of temporal trends of various POPs in anaerobically digested sewage sludge. Included in this study were PFOA and PFOS. Individual sludge samples were collected from eight WWTPs serving various population sizes in Switzerland in 1993, 2002, 2008, and 2012. It was determined that between 1993 and 2012, no statistically significant decreasing trend in PFOA and PFOS concentrations were present, despite an observation that the lowest concentrations of both compounds

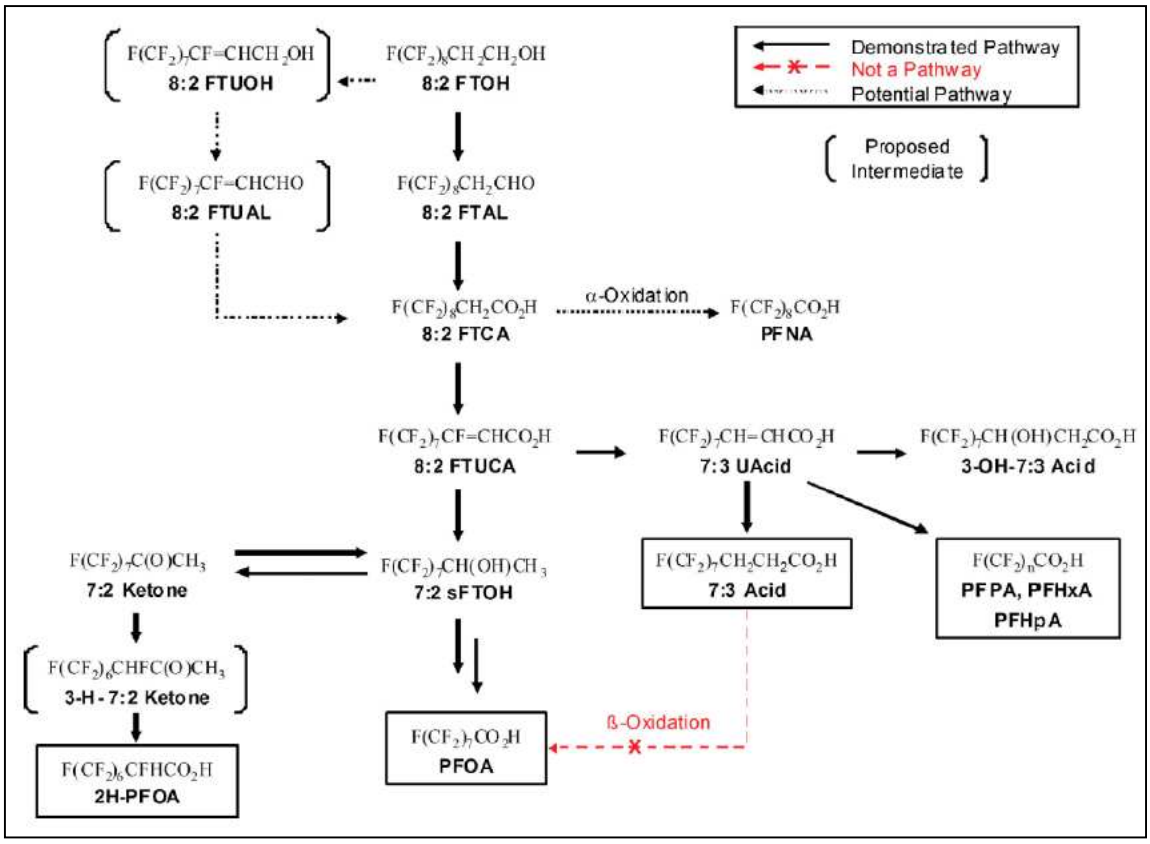

Figure 4-1: Aerobic Biodegradation Pathways of 8:2 FTOH – adapted from Wang et al (2009)

occurred in 2012. This is different to the observed spike in PFOA concentrations in 2008 and the decrease in PFOS concentrations beginning in 2006, likely due to the differences in sampling years/frequency between the two studies. PFOA concentrations among the 8 WWTPs during the study period ranged from 4 to 12 ng/g, which is within the lower range of concentrations detected in this study. PFOS concentrations were detected between 43 and 750 ng/g while concentrations in this study peaked at the lower end of this range (Zennegg et al. 2013) A similar study from Sweden, published in 2012, presented time trends of various organic compounds and metals from anaerobically digested sludge collected from 10 WWTPs every fall from 2004 to 2010. Thirteen

PFASs were analyzed but only 7 were detected (5 PFCAs, PFOS, and a PFOS precursor compound). None of the PFCA compounds analyzed showed statistically significant time trends of treated data, with the exception of one compound, perfluorododecane acid (PFDoDA) (not analyzed in this study), which showed a significant decreasing trend only prior to the exclusion of outliers. This is somewhat different to the results in this study, where slight, yet significant, decreases in PFHxA and PFHpA were observed. Additionally, contrary to the observations from the current study, PFOS showed no significant change in concentration over 7 years. Perfluorooctane sulfonamide (PFOSA), a PFOS precursor compound, was the only PFAS found to have a decreasing trend between 2004 and 2010. While ranges of concentrations for the PFCAs and PFOS were not available, the average concentration for PFOA, PFNA, PFDA, PFUnA, PFDoDA, and PFOS were provided and detected at 1.1, 0.4, 1.5, 0.8, 1.0, and 10 ng/g, respectively. (Olofsson et al. 2012) While these studies do give insight into temporal trends of PFCA and PFSA compounds in wastewater solids, it is important to note that they involve solids that are from a different geographic region (Europe) and that have undergone a different treatment process (anaerobic digestion) than those studied during this investigation.

