Investigation of Environmental Fate of Novel Perfluoroalkyl and Polyfluoroalkyl Substances in Soil and Biosolids

Presented by

Chen Liu

Department of Civil Engineering and Applied Mechanics

McGill University, Montreal

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ABSTRACT

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) have received great attention due to their ubiquitous detection in the environment. As PFASs are among the most persistent organic compounds, and some are toxic and bioaccumulative, it is important to understand concentrations, sources, and environmental fate of PFASs in different environmental matrices. To shed light on the sources and behaviours of PFASs observed in soil, this thesis focused on (1) evaluating biotransformation in aerobic soil of fluorotelomer phosphoric esters (PAPs), and (2) assessing the levels of identifiable and unidentified PFASs in municipal biosolids intended for land application.

In the first part of the thesis, biotransformation rates of two major PAPs (6:2 and 8:2 diPAPs) were evaluated in a semi-dynamic system over 112 days. Soil samples were prepared using an improved sample extraction preparation method, which has demonstrated the best recoveries for the diPAPs and the least propensity to cause undesirable solvent-enhanced hydrolysis. At the end of 112 days, the major polyfluoroalkyl carboxylic acids that were formed from 6:2 diPAPs were 9.34% 5:3 polyfluoroalkyl acid (5:3 acid), 6.42% perfluoropentanoic acid (PFPeA) and 5.99% perfluorohexanoic acid (PFHxA); the major one formed from 8:2 diPAPs was 2.14% perfluorooctanoic acid (PFOA). The half-lives of 6:2 diPAP and 8:2 diPAP were estimated to be 11.5 days and >1000 days, respectively. The approximate linear relationship between the half-lives of eleven PFASs (including 6:2 and 8:2 diPAPs) and their molecular weights suggested that the molecular weight is a good indicator of the general stability of low-molecular-weight PFAS-based compounds.

In the second part of the thesis, 12 municipal biosolid samples were collected in Canada. Concentrations of 28 PFASs, including 13 perfluoroalkyl carboxylic acids (PFCAs, C4-C14, 5:3 and 7:3 acids), 4 perfluoroalkyl sulfonic acids (C4, C6, C8, and C10), 4 fluorotelomer alcohols (8:2 FTOH, 6:2 FTOH, 7:2 sFTOH and 5:2 sFTOH), 3 fluorotelomer sulfonic acids (8:2 FTS, 6:2 FTS and 4:2 FTS), and 4 PAPs (8:2 diPAP, 6:2 diPAP, 8:2 monoPAP, and 6:2 monoPAP), were determined. The predominant PFASs in most of the samples were perfluorobutane sulfonic acid (PFBS), 5:3 acid, 6:2 diPAP, 8:2 FTOH and 6:2 FTOH, but not PFCAs or perfluorooctane sulfonic acid (PFOS). The significantly different PFAS profiles from those in the U.S. biosolids suggested the use of different PFAS products in Canada. A persulfate oxidation based assay was applied to determine the level of unidentified PFASs in biosolids. Further studies are needed to optimize the assay to determine whether the approach is feasible for biosolid samples. This study has contributed to a deeper understanding of environmental fate and potential sources of selected PFAS species in soil environment.

RÉSUMÉ

Les substances Perfluoroalkyl et polyfluoroalkyl (PFASs) ont reçu une grande attention en raison de leur omniprésence dans l'environnement. Comme les PFASs sont parmi les composés organiques les plus persistants dont certains sont toxiques et bioaccumulables, il est important de comprendre les concentrations, les sources et le devenir environnemental des PFASs dans différentes matrices environnementales. Pour faire la lumière sur les sources et les comportements des PFASs observées dans le sol, cette thèse portait sur l'évaluation (1) de la biotransformation des esters phosphoriques de fluorotélomériques (PAPs) dans le sol aérobie, et (2) des niveaux de PFASs identifiables et non identifiés dans les biosolides municipaux destinés à l'épandage.

Dans la première partie de la thèse, le taux de biotransformation de deux PAPs majeurs (6: 2 et 8: 2 diPAPs) ont été évalués dans un système semi-dynamique sur une période de 112 jours. Des échantillons de sol ont été préparés en utilisant une méthode d'extraction amélioré, qui a démontré une meilleure récupération des diPAPs et qui a le moins tendance à causer des effets indésirables d'hydrolyse de détergent renforcé. Au bout de 112 jours, les principaux acides polyfluoroalkyl carboxyliques qui se sont formés à partir de 6: 2 diPAPs étaient 9,34% 5: 3 d'acide polyfluoroalkyle (5: 3 d'acide), 6,42% d'acide

perfluoropentanoic (PFPeA),et 5,99% d'acide perfluorohexanoïque (PFHxA); la principale formée à partir de 8: 2 diPAPs était l'acide perfluorooctanoïque 2,14% (PFOA). Les demi-vies de 6: 2 diPAP et 8 diPAP ont été estimés à 11.5 jours et >1000 jours, respectivement. La relation linéaire approximative entre les demi-vies de onze PFASs (dont 6: 2 et 8: 2 diPAPs) et leurs poids moléculaires suggère que le poids moléculaire est un bon indicateur de la stabilité générale des molécules de faible poids moléculaire à base de PFAS. Dans la deuxième partie de la thèse, 12 échantillons de biosolides municipaux ont été recueillies au Canada. Les concentrations de 28 PFASs, y compris 13 perfluoroalkyl acides carboxyliques (APFC, C4-C14, 5: 3 et 7: 3 acides), 4 acides perfluoroalkyle sulfoniques (C4, C6, C8 et C10), 4 alcools télomères fluorés (8: 2 FTOH , 6: 2 FTOH, 7: 2 sFTOH, 5: 2 sFTOH), 3 acides sulfoniques fluorotélomériques (8: 2 FTS, 6:2 FTS, 4: 2 FTS), et 4 PAP (8: 2 diPAP, 6: 2 diPAP, 8: 2 monoPAP, et 6: 2 monoPAP), ont été déterminées. Les PFASs prédominants dans la plupart des échantillons étaient l'acide sulfonique perfluorobutane (PFBS), l'acide 5: 3, 6: 2 diPAP, 8: 2 FTOH et 6: 2 FTOH, mais pas APFC ou l'acide perfluorooctane sulfonique (SPFO). Les profils significativement différents des PFAS des biosolides américains ont suggéré l'utilisation de différents produits PFAS au Canada. Un essai d'oxydation à base de persulfate a été appliqué pour déterminer la quantité de PFASs non identifié dans les biosolides. D'autres études sont nécessaires pour optimiser le dosage afin de déterminer si l'approche est faisable pour les échantillons de biosolides. Cette étude a contribué à une compréhension plus profonde du devenir dans l'environnement et de sources potentielles d'espèces de PFAS sélectionnés dans le sol.

CONTRIBUTION OF AUTHORS

This thesis consists of a general introduction (Chapter I), a literature review (Chapter II), a manuscript of biodegradation study of polyfluoroalkyl phosphate esters in soil (Chapter III), a field study on PFAS substances in biosolids (Chapter IV), and a summary (Chapter V).