In 2013, Venkatesan and Halden presented the results of an analysis conducted on biosolids samples collected from the 2001 NSSS. In this study, 110 biosolids samples were split into five groups, composited, and analyzed for PFASs. Average short-chain PFCAs as well as PFNA concentrations, as analyzed by Venkatesan and Halden, resulted in concentrations slightly lower, but within the same range, as those in this study, while PFOA concentrations between the two studies were similar. The average PFOS concentration in the Venkatesan and Halden study was much higher (greater than 1 order

of magnitude) than the results of this study. PFDS was not analyzed by Venkatesan and Halden. While comparison between these two studies indicates many similarities between the samples collected in 2001 and from 2005 to 2013, direct comparisons are difficult since the Venkatesan and Halden study was conducted at a single time point and was comprised of biosolids samples from various WWTPs with different treatment processes, locations, and input types. However, the similarity in concentrations of all compounds analyzed in both studies, with the exception of PFOS, indicates that concentrations of these compounds has likely remained consistent over an extended period of time and are constant in biosolids. In fact, as a means to back this up, Venkatesan and Halden compared their results to other studies conducted on PFASs biosolids within the US between 2004 and 2007 and concluded that concentration differences between the studies were not statistically significant. (Venkatesan & Halden 2013) In general, data from this study appears to support Venkatesan and Halden's overall conclusions, with PFOS results being the primary exception, leading to concerns over whether more action may be needed other than the voluntary phase-out of various PFASs for the reduction in concentrations of these chemicals given that the samples analyzed in the Venkatesan and Halden study were collected prior to this action.

4.2 FULL DATA TABLES

Full data for all PFCA compounds is provided in Table 4-1. Full data for PFSA compounds analyzed is provided in Table 4-2.

Table 4-1: Full PFCA Data Tables

SAMPLE ID	Sample Loc	DATE	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
ABS091905B7	Bottom	09/19/05	BDL	27.938	7.404	BDL	1.860	1.835	BDL	BDL
ABS091905B8	Bottom	09/19/05	BDL	26.921	6.279	BDL	0.953	1.865	BDL	BDL
ABS091905B9	Bottom	09/19/05	BDL	28.222	7.914	BDL	2.936	2.122	BDL	BDL
ABS010506B7	Bottom	10/05/06	BDL	5.094	8.829	2.067	0.726	7.595	BDL	BDL
ABS010506B8	Bottom	10/05/06	BDL	4.471	4.378	BDL	6.000	6.642	BDL	BDL
ABS030606B7	Bottom	10/05/06	BDL	13.380	9.509	3.138	BDL	7.818	BDL	BDL
ABS030606B8	Bottom	03/06/06	BDL	14.677	6.679	0.296	2.735	8.283	BDL	BDL
ABS030606B9	Bottom	03/06/06	BDL	9.329	6.591	119.995	1.388	8.113	BDL	BDL
ABS052506B7	Bottom	03/06/06	BDL	11.207	7.006	0.174	31.937	19.740	BDL	BDL
ABS052506B8	Bottom	05/25/06	BDL	10.969	6.157	BDL	1.339	22.264	BDL	BDL
ABS052506B9	Bottom	05/25/06	BDL	12.747	7.912	1.283	0.620	19.505	BDL	BDL
ABS072506B4	Bottom	05/25/06	BDL	13.419	10.500	2.880	3.463	19.407	BDL	BDL
ABS072506B5	Bottom	07/25/06	BDL	14.280	10.783	3.270	6.752	18.479	BDL	BDL
ABS092806B4	Bottom	07/25/06	BDL	7.628	8.761	BDL	3.651	26.381	BDL	BDL
ABS092806B5	Bottom	09/28/06	BDL	5.724	7.877	0.985	2.702	22.865	BDL	BDL
ABS092806B6	Bottom	09/28/06	BDL	7.164	9.035	BDL	3.907	22.936	BDL	BDL
ABS112806B4	Bottom	11/28/06	BDL	2.723	6.634	0.342	2.465	27.826	BDL	BDL
ABS112806B5	Bottom	11/28/06	BDL	2.380	6.623	BDL	2.094	33.828	BDL	BDL
ABS112806B6	Bottom	11/28/06	BDL	3.100	6.183	BDL	2.893	33.908	BDL	BDL
ABS012907B4	Bottom	01/29/07	BDL	4.412	10.391	0.612	1.434	46.827	BDL	BDL
ABS012907B5	Bottom	01/29/07	BDL	3.456	11.180	2.142	3.620	34.455	BDL	BDL
ABS033007B4	Bottom	03/30/07	BDL	2.750	13.341	BDL	2.234	10.369	BDL	BDL
ABS033007B5	Bottom	03/30/07	BDL	3.579	11.955	0.482	2.407	13.547	BDL	BDL
ABS033007B6	Bottom	03/30/07	BDL	2.829	12.328	BDL	1.820	15.702	BDL	BDL
ABS053007B4	Bottom	05/30/07	BDL	5.158	5.457	0.147	2.172	18.906	BDL	BDL
ABS053007B5	Bottom	05/30/07	BDL	3.761	5.688	0.229	2.190	22.865	BDL	BDL
ABS053007B6	Bottom	05/30/07	BDL	4.121	6.482	BDL	1.423	15.456	BDL	BDL
ABS081007B4	Bottom	08/10/07	BDL	5.333	4.407	0.303	3.104	85.047	BDL	BDL
ABS081007B5	Bottom	08/10/07	BDL	6.904	4.970	1.203	2.828	76.203	BDL	BDL

Table 4-1 (Cont.)