The experiment work, data analysis, and manuscript writing were conducted by the candidate Chen Liu under the supervision of Dr. Jinxia Liu. Dr. Guo Li from Chongqing University (China) contributed to part of the experimental work in Chapter IV during his visit to McGill.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

ACRONYM	NAME	FORMULA
ACN	Acetonitrile	
AFFF	Aqueous film-forming foam	
EA	Ethyl acetate	
EtFOSA	*N*-Ethyl perfluorooctane sulfonamide	$C_8F_{17}SO_2NH(C_2H_5)$
EtFOSE	*N*-Ethyl perfluorooctane sulfonamidoethanol	$C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$
FTOH	Fluorotelomer alcohol	$F(CF_2CF_2)_nCH_2CH_2OH$
HAc	Acetic acid	
HMU	Hexamethylene 1,6-di-(8:2 fluorotelomer urethane	$(CH_2CH_2(CF_2)_7CF_3)_2(NH(CO)O)_2C_6H_{12}$
LC-MS/MS	Liquid chromatography–mass spectrometry	
K_{oc}	organic-carbon water partition coefficient	
K_{ow}	Octanol-water partition coefficient	
MeOH	Methanol	
MTBE	Methyl tert-butyl ether	
PAP	Polyfluoroalkyl phosphoric ester	
PBSF	Perfluorobutane sulfonyl fluoride	$C_4F_9SO_2F$
PFAA	Perfluoroalkyl acid	
PFAS	Perfluoroalkyl and polyfluoroalkyl substance	
PFBA	Perfluorobutanoic acid	C_3F_7COOH
PFBS	Perfluorobutane sulfonic acid	$C_4F_9SO_3H$
PFCA	Perfluoroalkyl carboxylic acid	$C_nF_{2n+1}CO_2H$

ACRONYM	NAME	FORMULA
PFDA	Perfluorodecanoic acid	$C_9F_{19}COOH$
PFDoA	Perfluorododecanoic acid	$C_{11}F_{23}COOH$
PFDS	Perfluorodecane sulfonic acid	$C_{10}F_{21}SO_3H$
PFHpA	Perfluoroheptanoic acid	$C_6F_{13}COOH$
PFHxA	Perfluorohexanoic acid	$C_5F_{11}COOH$
PFHxS	Perfluorohexane sulfonic acid	$C_6F_{13}SO_3H$
PFNA	Perfluorononanoic acid	$C_8F_{17}COOH$
PFOA	Perfluorooctanoic acid	$C_7F_{15}COOH$
PFOS	Perfluorooctane sulfonic acid	$C_8F_{17}SO_3H$
PFPeA	Perfluoropentanoic acid	C_4F_9COOH
PFSA	Perfluoroalkyl sulfonic acid	$C_nF_{2n+1}SO_3H$
PFTeA	Perfluorotetradecanoic acid	$C_{13}F_{27}COOH$
PFTrA	Perfluorotridecanoic acid	$C_{12}F_{25}COOH$
PFUdA	Perfluoroundecanoic acid	$C_{10}F_{21}COOH$
pK_a	Acid dissociation constant	
POSF	Perfluorooctane sulfonyl fluoride	$C_8F_{17}SO_2F$
TOP	Total oxidizable precursor	
WWTP	Wastewater treatment plant	
PAH	Polyaromatic hydrocarbon	
SamPAP diester	Bis(N-ethyl perfluorooctane sulfonamido ethanol) phosphate diester	$(C_8F_{17}SO_2N(C_2H_5)C_2H_4O_2)_2PO_2^-$
PFSIA	Perfluoroalkane sulfinic acid	$C_nF_{2n+1}SO_2H$

ACRONYM	NAME	FORMULA
PFPA	Perfluoroalkyl phosphonic acid	$O=P(OH)_2C_nF_{2n+1}$
PFPIA	Perfluoroalkyl phosphinic acid	$O=P(OH)(C_nF_{2n+1})(C_mF_{2m+1})$
4:2 FTS	4:2 Fluorotelomer sulfonic acid	$C_4F_9CH_2CH_2SO_3H$
5:2 sFTOH	5:2 Secondary fluorotelomer alcohol	$C_5F_{11}CH_2(OH)\ CH_2$
5:2 ketone	5:2 Fluorotelomer ketone	$C_5F_{11}CH_2COCH_3$
5:3 Acid	5:3 Polyfluoroalkyl carboxylic acid	$C_5F_{11}(CH_2)_2COOH$
6:2 diPAP	6:2 Fluorotelomer phosphate diester	$(O)P(OH)(OCH_2CH_2C_6F_{13})_2$
6:2 FTOH	6:2 Fluorotelomer alcohol	$C_6F_{13}CH_2CH_2OH$
6:2 FTS	6:2 Fluorotelomer sulfonic acid	$C_6F_{13}CH_2CH_2SO_3H$
6:2 monoPAP	6:2 Fluorotelomer phosphate monoester	$(O)P(OH)_2(OCH_2CH_2C_6F_{13})$
7:2 sFTOH	7:2 Secondary fluorotelomer alcohol	$C_7F_{15}CH_2(OH)\ CH_2$
7:3 Acid	7:3 Polyfluoroalkyl carboxylic acid	$C_7F_{15}(CH_2)_2COOH$
8:2 diPAP	8:2 Fluorotelomer phosphate diester	$(O)P(OH)(OCH_2CH_2C_8F_{17})_2$
8:2 FTAC	8:2 Fluorotelomer acrylate	$C_8F_{17}CH_2CH_2OC(O)CH=CH_2$
8:2 FTMAC	8:2 Fluorotelomer methacrylate	$C_8F_{17}CH_2CH_2OC(O)C(CH_3)=CH_2$
8:2 FTOH	8:2 Fluorotelomer alcohol	$C_8F_{17}CH_2CH_2OH$
8:2 FTS	8:2 Fluorotelomer sulfonic acid	$C_8F_{17}CH_2CH_2SO_3H$
8:2 FTSE	8:2 Fluorotelomer stearate ester	$C_8F_{17}CH_2CH_2O(CO)C_{17}H_{35}$
8:2 FTUA	8:2 Fluorotelomer unsaturated carboxylic acid	$C_7F_{15}CF=CHCOOH$
8:2 monoPAP	8:2 Fluorotelomer phosphate monoester	$(O)P(OH)_2(OCH_2CH_2C_8F_{17})$
8:2 TBC	8:2 Fluorotelomer citrate ester	$(OH)C(CH_2)_2(CO)_3(C_8F_{17}CH_2CH_2)_3$

CHAPTER I. INTRODUCTION

1.1 Perfluoroalkyl and polyfluoroalkyl substances (PFASs)

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a major family of organic fluorinated substances, which contain multiple C atoms that are fully fluorinated in an aliphatic structure. Due to hydrophobic and lipophobic properties, PFASs are widely used in industrial and commercial applications such as aqueous film-forming foams (AFFFs), textile coating, soil repellents, food-contact papers, insecticides, etc. (Moody and Field 2000, Key et al. 1997, Rao and Baker 1994a, U.S. EPA 1999). Since the C-F bond is extremely stable (Smart 1994), many species of PFASs are highly persistent in the environment.

Among more than a thousand species of PFASs (Buck et al. 2011), perfluoroalkyl acids (PFAAs) including perfluoroalkyl carboxylic acids (PFCAs, $C_nF_{2n+1}COOH$) and perfluoroalkyl sulfonic acids (PFSAs, $C_nF_{2n+1}SO_3H$) are particularly subject to regulatory and scientific scrutiny. Owing to extreme persistence, bioaccumulation potential, and toxicity demonstrated in animal lab testing, the long-chain PFAAs ($n \geq 6$ for PFSAs and $n \geq 7$ for PFCAs) have been banned or put to restricted use in many countries (ECHA 2013, U.S. EPA 2006a, UNEP 2009). Eight-carbon perfluorooctane carboxylic (PFOA) and sulfonic acids (PFOS) are the mostly used, detected, and investigated PFAAs. The short-chain

PFAAs and their derivatives are currently in production and use around the globe (Ritter 2010). They are also highly persistent, but they exhibit lower bioaccumulation potential and less toxic effects than the long-chained counterparts.

1.2 Precursors to perfluoroalkyl acids (PFAAs)

PFAAs in the environment come from direct and indirect sources. Direct sources refer to the discharge of PFAAs to the environment as such, whether it is intentional release or otherwise (Buck et al. 2011). Paul et al. (2008) and Prevedouros et al. (2006) argued that most of the inventory of PFOA and PFOS in the environment historically came from direct sources. Indirect sources refer to the formation of PFAAs via biotic or abiotic degradation of other PFASs, referred to as precursors to PFAA (pre-PFAAs), once they reach certain environmental compartments (Buck et al.,2011). Many believe the indirect sources significantly contribute to the burden of PFAAs observed in humans and the environment (Remde and Debus 1996, Key et al. 1998, Benskin et al. 2013a, Lee et al. 2010, Lee et al. 2013, Liu et al. 2007, Liu et al. 2010, Mejia Avendaño and Liu 2015, Wang et al. 2009). As most of PFAS-containing products in use today do not contain PFAAs, but pre-PFAAs (Herzke et al. 2012, Gebbink et al. 2013a), it is of primary importance to investigate the contributions to PFAAs from pre-PFAAs.

Fluorotelomer phosphoric esters (PAPs) are a major class of pre-PFAAs. They are phosphoric acid esters and contain one to three polyfluoroalkyl groups per molecule. Eriksson and Karrman (2015) have shown that PAPs are by far the most dominant class of PFASs found in indoor dust. PAPs are also detected in drinking water (Ding et al. 2012), sewage sludge (Liu et al. 2013), marine sediment (Benskin et al. 2012), and even in human sera (D’eon et al. 2009). D'Eon and Mabury (2011) found in a rat model that that diPAPs (PAPs with two polyfluoroalkyl groups per molecule) could be biotransformed into PFCAs. The researchers further inferred that PAPs exposure could be a significant indirect source of PFCAs in human sera (D'Eon and Mabury 2011). Lee et al. (2010) showed that PAPs can be transformed into PFCAs microbially in activated sludge. Lee et al. (2013) also showed that endogenous diPAPs present in wastewater treatment plants (WWTPs) and paper fiber biosolids can be biotransformed into PFCAs in soil, and diPAPs and their degradation products can be taken up by plants.

Even though the qualitative correlation between PAPs and PFCAs has been established, there lacks quantitative information about how significant PAPs are as precursors to PFCAs and how fast PAPs degrade in the environment. The information is of particular importance for soil, which is one of the major reservoirs of pollutants. The major experimental challenges are to establish efficient and reproducible recovery methods of PAPs from soil (Lee et al. 2013), and to minimize solvent-enhanced hydrolysis of PAPs during sample extraction (Dasu et al. 2010).

1.3 Unknown PFAS species in biosolids

Land application of municipal biosolids represents a major route through which PFASs in sewage are returned to the soil environment. Investigating levels and types of PFASs in biosolids can help understand the magnitude of PFAS input to the areas that receive biosolids. The recent literature has suggested that in selected environment samples (e.g., sediments and urban runoff), there exist unknown PFAS species that are missed by the current target monitoring of known PFAS species (Houtz and Sedlak 2012, Yeung et al. 2013a). Some of the unknown PFAS species might have the potential to break down to generate PFAAs. Thus, it is hypothesized that similar to sediment and urban runoff samples reported in the literature, biosolids also contain a significant fraction of unidentified PFASs. As little information is available regarding the levels of PFASs in biosolids in Canada, there is a need to determine the levels of identifiable and unidentified PFAS species in these samples.

1.4 Objectives and approaches

The overall goal of this thesis was to assess the environmental significance and fate of pre-PFAAs and unidentified PFAS species in soil and biosolids.

The objective of the soil biotransformation study (Chapter III) was to investigate with improved experimental methods the half-lives of PAPs in soil and to establish the production yields of PFCAs from PAPs quantitatively. The key points of the study approaches were:

- Two mostly detected diPAPs (6:2 and 8:2) were chosen as model test compounds;
- Biotransformation of the diPAPs were conducted in a semi-dynamic soil system, and both the air and soil phases were monitored;
- Six different soil extraction methods were evaluated to obtain the best extraction scheme for the PAPs with high efficiency and low propensity for solvent-enhanced hydrolysis;
- Production of PFCAs and other degradation products were monitored for up to 4 months.

The objective of the biosolids monitoring study (Chapter IV) was to examine the significance of unidentified PFAS species in comparison to 28 identifiable ones in biosolids from WWTPs in Canada. The key points of the study approaches were:

- 12 biosolids samples were collected from major WWTPs in Quebec and the rest of Canada for testing;
- Improved extraction and cleanup methods were developed for monitoring 13 PFCAs, 4 PFSAs, 3 fluorotelomer sulfonic acids (FTSs), 4 fluorotelomer alcohols (FTOHs), and 4 PAPs;
- A total oxidizer precursor (TOP) assay was tested for its applicability to quantitively measure the level of unidentifiable PFASs in biosolid samples.

Central to all the work is a state-of-the-art liquid chromatography tandem mass spectrometry (LC-MS/MS) system at the Department of Civil Engineering at McGill University. The system is capable of ultra-low level detection of PFASs in complex environmental samples.

CHAPTER II. LITERATURE REVIEW

2.1 Perfluoroalkyl acids (PFAAs)

Perfluoroalkyl acids (PFAAs) including perfluoroalkyl carboxylic (PFCAs), sulfonic (PFSAs), sulfinic (PFSIA), phosphonic (PFPA) and phosphinic acids (PFPIA), have been the focus of the PFAS research. Due to the lack of the structures that are susceptible to electrophilic or nucleophilic attack, PFAAs do not hydrolyze, photolyze or biodegrade in the environment and are highly persistent. PFAAs can be emitted directly into the environment during manufacturing and use or indirectly from degradation of polyfluoroalkyl substances (so-called precursors or pre-PFAAs). Among the PFAAs, long-chain PFSAs ($n \geq 6$) and PFCAs ($n \geq 7$) are being regulated because of high bioaccumulation potential and toxicity exhibited in laboratory animals (U.S. EPA 2006b, UNEP 2006). Currently, the short-chain PFAAs are widely used as replacements for the long-chain counterparts. Though limited studies have shown that the short-chain PFAAs are not bioaccumulative and have low toxicity, many people call for a complete ban of PFAAs and their precursors and derivatives (Blum et al. 2015).

The two most widely known and distributed PFAAs contain an eight-carbon backbone and include PFOA and PFOS. There is a large body of literature regarding toxicology and health impact of PFOS and PFOA (Cui et al. 2009, Hagenaars et al. 2008, Hanson et al. 2005, Huang et al. 2010, Inoue et al. 2004, Lau et al. 2004, Nakayama et al. 2004, Olson and Andersen 1983, Shi et al. 2008). The two compounds are readily absorbed after oral exposure and accumulate primarily in the serum, kidney, and liver of animals or humans (Lau et al. 2004). Toxicological studies on animals show potential developmental, reproductive and systemic effects (Lau et al. 2004). PFOS and PFOA have long half-lives in humans ranging from 2 to 9 years (Olsen et al. 2007). A variety of regulations, guidelines, and health standards have been proposed. For instance, Health Canada issued provisional drinking water guidelines values of 0.3 μg/L for PFOS and 0.7 μg/L for PFOA (Health Canada 2011). The state of New Jersey has established the most stringent health-based guidance value of 0.04 μg/L for PFOA in drinking water (NJDEP 2013).