SAMPLE ID	Sample Loc	DATE	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
ABS100207B4	Bottom	10/02/07	BDL	5.358	7.241	118.430	1.456	7.702	BDL	BDL
ABS100207B5	Bottom	10/02/07	BDL	6.745	11.488	1.613	254.350	21.995	BDL	BDL
ABS100207B6	Bottom	10/02/07	BDL	8.436	10.318	BDL	6.683	12.077	BDL	BDL
ABS120307B5	Bottom	12/03/07	BDL	6.351	5.591	BDL	4.451	15.507	BDL	BDL
ABS120307B6	Bottom	12/03/07	BDL	7.373	7.693	BDL	1.340	4.426	BDL	BDL
ABS031308B4	Bottom	03/13/08	BDL	11.232	7.886	BDL	2.490	BDL	BDL	BDL
ABS031308B5	Bottom	03/13/08	BDL	11.225	8.327	1.368	2.459	BDL	BDL	BDL
ABS060508B4	Bottom	06/05/08	BDL	3.606	12.929	1.317	7.611	16.961	BDL	BDL
ABS060508B5	Bottom	06/05/08	BDL	3.385	9.093	0.937	6.756	12.798	BDL	BDL
ABS060508B6	Bottom	06/05/08	BDL	3.359	10.667	67.812	6.094	19.323	BDL	BDL
ABS103008B4	Bottom	10/30/08	BDL	6.897	13.531	2.809	124.793	58.200	BDL	BDL
ABS103008B5	Bottom	10/30/08	BDL	4.745	9.251	1.189	11.866	24.898	BDL	BDL
ABS103008B6	Bottom	10/30/08	BDL	6.449	12.039	BDL	14.252	24.808	BDL	BDL
ABS121608B4	Bottom	12/16/08	BDL	11.810	27.452	123.103	1098.693	386.919	BDL	BDL
ABS121608B5	Bottom	12/16/08	BDL	6.791	14.800	12.331	102.400	49.163	BDL	BDL
ABS022609B4	Bottom	12/16/08	BDL	20.251	11.878	BDL	17.689	33.445	BDL	BDL
ABS022609B5	Bottom	02/26/09	BDL	21.105	11.255	BDL	15.563	27.633	BDL	BDL
ABS022609B6	Bottom	02/26/09	BDL	16.159	10.698	BDL	7.368	28.847	BDL	BDL
ABS052009B4	Bottom	05/20/09	BDL	7.948	7.818	BDL	4.831	13.566	BDL	BDL
ABS052009B5	Bottom	05/20/09	BDL	8.939	11.389	BDL	6.220	17.707	BDL	BDL
ABS052009B6	Bottom	05/20/09	BDL	8.808	8.076	BDL	6.515	24.520	BDL	BDL
ABS082809B4	Bottom	08/28/09	BDL	17.267	23.230	18.532	206.928	183.336	BDL	BDL
ABS082809B5	Bottom	08/28/09	BDL	13.092	17.409	4.922	53.465	51.941	BDL	BDL
ABS011110B4	Bottom	01/11/10	BDL	2.515	5.406	BDL	2.369	11.714	BDL	BDL
ABS011110B5	Bottom	01/11/10	BDL	3.452	4.951	0.525	2.340	18.325	BDL	BDL
ABS011110B6	Bottom	01/11/10	BDL	2.827	6.581	BDL	1.899	10.359	BDL	BDL
ABS031610B4	Bottom	03/16/10	BDL	2.057	6.870	0.098	3.737	8.908	BDL	BDL
ABS031610B5	Bottom	03/16/10	BDL	2.976	7.271	BDL	3.057	8.027	BDL	BDL
ABS031610B6	Bottom	03/16/10	BDL	4.175	7.370	BDL	2.908	7.634	BDL	BDL

Table 4-1 (Cont.)