Physical-chemical properties control the behaviors of PFAAs in the environment. Because acid dissociation constants (pK_a) of PFCAs are in the range of 0 to 1, PFCAs are present in environmental media primarily as organic anions. Similarly, PFSAs with pK_a values of less than 0 are also fully dissociated as anions in environment media. DuPont and 3M reported that the logarithm organic-carbon water partition coefficients ($\text{Log}K_{oc}$) of PFOA and PFOS were in the range of 1.90 - 2.17 (Dekleva 2003) and 2.75 - 3.1 (3M 2000), respectively, which are lower than those for typical persistent organic substances of similar sizes. Thus the relative high water solubility and low sorption to solid matrix determine that PFCAs and PFSAs can be transported over long distance through river and ocean flows around the globe (Yamashita et al. 2005, Young et al. 2007). The wide distribution of PFAAs is also attributed to their precursors such as volatile fluorotelomer alcohols (FTOHs) and perfluorooctane sulfonamidoethanol (Benskin et al. 2013a, Liu et al. 2007, Liu et al. 2010, Wang et al. 2009, Rhoads et al. 2008, Wang et al. 2011). These volatile compounds are capable of long-distance transport. Their atmospheric and microbial degradation is hypothesized to contribute to the widespread dissemination of PFAAs (Young et al. 2007, Ellis et al. 2004).

Most of the current commercial products contain very little PFAAs but other PFAS which have the potential to break down into PFAAs (Lee et al. 2010, Liu et al. 2007, Wang et al. 2009, Rhoads et al. 2008, Ellis et al. 2004, Anumol et al. 2014, Benskin et al. 2013b). Thus, it is of great scientific and regulatory interests to evaluate the significance of precursors in contributing to the load of PFAAs in humans, wildlife, and different environmental media.

2.2 Polyfluoroalkyl phosphate esters (PAPs)

2.2.1 Environmental presence

Polyfluoroalkyl phosphate esters (PAPs) are one class of PFAS containing phosphate ester functional groups. PAPs containing one, two, and three polyfluoroalkyl groups are abbreviated as monoPAP, diPAP, and triPAP, respectively. For the PAPs synthesized using fluorotelomerization process, they are named as n:2 fluorotelomer phosphate esters, where “n” is the number of carbon atoms on which F atoms have replaced all the H substituents. PAPs are commonly used as grease-proofing agents for food contact paper (Begley et al. 2008). In a recent study, PAPs have even been observed in personal care products (Fujii et al. 2013). PAPs have been widely detected in different environments, such as drinking water (Ding et al. 2012), sewage sludge (Liu et al. 2013), biosolids (Lee et al. 2013), indoor dust (Eriksson and Karrman 2015, De Silva et al. 2012), human serum (D’eon et al. 2009), and human milk (Kubwabo et al. 2013). Eriksson and Karrman (2015) found that PAPs have surpassed PFAAs as the most dominant classes of PFAS in indoor dust.

2.2.2 Toxicity

There are very limited toxicological studies of PAPs. Rosenmai et al. (2013) showed that 8:2 diPAP and 8:2 monoPAP could affect the progestogen and androgen synthesis *in vivo*. Toxicity of PAPs is also partly attributed to their metabolites. Rand and Mabury (2014) found that after rats were dosed with 6:2

diPAP, protein binding of metabolites would occur in the plasma, liver, and kidney. However, the consequence of protein binding is still unknown.

2.2.3 Biotransformation and bioavailability

Biotransformation of PAPs and subsequent formation of PFCAs in various biological systems has been reported (Lee et al. 2010, Lee et al. 2013, D'Eon and Mabury 2011, D'eon and Mabury 2007). D'Eon and Mabury (2011) studied the biotransformation of 4:2, 6:2 and 8:2 monoPAPs and diPAPs in rats. The results revealed that diPAPs were bioavailable with bioavailability decreasing with increasing chain length from 4 to 10 perfluorinated carbons. Both monoPAPs and diPAPs could be biotransformed into PFCAs. The microbial transformation of PAPs was first studied in a WWTP-simulated system (Lee et al. 2010). Lee et al. (2013) recently examined biotransformation of diPAPs in biosolid-amended soil and plant uptake of diPAPs and their degradation products. It was found that both plant uptake and biotransformation of diPAPs could contribute to the decline of diPAPs in soil over time.

Biotransformation of fluorotelomer-based PAPs begins with hydrolysis of the phosphate ester groups to produce fluorotelomer alcohols (FTOHs). **Figure 2.1** illustrates the biotransformation pathways of 6:2 diPAP and 6:2 monoPAP. Though phosphate ester hydrolysis can occur both abiotically and biotically, current studies have mainly examined microbial or enzymatic hydrolysis (Lee et al. 2010, D'Eon and Mabury 2011, D'eon and Mabury 2007, Liu and Mejia Avendaño 2013, Jackson and Mabury 2012). The produced 6:2 FTOH then undergoes a series of biotransformation processes, which have been well elucidated (Liu et al. 2007, Liu et al. 2010, Wang et al. 2009). PFCAs of several chain lengths are the terminal products of 6:2 FTOH, but there is a range of other degradation products and intermediates involved (**Figure 2.1**), such as 5:2 secondary fluorotelomer alcohol (5:2 sFTOH), 5:2 fluorotelomer ketone (5:2 ketone), and 5:3 polyfluoroalkyl carboxylic acid (5:3 acid). Other PAPs follow similar pathways and vary slightly in the types of degradation products or metabolites (Liu and Mejia Avendaño 2013). As FTOHs, in general, have short half-lives in biological systems (Liu and Avendaño 2013), the hydrolysis rates of PAPs become a major factor in controlling the overall formation rates from PAPs to PFCAs. Hydrolysis rates of PAPs and yields of PFCAs in soil are not well elucidated, which is partly due to the challenges in extracting PAPs from environmental media with satisfactory recovery.

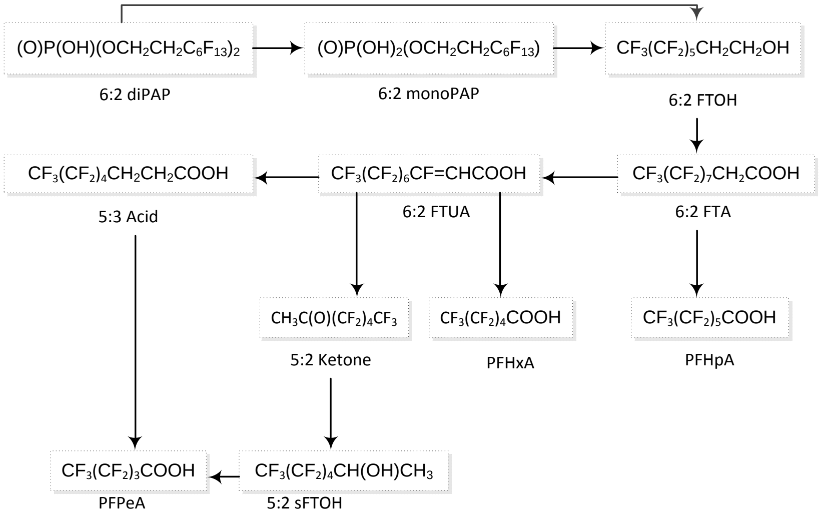

Figure 2.1: Biotransformation pathways of 6:2 diPAP and 6:2 monoPAP (Lee et al. 2010). Pathways from 6:2 FTOH to the other degradation products were proposed in another study (Liu et al. 2010).