SAMPLE ID	Sample Loc	DATE	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
ABS060110B4	Bottom	06/01/10	BDL	7.425	12.994	0.277	4.202	21.662	BDL	BDL
ABS060110B5	Bottom	06/01/10	BDL	8.612	10.382	59.712	6.687	24.065	BDL	BDL
ABS060110B6	Bottom	06/01/10	BDL	9.378	10.805	0.253	160.173	40.941	BDL	BDL
ABS080510B4	Bottom	08/05/10	BDL	10.355	10.922	0.380	5.660	42.326	BDL	BDL
ABS080510B5	Bottom	08/05/10	BDL	9.135	9.325	0.177	4.196	38.979	BDL	BDL
ABS080510B6	Bottom	08/05/10	BDL	9.094	9.668	0.180	6.877	47.433	BDL	BDL
ABS101210B4	Bottom	10/12/10	BDL	19.282	7.355	0.185	2.793	BDL	BDL	BDL
ABS101210B5	Bottom	10/12/10	BDL	25.261	8.216	BDL	3.846	BDL	BDL	BDL
ABS101210B6	Bottom	10/12/10	BDL	17.407	6.361	BDL	2.896	BDL	BDL	BDL
ABS120710B4	Bottom	12/07/10	BDL	11.067	6.345	BDL	3.249	4.690	BDL	BDL
ABS120710B5	Bottom	12/07/10	BDL	6.466	6.839	2.066	3.242	3.237	BDL	BDL
ABS020911B4	Bottom	02/09/11	BDL	7.075	6.914	BDL	3.255	17.546	BDL	BDL
ABS020911B5	Bottom	02/09/11	BDL	11.766	8.075	BDL	4.998	25.225	BDL	BDL
ABS020911B6	Bottom	02/09/11	BDL	9.172	7.840	BDL	5.405	18.358	BDL	BDL
ABS040611B4	Bottom	04/06/11	BDL	13.738	7.069	4.938	8.359	12.400	BDL	BDL
ABS040611B5	Bottom	04/06/11	BDL	15.931	6.372	0.884	16.984	9.100	BDL	BDL
ABS060611B4	Bottom	06/06/11	BDL	6.963	5.531	0.502	1.884	28.297	BDL	BDL
ABS060611B5	Bottom	06/06/11	BDL	10.430	5.574	0.155	2.262	21.373	BDL	BDL
ABS060611B6	Bottom	06/06/11	BDL	9.708	4.819	3.435	2.989	21.257	BDL	BDL
ABS080511B4	Bottom	08/05/11	BDL	3.273	5.998	0.905	8.852	16.966	BDL	BDL
ABS080511B5	Bottom	08/05/11	BDL	3.695	5.371	1.144	4.038	12.048	BDL	BDL
ABS080511B6	Bottom	08/05/11	BDL	2.536	3.991	0.357	3.757	23.159	BDL	BDL
ABS101811B4	Bottom	10/18/11	BDL	5.949	3.056	0.435	1.583	20.452	BDL	BDL
ABS101811B5	Bottom	10/18/11	BDL	3.212	2.245	0.356	1.513	19.008	BDL	BDL
ABS101811B6	Bottom	10/18/11	BDL	3.028	2.058	BDL	1.058	13.860	BDL	BDL
ABS111611B4	Bottom	11/16/11	BDL	6.574	2.683	0.650	2.044	12.468	BDL	BDL
ABS111611B5	Bottom	11/16/11	BDL	5.793	2.130	1.693	1.269	12.299	BDL	BDL
ABS121411B4	Bottom	12/14/11	BDL	4.692	3.329	BDL	0.490	2.062	BDL	BDL
ABS121411B5	Bottom	12/14/11	BDL	4.224	2.368	BDL	0.305	2.498	BDL	BDL

Table 4-1 (Cont.)

SAMPLE ID	Sample Loc	DATE	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
ABS121411B6	Bottom	12/14/11	BDL	2.752	2.511	BDL	0.549	3.215	BDL	BDL
ABS022312B4	Bottom	02/23/12	BDL	8.704	2.936	BDL	0.118	2.155	BDL	BDL
ABS022312B5	Bottom	02/23/12	BDL	8.229	4.775	BDL	1.044	0.646	BDL	BDL
ABS022312B6	Bottom	02/23/12	BDL	11.306	5.533	BDL	0.276	5.916	BDL	BDL
ABS041012B4	Bottom	04/10/12	BDL	6.978	9.283	BDL	0.745	13.030	BDL	BDL
ABS041012B5	Bottom	04/10/12	BDL	7.271	9.395	BDL	1.003	12.358	BDL	BDL
ABS041012B6	Bottom	04/10/12	BDL	6.440	15.742	BDL	0.743	18.109	BDL	BDL
ABS062512B4	Bottom	06/25/12	BDL	12.576	20.592	BDL	0.535	16.879	BDL	BDL
ABS062512B5	Bottom	06/25/12	BDL	11.636	18.634	BDL	1.564	20.997	BDL	BDL
ABS062512P4	HDPE	06/25/12	BDL	2.712	4.831	BDL	0.652	27.326	BDL	BDL
ABS062512P5	HDPE	06/25/12	BDL	3.449	5.708	0.884	1.363	27.656	BDL	BDL
ABS080212B4	Bottom	08/02/12	BDL	1.968	3.909	1.051	0.155	15.147	BDL	BDL
ABS080212B5	Bottom	08/02/12	BDL	2.165	4.432	1.653	1.673	14.456	BDL	BDL
ABS080212B6	Bottom	08/02/12	BDL	2.077	3.367	1.048	0.532	14.628	BDL	BDL
ABS080212P4	HDPE	08/02/12	BDL	2.576	3.878	0.841	0.486	16.379	BDL	BDL
ABS080212P5	HDPE	08/02/12	BDL	2.223	5.146	0.720	0.332	16.343	BDL	BDL
ABS080212P6	HDPE	08/02/12	BDL	2.578	3.164	0.928	0.499	13.916	BDL	BDL
ABS101112B4	Bottom	10/11/11	BDL	4.406	2.732	1.124	0.671	BDL	BDL	BDL
ABS101112B5	Bottom	10/11/11	BDL	3.785	2.759	1.226	0.572	BDL	BDL	BDL
ABS101112B6	Bottom	10/11/11	BDL	3.742	4.614	2.157	1.914	BDL	BDL	BDL
ABS101112P4	HDPE	10/11/11	BDL	5.745	6.979	1.218	0.706	6.970	BDL	BDL
ABS101112P5	HDPE	10/11/11	BDL	5.584	2.587	0.811	0.373	7.609	BDL	BDL
ABS101112P6	HDPE	10/11/11	BDL	3.769	5.783	0.888	0.849	5.208	BDL	BDL
ABS121712B4	Bottom	12/17/12	BDL	13.109	3.442	0.422	BDL	BDL	BDL	BDL
ABS121712B5	Bottom	12/17/12	BDL	17.653	2.515	0.389	0.100	BDL	BDL	BDL
ABS121712B6	Bottom	12/17/12	BDL	14.942	2.406	0.429	BDL	BDL	BDL	BDL
ABS121712P4	HDPE	12/17/12	BDL	15.260	3.103	2.416	4.322	BDL	BDL	BDL
ABS121712P5	HDPE	12/17/12	BDL	14.000	3.033	0.314	BDL	BDL	BDL	BDL
ABS021513B4	Bottom	02/15/13	BDL	12.524	6.948	1.265	0.053	14.905	BDL	BDL

Table 4-1 (Cont.)

SAMPLE ID	Sample Loc	DATE	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
ABS021513B5	Bottom	02/15/13	BDL	11.307	6.692	1.322	0.238	13.769	BDL	BDL
ABS021513P4	HDPE	02/15/13	BDL	14.587	5.565	1.135	BDL	3.772	BDL	BDL
ABS021513P5	HDPE	02/15/13	BDL	13.685	4.203	0.450	BDL	BDL	BDL	BDL
ABS021513P6	HDPE	02/15/13	BDL	17.503	6.157	1.606	BDL	BDL	BDL	BDL
ABS032213B4	Bottom	03/22/13	BDL	5.309	4.574	BDL	1.109	14.645	BDL	BDL
ABS032213B5	Bottom	03/22/13	BDL	7.126	3.810	BDL	1.102	17.068	BDL	BDL
ABS032213B6	Bottom	03/22/13	BDL	4.196	2.964	BDL	1.682	21.213	BDL	BDL
ABS032213P4	HDPE	03/22/13	BDL	8.133	2.278	2.122	1.441	7.396	BDL	BDL
ABS032213P5	HDPE	03/22/13	BDL	9.454	4.301	2.308	1.912	7.553	BDL	BDL
ABS032213T4	Top	03/22/13	BDL	9.341	4.939	BDL	BDL	15.181	BDL	BDL
ABS032213T5	Top	03/22/13	BDL	11.735	5.878	BDL	BDL	17.064	BDL	BDL
ABS032213T6	Top	03/22/13	BDL	10.954	4.741	BDL	BDL	23.533	BDL	BDL
ABS040513B4	Bottom	04/05/13	BDL	6.608	1.837	0.106	0.561	25.930	BDL	BDL
ABS040513B5	Bottom	04/05/13	BDL	7.083	2.438	0.183	0.902	31.823	BDL	BDL
ABS040513B6	Bottom	04/05/13	BDL	6.174	1.955	0.177	1.147	37.240	BDL	BDL
ABS040513P4	HDPE	04/05/13	BDL	13.435	3.889	0.892	BDL	23.344	BDL	BDL
ABS040513P5	HDPE	04/05/13	BDL	15.531	5.684	83.599	168.626	42.085	BDL	BDL
ABS040513P6	HDPE	04/05/13	BDL	13.483	3.944	5.083	5.653	25.530	BDL	BDL
ABS040513T4	Top	04/05/13	BDL	8.340	2.679	0.003	0.981	25.273	BDL	BDL
ABS040513T5	Top	04/05/13	BDL	7.992	2.743	0.003	1.042	21.293	BDL	BDL
ABS040513T6	Top	04/05/13	BDL	5.977	2.285	0.163	1.024	30.332	BDL	BDL
ABS060413B1	Bottom	06/04/13	BDL	6.251	2.790	0.081	2.017	27.198	BDL	BDL
ABS060413B2	Bottom	06/04/13	BDL	5.836	1.519	0.202	2.847	22.812	BDL	BDL
ABS060413B3	Bottom	06/04/13	BDL	4.083	0.797	BDL	2.211	23.695	BDL	BDL
ABS060413P1	HDPE	06/04/13	BDL	3.584	1.778	0.395	1.256	25.520	BDL	BDL
ABS060413P2	HDPE	06/04/13	BDL	3.030	0.760	0.478	2.216	27.702	BDL	BDL
ABS060413P3	HDPE	06/04/13	BDL	3.413	1.256	0.517	1.562	18.506	BDL	BDL
ABS060413T1	Top	06/04/13	BDL	4.085	4.112	0.656	1.343	27.598	BDL	BDL
ABS060413T2	Top	06/04/13	BDL	5.254	2.521	1.186	1.978	37.246	BDL	BDL

Table 4-1 (Cont.)

SAMPLE ID	Sample Loc	DATE	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
ABS060413T3	Top	06/04/13	BDL	3.776	1.982	0.505	1.358	28.201	BDL	BDL
ABS083013B1	Bottom	08/30/13	BDL	1.946	BDL	0.503	2.967	24.428	BDL	BDL
ABS083013B2	Bottom	08/30/13	BDL	1.539	BDL	0.128	2.145	23.698	BDL	BDL
ABS083013B3	Bottom	08/30/13	BDL	1.137	BDL	0.460	2.134	32.587	BDL	BDL
ABS083013P1	HDPE	08/30/13	BDL	0.116	BDL	0.606	3.177	34.370	BDL	BDL
ABS083013P2	HDPE	08/30/13	BDL	0.257	BDL	0.903	5.340	36.327	BDL	BDL
ABS083013T1	Top	08/30/13	BDL	1.300	BDL	0.441	4.908	21.026	BDL	BDL
ABS083013T2	Top	08/30/13	BDL	1.282	BDL	0.492	2.665	22.802	BDL	BDL
ABS083013T3	Top	08/30/13	BDL	1.497	BDL	0.338	2.777	23.600	BDL	BDL
ABS103013B1	Bottom	10/30/13	BDL	3.312	9.102	2.656	4.066	32.671	BDL	BDL
ABS103013B2	Bottom	10/30/13	BDL	3.698	9.828	1.180	2.680	32.056	BDL	BDL
ABS103013B3	Bottom	10/30/13	BDL	2.874	9.710	2.388	5.374	36.611	BDL	BDL
ABS103013P1	HDPE	10/30/13	BDL	2.909	9.803	1.076	2.797	27.697	BDL	BDL
ABS103013P2	HDPE	10/30/13	BDL	2.903	9.654	1.338	3.051	32.388	BDL	BDL
ABS103013T1	Top	10/30/13	BDL	3.237	10.619	0.415	1.246	10.979	BDL	BDL
ABS103013T2	Top	10/30/13	BDL	3.539	11.331	1.011	1.951	16.427	BDL	BDL
ABS103013T3	Top	10/30/13	BDL	3.347	10.636	0.858	2.010	22.712	BDL	BDL