2.2.4 Extraction methods and chemical analysis

Environmental monitoring of PAPs and laboratory investigation of their environmental fate (e.g., biodegradation and sorption) require knowledge of their physical and chemical properties. However, such knowledge is greatly lacking. In the meantime, challenges in handling and chemical analysis of PAPs and their degradation products have hampered the research on PAPs. For instance, monoPAPs are found to form monolayers on the solid substrates surfaces such as metal and glasses (Riddell et al. 2011). Analysis of PAPs requires stringent chromatographic conditions to achieve satisfactory chromatographic separation.

2.2.4.1 Extraction methods for solid matrices

Extraction and analytical methods reported in the literature for PAPs are summarized in **Table 2.1**. In most studies, methanol (MeOH) was chosen as the extraction solvent. In some studies, ammonium hydroxide (NH_4OH) (Lee et al. 2013, Benskin et al. 2013b) or sodium hydroxide (NaOH) (Eriksson and Karrman 2015) was added into MeOH to raise pH. In most PAPs studies, solvent extraction was followed by a clean-up step using black carbons (e.g. ENVI Carb cartridges or pellet) to reduce potential matrix effects. However, Benskin et al. (2013a) found that a significant portion of bis(N-ethyl perfluorooctane sulfonamido ethanol) phosphate diesters (SamPAP diesters) could absorb to ENVI Carb pellet to reduce mass recovery. With additional solvent rinsing of the ENVI Carb pellet, the recovery of SamPAP diester was improved from 4.5% to 87% **(Table 2.1)**. Lee et al. (2013) did not use ENVI Carb to clean up soil extracts; instead, 0.2 µm syringe filters were applied to remove ultrafine particles **(Table 2.1)**. In several studies, it was noted that recovery of PAPs suffered poor reproducibility among replicates (D’eon et al. 2009, De Silva et al. 2012). In the meantime, monoPAPs generally showed very low recoveries and sometimes were omitted in study results Lee et al. (2013).

If the concentrations of PAPs were lower than the detection limits of analytical instruments, sample concentration through nitrogen evaporation or solid phase extraction (SPE) is necessary. SPE can also reduce matrix effects. Oasis® WAX is the most commonly used cartridge for the purpose (Eriksson and Karrman 2015, Liu et al. 2013, Gebbink et al. 2013b).

2.4.4.2 Extraction methods for water and sludge samples

A range of organic solvents have been applied to extract PAPs from water or sludge samples, including acetonitrile (ACN), MeOH with NH_4OH, tetrahydrofuran with acetic acid (THF/HAc), and ACN/THF (Ding et al. 2012, Liu et al. 2013, Benskin et al. 2012) **(Table 2.1)**. Liu et al. (2013) investigated the impact of different extraction solvents on the recoveries of PAPs. It was found that MeOH, THF, and ACN had very low recoveries (e.g., 20%) for 6:2 diPAP, and it was postulated to be caused by insufficient polar and nonpolar interactions with PAPs. In addition, SPE is among the commonly used methods to concentrate and clean up target analytes in liquid samples. Liu et al. (2013) compared the performances of different cartridges. It was found that Oasis® WAX cartridges performed the best for diPAPs, and Oasis® MAX cartridges for triPAPs.

An ion pairing method using MTBE and ion pairing agents was also employed for extraction of PAPs from environmental samples. D'eon et al. (2009) used the method to extract diPAPs from WWTP sludge.

2.4.4.3 Analytical methods

PAPs are commonly analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS). A range of mobile phases and columns have been tested (**Table 2.1**). Riddell et al. (2011) found that pH of aqueous mobile phases could substantially affect the chromatographic separation and response of PAPs, and recommended adding NH_4OH to the aqueous mobile phase. Ding et al. (2012) compared the response of PAPs in four different aqueous mobile phases, including water containing 0.5% formic acid (pH = 2.3), water containing 0.1% formic acid (pH = 2.81), pure water (pH = 7), water containing 0.1% NH_4OH (pH = 10.47), and water containing 0.5% NH_4OH (pH = 10.72). They reached the same conclusion as Riddell et al. (2011) that water containing 0.1% NH_4OH could not only increase the signal intensity of PAPs but also reduce monoPAPs tailings. The performances of UPLC BEH C18 and C8 column were compared by Ding et al. (2012). It was found that BEH C8 column could improve peak shape of monoPAPs, and increase the signal intensity of most monoPAPs, except for 6:2 monoPAP.

Table 2.1: Summary of extraction methods, analytical methods and recoveries of PAPs applied in previous studies

Source	Matrix	Extraction method		Recovery (%)				Analytical method	
		Extraction solvent	Clean-up or enrichment					LC mobile phase	LC column
Lee et al. (2013)				4:2 diPAP	6:2 diPAP	8:2 diPAP	10:2 diPAP	50 mM ammonium acetate in MeOH 50 mM ammonium acetate in Water	GeminiNX C18 column (4.6 X 50 mm,3 µm)
	soil	1% NH_4OH in MeOH (v/v)	Filtered with 0.2 µm syringe filters	97 ± 14	91 ± 7	115 ± 37	83 ± 38		
	Catch plate	1% NH_4OH in MeOH (v/v)	Filtered with 0.2 µm syringe filters	78 ± 7	37 ± 9	55 ± 4	110 ± 14		
	plants	1% NH_4OH in MeOH (v/v)	ENVI Carb cartridges	117 ± 23	83 ± 16	39 ± 11			
De Silva et al. (2012)	Dust	MeOH	ENVI Carb cartridges	4:2 diPAP	6:2 diPAP	8:2 diPAP	10:2 diPAP	100 mM ammonium acetate in MeOH 100 mM ammonium acetate in Water	GeminiNX C18 column (4.6 X 50 mm,3 µm)
				110.6	93 ± 37	82 ± 37	100 ± 28		
Benskin et al. (2012)				SamPAP				0.1% ammonium formate/0.3% formic acid in water MeOH	Ascentis Express F5 (100 mm x 2.1 mm, 2.7 µm) equipped with F5 guard (50 mm x 2.1 mm, 2.7 µm). Delay column: Two Water Xterra C18 (4.6 mm x 30 mm, 5 µm)
	Marine sediment	MeOH	ENVI Carb	134 ± 4					
	seawater	SPE: load samples on Oasis WAX cartridge and elute by ACN and 1% NH4OH (v/v) in MeOH in sequence.		115 ± 7					
Benskin et al. (2013b)	Marine sediment	1% NH4OH in MeOH (v/v)	ENVI Carb	SamPAP diester				0.1% ammonium formate/0.1% formic acid in water MeOH	Water Xterra C18 (4.6 mm x 30 mm, 5ter Two Water Xterra (4.6 mm x 30 mm, 5 µm)
				4.5 ±1.1 (Without ACN rinsing ENVI Carb pellet)					
				87 ± 5.2 (With ACN rinsing ENVI Carb pellet)					
D’eon et al. (2009)				4:2 diPAP	6:2 diPAP	8:2 diPAP	10:2 diPAP	10 mM ammonium acetate in MeOH 10 mM ammonium acetate in Water	Ascentis Express C18 LC column (50 mm x 4.6 mm, 2.7 µm) GeminiNX C18 column (4.6 mm x 50 mm, 3 µm)
	WWTP sludge	MTBE ion pairing	Filtered with 0.2 µm nylon filters	38 ± 81	52 ± 21	53 ± 31	45 ± 51		
	Paper fiber	MTBE ion pairing	Filtered with 0.2 µm nylon filters	113 ± 1	104 ± 04	61 ± 16	86 ± 64		
	Human sera	MTBE ion pairing	Filtered with 0.2 µm nylon filters	89 ± 9l	116 ± 16	99 ± 9	110 ± 10		