BDL = Below detection limit

Table 4-2: Full PFSA Data Tables

SAMPLE ID	Sample Loc	DATE	PFBS	PFHxS	PFOS	PFDS
ABS091905B7	Bottom	09/19/05	BDL	BDL	0.892	1.273
ABS091905B8	Bottom	09/19/05	BDL	BDL	1.500	2.048
ABS091905B9	Bottom	09/19/05	BDL	BDL	0.995	1.430
ABS010506B7	Bottom	10/05/06	BDL	BDL	39.364	11.296
ABS010506B8	Bottom	10/05/06	BDL	BDL	19.561	5.884
ABS030606B7	Bottom	10/05/06	BDL	BDL	30.983	6.387
ABS030606B8	Bottom	03/06/06	BDL	BDL	50.781	8.844
ABS030606B9	Bottom	03/06/06	BDL	BDL	37.709	7.351
ABS052506B7	Bottom	03/06/06	BDL	BDL	58.819	6.535
ABS052506B8	Bottom	05/25/06	BDL	BDL	70.016	7.745
ABS052506B9	Bottom	05/25/06	BDL	BDL	75.584	7.183
ABS072506B4	Bottom	05/25/06	BDL	BDL	50.099	10.142
ABS072506B5	Bottom	07/25/06	BDL	BDL	62.465	13.360
ABS092806B4	Bottom	07/25/06	BDL	BDL	71.336	12.281
ABS092806B5	Bottom	09/28/06	BDL	BDL	61.769	14.687
ABS092806B6	Bottom	09/28/06	BDL	BDL	67.293	15.539
ABS112806B4	Bottom	11/28/06	BDL	BDL	48.388	6.263
ABS112806B5	Bottom	11/28/06	BDL	BDL	42.852	4.792
ABS112806B6	Bottom	11/28/06	BDL	BDL	40.090	5.184
ABS012907B4	Bottom	01/29/07	BDL	BDL	41.610	10.939
ABS012907B5	Bottom	01/29/07	BDL	BDL	37.311	10.362
ABS033007B4	Bottom	03/30/07	BDL	BDL	32.404	7.294
ABS033007B5	Bottom	03/30/07	BDL	BDL	26.186	5.553
ABS033007B6	Bottom	03/30/07	BDL	BDL	28.801	5.144
ABS053007B4	Bottom	05/30/07	BDL	BDL	29.220	4.642
ABS053007B5	Bottom	05/30/07	BDL	BDL	27.526	5.738
ABS053007B6	Bottom	05/30/07	BDL	BDL	28.814	4.425
ABS081007B4	Bottom	08/10/07	BDL	BDL	46.663	10.300
ABS081007B5	Bottom	08/10/07	BDL	BDL	39.069	7.902
ABS100207B4	Bottom	10/02/07	BDL	BDL	8.097	5.137
ABS100207B5	Bottom	10/02/07	BDL	BDL	13.351	5.585
ABS100207B6	Bottom	10/02/07	BDL	BDL	7.885	3.532
ABS120307B5	Bottom	12/03/07	BDL	BDL	19.106	4.860
ABS120307B6	Bottom	12/03/07	BDL	BDL	24.194	7.640
ABS031308B4	Bottom	03/13/08	BDL	BDL	20.215	9.620
ABS031308B5	Bottom	03/13/08	BDL	BDL	23.267	12.321
ABS060508B4	Bottom	06/05/08	BDL	BDL	26.020	5.008
ABS060508B5	Bottom	06/05/08	BDL	BDL	23.928	5.617
ABS060508B6	Bottom	06/05/08	BDL	BDL	21.810	4.622
ABS103008B4	Bottom	10/30/08	BDL	BDL	17.750	4.164
ABS103008B5	Bottom	10/30/08	BDL	BDL	17.423	3.469
ABS103008B6	Bottom	10/30/08	BDL	BDL	16.684	3.216
ABS121608B4	Bottom	12/16/08	BDL	BDL	25.201	4.280
ABS121608B5	Bottom	12/16/08	BDL	BDL	20.355	2.790
ABS022609B4	Bottom	12/16/08	BDL	BDL	21.420	5.847
ABS022609B5	Bottom	02/26/09	BDL	BDL	21.393	5.516

Table 4-2 (Cont.)