Table 2.1 Continued

Source	Matrix	Extraction method			Extraction method					Analytical method	
		Extraction solvent		Clean-up or enrichment						LC mobile phase	column
Ding et al. (2012)	Drinking water	SPE: load samples on WAX cartridge and elute with 0.5% NH4OH in MeOH			4:2 monoPAP	6:2 monoPAP	8:2 monoPAP	10:1 monoPAP	10:2 monoPAP	0.1% NH$_4$OH in water MeOH	Waters ACQUITY UPLC BEH C8 (100 mm x 2.1 mm, 1.7 μm)
					73 ± 2	75 ± 10	95 ± 13	90 ± 10	65 ± 7		
					4:2 diPAP	6:2 diPAP	8:2 diPAP	10:1 diPAP	10:2 diPAP		
					11 ± 5	78 ± 10	104 ± 2	91 ± 10	85 ± 5		
					4:2/6:2 diPAP	6:2/8:2 diPAP	8:2/10:2 diPAP				
					80 ± 4	85 ± 12	82 ± 9				
Liu et al. (2013)	Sewage sludge	MonoPAPs and diPAPs	THF/HAc (1:1; v/v) (HAc = 1 M)	WAX coupled with ENVI Carb		6:2 monoPAP	8:2 monoPAP	10:2 monoPAP	6:2 diPAP	0.5% formic acid in MeOH 0.5% formic acid in water	Kinetex C18 (50 mm x 4.6 mm, 3 μm)
					16.7 ng/g spiking	29	48	69	41		
					167 ng/g spiking	24	37	40	79		
		TriPAPs	ACN/THF (1:1; v/v)	MAX coupled with ENVI Carb		8:2 diPAP	10:2 diPAP	6:2 triPAP	8:2 triPAP		
					16.7 ng/g spiking	78	93	56	50		
					167 ng/g spiking	54	100	64	85		
Gebbink et al. (2013b)	Packaging material	MeOH		SPE: load on Oasis WAX and elute with MeOH and 1% NH4OH in MeOH	From food samples: ranged from 0 (13C-6:2 monoPAP and 13C-8:2 monoPAP) – 271 (13C -6:2 diPAP)					2 mM ammonium acetate and 5 mM 1-methyl piperifine (1-MP) in 95% water and 5% MeOH	Waters ACQUITY UPLC BEH C8 (50 mm x 2.1 mm, 1.7 μm)
	Food	ACN			From fish homogenate: ranged from 26 (10:2/12:2 diPAP) to 97 (6:2/10:2 diPAP)					2 mM ammonium acetate and 5 mM 1-MP in 75% MeOH, 20% ACN and 5% water	
Eriksson and Karrman (2015)	Household dust	Soak with NaOH Extract with MeOH Neutralize with HCl		Oasis WAX coupled with Oasis HLB, elute with 25 mM acetate in 2:8 MeOH/water, MeOH and 2% NH4OH in MeOH, Clean up with ENVI Carb	6:2 diPAP	8:2 diPAP	6:2 monoPAP	8:2 monoPAP		1 mM 1-MP in water 1 mM 1-MP in MeOH	Waters ACQUITY UPLC BEH C8 (50 mm x 2.1 mm, 1.7 μm)
					84	103	96	123			

2.2.5 Solvent-enhanced hydrolysis

One of the challenges to satisfactorily recovering fluorotelomer-based compounds with hydrolysable functional groups (e.g., carboxylic acid and phosphate esters) from soil is solvent-enhanced hydrolysis, which was first reported by Dasu et al. (2010). The researchers examined the rare phenomenon by testing the stability of fluorotelomer carboxylic acid esters and fluorotelomer acrylates in different organic solvents including ACN, MeOH, MTBE and ethyl acetate (EA). Recoveries were examined for solvent extraction of live, autoclaved, γ-irradiated, and heat-treated soil. It was found that rapid and extensive hydrolysis occurred to a fluorotelomer monoester stearate in live and γ-irradiated soil and in solvents with higher dielectric constants (ACN and MeOH). Slight hydrolysis still could occur in MTBE and EA of low dielectric constants. It was hypothesized that microbial and enzymatic activities were the cause of the solvent-enhanced hydrolysis. In organic solvents, microbial and enzymatic activities towards those ester bonds were enhanced rather than deactivated Dasu et al. (2010).

Such solvent-enhanced hydrolysis would lead to significant overestimation of hydrolysis rates of the compounds with hydrolysable functional groups. Measures to eliminate or greatly minimize the phenomenon must be implemented to allow assessment of the environmental stability of fluorotelomer precursors and their formation rates and yields to PFAAs.

2.3 Unidentified PFAS

It has long suspected that current targeted analysis cannot detect all the PFASs present in the environment. According to an OECD report, more than 1000 differents PFASs have been produced and used (OECD 2007), but the routine environmental analysis only covers about ten to dozens of PFASs. In addition, studies on biotransformation of selected PFASs have shown that each PFAS could degrade to more than a dozen of degradation products, and many of them have not been included in environmental monitoring. The significance of the unidentified PFASs in the environment is yet to be understood.

2.3.1 Presence of unidentified PFASs in humans

Yeung et al. (2013b) first investigated the unidentified PFASs in the human plasma collected in Germany in 1982 to 2009 and the human blood collected in China in 2004. Fifty-two known PFASs and the extractable organofluorine (EOF) were measured in these samples. In the Chinese samples, the known PFASs accounted for 31% - 86% of the EOF. In the German samples, the known PFASs accounted for 52% - 100% and 57% - 100% of the EOF in two different samples collected from two different cities. Yeung et al. (2013b) found that the samples collected after the year 2000 had increasing proportions of unidentified PFASs, suggesting that humans have been exposed to newer unknown organofluorine products.

2.3.2 Presence of unidentified PFAS in the environment

Multiple earlier studies on mass flow of PFOA and PFOS in WWTPs have shown that the levels of PFOS and PFOA were often higher in the effluents than the influents (Becker et al. 2008, Guo et al. 2010, Kunacheva et al. 2011, Sinclair and Kannan 2006). This indicated that there were some unknown PFASs or PFOS/PFOA precursors present in waste streams. Those compounds can break down during biological wastewater treatment processes to form PFOS and PFOA. However, identities of most of these PFOS/PFOA precursors are not known.

Recently, Weiner et al. (2013) studied the known and unknown PFASs in commercially available aqueous film-forming foams (AFFFs). AFFFs containing fluorinated surfactants are commonly used for fire-fighting training activities and emergency responses. The results showed that the known PFCAs (C4-C14) and PFSAs (C4, C6, C8, C10) only accounted for 0.04% to 54% of the total organic fluorine (TOF) in the AFFF samples. Yeung et al. (2013a) investigated the extent of unidentified organic fluorine in Lake Ontario sediment samples. It was found that the known PFCAs and PFSAs only accounted for 2 - 44% of TOF, suggesting that there was a high proportion of unknown fluorine existed in the samples. Houtz and Sedlak (2012) reached a similar conclusion that a significant fraction of unidentified PFAS was present in urban runoff.

2.3.3 Methods to estimate unidentified PFAS

Two methods have been developed to determine the content of unidentified PFASs. The first method is named Total Oxidizable Precursor (TOP) assay, and it is based on the high chemical stability of perfluoroalkyl chains (Houtz and Sedlak 2012). When polyfluoroalkyl substances with perfluoroalkyl chains are exposed to hydroxyl and sulfate radicals generated by thermolysis of persulfate under high temperature and high pH conditions, they are converted to PFCAs of various chain lengths **(Figure 2.2)**. PFAAs mostly remain unchanged under the same conditions. The levels of unidentified PFASs thus can be evaluated by comparing the concentrations of PFCAs before and after the TOP assay (Houtz and Sedlak 2012). The assay does not require the use of another analytical instrument except for LC-MS/MS; however, the oxidation efficiency is impacted by the type of precursors and other organic and inorganic substances co-existing in environmental samples.

The second method assesses the levels of total PFAS by measuring the content of total or extractable organic fluorine in a sample. It requires the use of a dedicated combustion ion chromatography (CIC) system. Samples are first extracted using organic solvents or by solid phase extraction, and inorganic fluorine content is removed. Then the sample extract is subject to high-temperature pyrolysis, and all the organofluorine is converted to inorganic fluorine. The inorganic fluorine in the form of HF is absorbed into

NaOH solution, and finally the concentration of F^- in the solution will be analyzed using ion chromatography (Yeung et al. 2013a). The method cannot differentiate the origin of organic fluorine atoms unless PFAS-specific extraction method is applied.

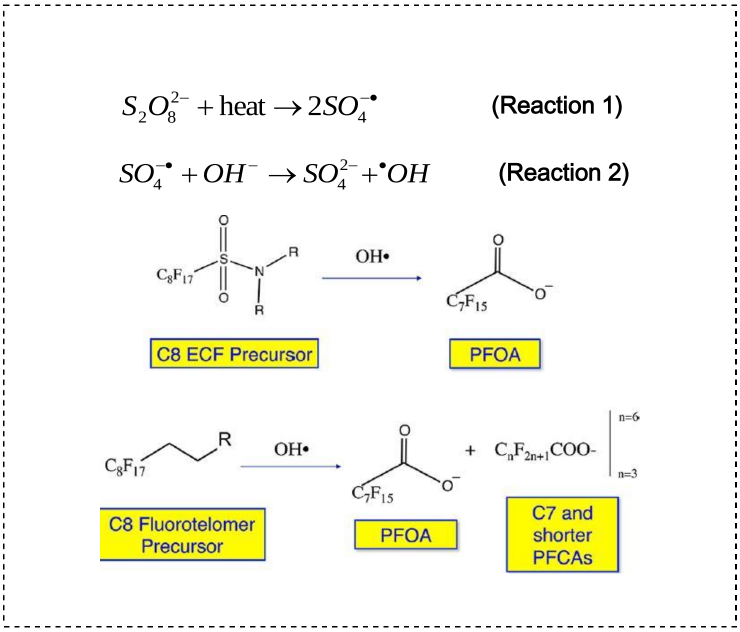

Figure 2.2: Illustration of working principles of the Total Oxidizable Precursor (TOP) assay (Houtz and Sedlak 2012).

2.3.4 PFASs in biosolids

Biosolids are treated sewage sludge and refer to the solid fraction of final treatment product in wastewater treatment processes. As they are nutrient-rich organic materials, they are often applied as fertilizer for agriculture purposes or for reclaiming nutrient-poor soils. Recycling biosolids represent a major route by which organic and inorganic pollutants, including PFASs, re-enter the environment.

Several studies have reported the levels of PFASs in biosolids. Sepulvado et al. (2011) monitored the concentration levels of PFASs in the USA in agricultural soils amended with the biosolids from municipal WWTPs without known PFAS industrial inputs. The results showed that PFOS was the predominant PFASs with the concentrations of 80 – 219 ng/g and 2 – 483 ng/g in biosolids and biosolid-amended soil, respectively. Chen et al. (2012) investigated the concentrations of PFOA and PFOS in municipal wastewater treatment biosolids in China. The concentrations of PFOA and PFOS ranged from 0.5 – 158 ng/g and 0.5 – 19.8 ng/g, respectively. Yoo et al. (2010) measured the concentrations of FTOHs, which are precursors to PFCAs, in biosolid amended soils. Elevated levels of 8:2 FTOH (5 – 73 ng/g) and 10:2 FTOH (< 5.6 – 166 ng/g) were observed in biosolid-amended soil compared to the soil (0 ng/g) which did not receive biosolids. Yoo et al. (2011) suggested that the land application of biosolids can result in not only observable levels of PFASs in soil, but also in plants.

The levels of PFASs in Canadian biosolids have not been reported. As it is known that targeted analysis only measure the levels of known PFASs, future studies should employ approaches to estimating the content of unidentified PFASs to reflect more accurately the levels of total PFASs.

CHAPTER III. AEROBIC BIOTRANSFORMATION OF POLYFLUOROALKYL PHOSPHATE ESTERS (PAPs) IN SOIL

(To be submitted to *Environmental Pollution)*

3.1 Abstract

Microbial transformation of polyfluoroalkyl phosphate esters (PAPs) into perfluorocarboxylic acids (PFCAs) has recently been confirmed to occur in activated sludge and soil. However, there lacks quantitative information about the half-lives of the PAPs and their significance as the precursors to PFCAs. In the present study, the biotransformation of 6:2 and 8:2 diPAP in aerobic soil in semi-dynamics reactors was investigated using improved sample preparation methods. To develop an efficient extraction method for PAPs, six different extraction solvents were compared, and the phenomenon of solvent-enhanced hydrolysis was investigated. It was found that adding acetic acid could enhance the recoveries of diPAPs and inhibit undesirable hydrolysis during solvent extraction of soil. However, extensive hydrolysis of 6:2 and 8:2 monoPAPs occurred no matter what solvent was used. The apparent biotransformation half-lives of 6:2 diPAP and 8:2 diPAP were estimated to be 11.5 days and >1000 days, respectively. At day 112, the major degradation products of 6:2 diPAP were 5:3 fluorotelomer carboxylic acid (5:3 acid, 9.34%), perfluoropentanoic acid (PFPeA, 6.42%) and perfluorohexanoic acid (PFHxA, 5.99%). The major product of 8:2 diPAP was

perfluorooctanoic acid (PFOA, 2.14%). The approximate linear relationship between the half-lives of eleven PFASs (including 6:2 and 8:2 diPAPs) and their molecular weights suggested that the molecular weight is a good indicator of the general stability of low-molecular-weight PFAS-based compounds in soil.