SAMPLE ID	Sample Loc	DATE	PFBS	PFHxS	PFOS	PFDS
ABS022609B6	Bottom	02/26/09	BDL	BDL	16.523	6.290
ABS052009B4	Bottom	05/20/09	BDL	BDL	14.254	7.615
ABS052009B5	Bottom	05/20/09	BDL	BDL	14.356	7.750
ABS052009B6	Bottom	05/20/09	BDL	BDL	16.596	6.637
ABS082809B4	Bottom	08/28/09	BDL	BDL	25.665	8.989
ABS082809B5	Bottom	08/28/09	BDL	BDL	24.564	7.911
ABS011110B4	Bottom	01/11/10	BDL	BDL	17.707	1.696
ABS011110B5	Bottom	01/11/10	BDL	BDL	21.265	1.997
ABS011110B6	Bottom	01/11/10	BDL	BDL	18.816	1.856
ABS031610B4	Bottom	03/16/10	BDL	BDL	25.684	2.745
ABS031610B5	Bottom	03/16/10	BDL	BDL	19.483	1.757
ABS031610B6	Bottom	03/16/10	BDL	BDL	22.960	3.256
ABS060110B4	Bottom	06/01/10	BDL	BDL	23.357	3.568
ABS060110B5	Bottom	06/01/10	BDL	BDL	20.622	2.082
ABS060110B6	Bottom	06/01/10	BDL	BDL	23.090	4.242
ABS080510B4	Bottom	08/05/10	BDL	BDL	22.149	5.126
ABS080510B5	Bottom	08/05/10	BDL	BDL	16.932	8.357
ABS080510B6	Bottom	08/05/10	BDL	BDL	18.513	6.783
ABS101210B4	Bottom	10/12/10	BDL	BDL	1.327	0.384
ABS101210B5	Bottom	10/12/10	BDL	BDL	1.696	0.621
ABS101210B6	Bottom	10/12/10	BDL	BDL	1.573	0.597
ABS120710B4	Bottom	12/07/10	BDL	BDL	5.393	1.809
ABS120710B5	Bottom	12/07/10	BDL	BDL	5.375	2.210
ABS020911B4	Bottom	02/09/11	BDL	BDL	15.995	1.952
ABS020911B5	Bottom	02/09/11	BDL	BDL	20.147	3.052
ABS020911B6	Bottom	02/09/11	BDL	BDL	19.428	2.089
ABS040611B4	Bottom	04/06/11	BDL	BDL	7.338	3.316
ABS040611B5	Bottom	04/06/11	BDL	BDL	7.490	2.689
ABS060611B4	Bottom	06/06/11	BDL	BDL	30.897	3.814
ABS060611B5	Bottom	06/06/11	BDL	BDL	31.887	5.175
ABS060611B6	Bottom	06/06/11	BDL	BDL	36.638	3.762
ABS080511B4	Bottom	08/05/11	BDL	BDL	3.589	1.679
ABS080511B5	Bottom	08/05/11	BDL	BDL	2.577	1.245
ABS080511B6	Bottom	08/05/11	BDL	BDL	13.001	6.089
ABS101811B4	Bottom	10/18/11	BDL	BDL	15.178	3.347
ABS101811B5	Bottom	10/18/11	BDL	BDL	17.835	3.075
ABS101811B6	Bottom	10/18/11	BDL	BDL	13.239	2.108
ABS111611B4	Bottom	11/16/11	BDL	BDL	14.401	3.930
ABS111611B5	Bottom	11/16/11	BDL	BDL	13.518	3.785
ABS121411B4	Bottom	12/14/11	BDL	BDL	11.983	2.024
ABS121411B5	Bottom	12/14/11	BDL	BDL	11.323	1.749
ABS121411B6	Bottom	12/14/11	BDL	BDL	9.473	1.912
ABS022312B4	Bottom	02/23/12	BDL	BDL	11.359	4.673
ABS022312B5	Bottom	02/23/12	BDL	BDL	14.514	5.592
ABS022312B6	Bottom	02/23/12	BDL	BDL	12.242	5.005
ABS041012B4	Bottom	04/10/12	BDL	BDL	10.450	5.818

Table 4-2 (Cont.)

SAMPLE ID	Sample Loc	DATE	PFBS	PFHxS	PFOS	PFDS
ABS041012B5	Bottom	04/10/12	BDL	BDL	12.404	4.542
ABS041012B6	Bottom	04/10/12	BDL	BDL	11.166	2.605
ABS062512B4	Bottom	06/25/12	BDL	BDL	14.802	3.681
ABS062512B5	Bottom	06/25/12	BDL	BDL	12.104	3.859
ABS062512P4	HDPE	06/25/12	BDL	BDL	17.046	4.650
ABS062512P5	HDPE	06/25/12	BDL	BDL	12.958	4.346
ABS080212B4	Bottom	08/02/12	BDL	BDL	15.332	4.885
ABS080212B5	Bottom	08/02/12	BDL	BDL	13.148	6.449
ABS080212B6	Bottom	08/02/12	BDL	BDL	14.462	6.397
ABS080212P4	HDPE	08/02/12	BDL	BDL	17.296	6.715
ABS080212P5	HDPE	08/02/12	BDL	BDL	13.950	5.109
ABS080212P6	HDPE	08/02/12	BDL	BDL	12.612	6.135
ABS101112B4	Bottom	10/11/11	BDL	BDL	8.508	10.275
ABS101112B5	Bottom	10/11/11	BDL	BDL	4.721	5.280
ABS101112B6	Bottom	10/11/11	BDL	BDL	9.218	10.314
ABS101112P4	HDPE	10/11/11	BDL	BDL	6.395	6.784
ABS101112P5	HDPE	10/11/11	BDL	BDL	11.873	11.329
ABS101112P6	HDPE	10/11/11	BDL	BDL	3.863	2.861
ABS121712B4	Bottom	12/17/12	BDL	BDL	15.069	5.275
ABS121712B5	Bottom	12/17/12	BDL	BDL	14.329	4.292
ABS121712B6	Bottom	12/17/12	BDL	BDL	9.358	6.233
ABS121712P4	HDPE	12/17/12	BDL	BDL	14.844	5.181
ABS121712P5	HDPE	12/17/12	BDL	BDL	12.970	4.563
ABS021513B4	Bottom	02/15/13	BDL	BDL	24.009	7.638
ABS021513B5	Bottom	02/15/13	BDL	BDL	24.265	7.323
ABS021513P4	HDPE	02/15/13	BDL	BDL	24.470	7.253
ABS021513P5	HDPE	02/15/13	BDL	BDL	21.779	6.640
ABS021513P6	HDPE	02/15/13	BDL	BDL	21.143	5.585
ABS032213B4	Bottom	03/22/13	BDL	BDL	13.306	6.675
ABS032213B5	Bottom	03/22/13	BDL	BDL	9.603	5.795
ABS032213B6	Bottom	03/22/13	BDL	BDL	9.507	8.015
ABS032213P4	HDPE	03/22/13	BDL	BDL	11.990	7.200
ABS032213P5	HDPE	03/22/13	BDL	BDL	12.018	5.744
ABS032213T4	Top	03/22/13	BDL	BDL	17.026	7.977
ABS032213T5	Top	03/22/13	BDL	BDL	13.675	5.997
ABS032213T6	Top	03/22/13	BDL	BDL	15.312	6.109
ABS040513B4	Bottom	04/05/13	BDL	BDL	32.981	4.920
ABS040513B5	Bottom	04/05/13	BDL	BDL	38.073	6.577
ABS040513B6	Bottom	04/05/13	BDL	BDL	36.999	5.376
ABS040513P4	HDPE	04/05/13	BDL	BDL	39.884	7.404
ABS040513P5	HDPE	04/05/13	BDL	BDL	40.118	7.448
ABS040513P6	HDPE	04/05/13	BDL	BDL	34.336	5.974
ABS040513T4	Top	04/05/13	BDL	BDL	35.136	5.606
ABS040513T5	Top	04/05/13	BDL	BDL	39.698	5.605
ABS040513T6	Top	04/05/13	BDL	BDL	37.800	5.688
ABS060413B1	Bottom	06/04/13	BDL	BDL	24.024	6.798

Table 4-2 (Cont.)

SAMPLE ID	Sample Loc	DATE	PFBS	PFHxS	PFOS	PFDS
ABS060413B2	Bottom	06/04/13	BDL	BDL	28.446	7.299
ABS060413B3	Bottom	06/04/13	BDL	BDL	26.237	7.740
ABS060413P1	HDPE	06/04/13	BDL	BDL	25.829	9.936
ABS060413P2	HDPE	06/04/13	BDL	BDL	19.488	9.360
ABS060413P3	HDPE	06/04/13	BDL	BDL	22.557	9.938
ABS060413T1	Top	06/04/13	BDL	BDL	25.765	6.835
ABS060413T2	Top	06/04/13	BDL	BDL	25.627	7.852
ABS060413T3	Top	06/04/13	BDL	BDL	27.490	6.829
ABS083013B1	Bottom	08/30/13	BDL	BDL	11.564	4.822
ABS083013B2	Bottom	08/30/13	BDL	BDL	12.393	4.933
ABS083013B3	Bottom	08/30/13	BDL	BDL	8.578	6.871
ABS083013P1	HDPE	08/30/13	BDL	BDL	16.452	6.410
ABS083013P2	HDPE	08/30/13	BDL	BDL	16.973	6.074
ABS083013T1	Top	08/30/13	BDL	BDL	14.885	4.650
ABS083013T2	Top	08/30/13	BDL	BDL	13.275	5.011
ABS083013T3	Top	08/30/13	BDL	BDL	12.366	5.363
ABS103013B1	Bottom	10/30/13	BDL	BDL	13.090	6.380
ABS103013B2	Bottom	10/30/13	BDL	BDL	13.044	5.864
ABS103013B3	Bottom	10/30/13	BDL	BDL	13.018	5.387
ABS103013P1	HDPE	10/30/13	BDL	BDL	15.690	5.836
ABS103013P2	HDPE	10/30/13	BDL	BDL	13.783	5.591
ABS103013T1	Top	10/30/13	BDL	BDL	14.635	6.541
ABS103013T2	Top	10/30/13	BDL	BDL	13.428	6.230
ABS103013T3	Top	10/30/13	BDL	BDL	14.205	5.369

BDL = Below detection limit

CHAPTER 5: OVERALL CONCLUSIONS

PFCA and PFSA compounds are persistent and present in environmental samples worldwide. The use of these compounds, as well as their precursor compounds, in numerous consumer and industrial applications has led to their presence in the wastewater treatment process and, eventually, wastewater effluent and solids. Analysis of PFCAs and PFSAs in biosolids over time can help give insight into any changes in use of these compounds as well as determine the potential of introducing these compounds into the environment due to biosolids land application. The PFCA compounds that could be analyzed indicated either no statistically significant trend or only a slight, yet significant, decrease in concentrations between 2005 and 2013. Averaging biosolids concentrations by years demonstrated spikes in PFPeA and PFHxA levels occurring in 2009 while spikes in PFOA and PFNA levels occurred in 2008. Both PFOS and PFDS saw significant decreases between 2005 and 2013, with the change in PFOS being the most apparent. In particular, PFOS demonstrated a large decrease between 2006 and 2007. While concentrations of the PFCA and PFSA compounds detected in this study are considered to be within the trace range, their ability to persist in environmental and human samples, as well as their toxicological and health concerns, denotes the significance that even low concentrations may have. Additionally, the lack of decrease of concentrations in of many of the analyzed compounds in biosolids suggests the need for further action regarding their regulation and reduction of use.

Finally, while PTFE may contain PFCA compounds as impurities, a comparison in storage methods indicated that the material did not have a significant impact on PFCA concentrations in stored biosolids samples. While variability amongst the samples was

evident, this was likely due to the heterogeneity of the biosolids themselves rather than any effects from differences in storage methods. It can be concluded that the use of storage containers containing PTFE-lined caps are suitable for biosolids samples to be analyzed for PFCAs.

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