3.2 Introduction

Polyfluoroalkyl phosphate esters (PAPs), as an important class of anionic fluorinated surfactants, have received considerable attention as contaminants of emerging concerns. They have been widely detected in the environment and shown the potential to degrade into perfluoroalkyl carboxylic acids (PFCAs) (Eriksson and Karrman 2015, De Silva et al. 2012). Long-chain PFCAs containing more than seven perfluorinated carbons are subject to strict regulatory scrutiny and use restrictions due to persistence, toxicity, and bioaccumulation potential (Conder et al. 2008). PAPs are formulated into oil- and water-resistant papers and synthetic fibers (Funaki et al. 2000, Yoshida et al. 1998), and used in semiconductor materials (Schultz 2007) and personal care products including shampoos and cosmetics (Fujii et al. 2013). A PAP molecule comprises a phosphate hydrophile and fluorine-containing hydrophobic chains (Kissa 2001). For the PAPs reported in the recent literature, the fluorine-containing chains are either fluorotelomer-based perfluoroethyl groups [$F(CF_2CF_2)_nCH_2CH_2-$, n = 2 - 9] (Eriksson and Karrman

2015) or *N*-ethyl perfluorooctane sulfonamido groups [$F(CF_2)_8SO_2NCH_2CH_2-$] (Benskin et al. 2013b). Fluorotelomer-based PAPs have exhibited many variations in the number of fluorine-containing chains (i.e., mono-, di-, and tri-ester) and the size of the chains (i.e., m = 2 - 9) (Gebbink et al. 2012). The fluorine-containing chains within the same molecules are not necessarily the same. For instance, two different sizes of perfluoroethyl groups have been detected many diPAP species, such as 6:2/8:2 diPAP and 8:2/10:2 diPAP (Eriksson and Karrman 2015, De Silva et al. 2012).

Detection of a range of PAPs in human serum samples was attributed to migration of PAPs from treated food package paper (D'Eon and Mabury 2011, D'eon and Mabury 2007). The recent discovery of the ubiquitous presence of PAPs in indoor dust in private households suggested dust inhalation and ingestion another possible significant exposure route to humans (Eriksson and Karrman 2015, De Silva et al. 2012). With median levels of $\sum$monoPAPs and $\sum$diPAPs ranging from 3.7 to 1023 ng/g and 3.6 to 692 ng/g, respectively, PAPs are so far the most dominant class of polyfluoroalkyl and perfluoroalkyl substances (PFASs) found in indoor dust (Eriksson and Kaerrman 2014). The findings suggest the possible significant presence of PAPs in household products and subsequently high levels in waste streams, sewage, and biosolids. DiPAPs (6:2, 6:2/8:2, and 8:2) have been detected at ppb levels in sewage sludge, sub-ppb levels in wastewater influent and effluent, as well as sub-ppt levels in ocean surface waters (D’eon et al. 2009, Loi et al. 2013) while monoPAPs (8:2 and 10:2) were identified at ppb level in sewage sludge (Liu et al. 2013). Human exposure to PAPs has raised concerns about their impact on human health. Rosenmai et al. (2013) observed that the presence of 8:2 diPAP and 8:2 monoPAP led to decreased levels of androgens *in vivo*, and, therefore, suggested that these diPAPs could inhibit male sex hormone synthesis. Toxicity rising from PAPs is also associated with their degradation products or metabolites. Metabolism of 6:2 diPAP in rats lead to covalent protein binding with intermediate metabolites in plasma, liver, and kidney, which could induce toxicity (Rand and Mabury 2014).

The metabolism or microbial transformation of PAPs leading to persistent PFAAs has been reported in rats (D'eon and Mabury 2007), activated sludge (Lee et al. 2010), and aerobic soil (Lee et al. 2013). The biotransformation process begins with hydrolysis of the phosphate ester bonds to produce fluorotelomer alcohols (FTOHs) (**Fig 2.1**). Then biotransformation of FTOHs leads to the formation of not only PFCAs of several chain lengths, but also a number of characteristic fluorotelomer carboxylic acids and volatile intermediates (**Fig 2.1**) (Liu et al. 2010, Wang et al. 2009). Biotransformation pathways and kinetics of 6:2 and 8:2 FTOHs have been well elucidated (Liu and Avendaño 2013). As FTOHs generally exhibit very short half-lives (e.g., < 2 days for 6:2 FTOH and < 7 days for 8:2 FTOH in aerobic soils), the stability of the phosphate ester bonds and the ester hydrolysis rates become the key factors in determining how fast PFCAs would be generated from PAPs. Lee et al. (2013) have qualitatively demonstrated soil biodegradation of diPAPs and subsequent uptake of their degradation products by plants; however, reliable estimation of PAP hydrolysis rates or half-lives and the formation yields to PFCAs are still lacking. The information is particularly lacking for soil, which is one of the major reservoirs of pollutants. Such data is critical in the estimation of the contributions of indirect sources of PFCAs, such as from precursor substances like PAPs, and in predicting with high confidence the future trends of PFCAs in the environment.

One of the challenges of investigating the environmental fate in soil of fluorotelomer-based substances with hydrolysable function groups (e.g., carboxylic acid and phosphate esters) is solvent-enhanced hydrolysis during solvent extraction of the environmental samples. Dasu et al. (2010) first reported the rare but important phenomenon. They discovered that 8:2 fluorotelomer stearate ester (8:2 FTSE) can undergo rapid hydrolysis in certain organic solvents (e.g., acetonitrile and methanol) during solvent extraction of live or even γ-irradiated soils. Such experimental artifact would lead to substantial overestimation of the rate of hydrolysis and underestimation of the environmental stability of fluorotelomer-based ester compounds. Though the fundamental mechanisms behind the phenomenon are still elusive, it appears that residual microbial enzymes are not deactivated in those organic solvents, rather they exhibit greatly enhanced hydrolytic activity towards the ester bonds. We have noticed that several distinct solvents (e.g., methanol, methanol containing ammonium hydroxide, and tetrahydrofuran containing acetic acid) have been used for extracting PAPs from solid environmental matrices. However, there was no investigation whether any solvent-enhanced hydrolysis would occur to PAPs (Lee et al. 2013, De Silva et al. 2012, Liu and Avendaño 2013). Given that hydrolysis rates of phosphorous esters are likely to be sensitive to pH (Larson and Weber 1994), it is unlikely that a broad range of pH used in various studies would be equally suitable for PAP extraction unless PAPs are highly resistant to hydrolysis irrelevant of pH.

The overall goal of this study was to determine the rate of hydrolysis of PAPs in soil, their half-lives, and the formation yields to PFCAs. It is of primary importance to evaluate the suitability and efficiency of different solvents for PAP extraction without leading to the undesirable solvent-enhanced hydrolysis. 6:2 and 8:2 diPAPs, which are most dominant PAPs in the environment, were chosen as test compounds. In the end, we compare the stability of diPAPs in soil to that of other fluorotelomer derivatives with hydrolysable functional groups to evaluate the factors that impact their environmental recalcitrance. Such information would be useful for predicting the stability of many fluorotelomers that have not been subject to experimental studies.

3.3 Materials and methods

3.3.1 Chemicals and reagents

6:2 and 8:2 diPAPs (>97%) were purchased from Toronto Research Chemicals (Toronto, Canada). The rest of PFAS standards and isotopically labeled internal standards were obtained from a variety of sources as listed in **Table S3.1** in the Supplementary Information (SI). HPLC-grade solvents including acetonitrile (ACN), methanol (MeOH), methyl tert-butyl ether (MTBE), and ethyl acetate (EA), LC/MS-grade water and acetic acid (HAc), certified sodium hydroxide (5 N) and hydrochloric acid (6 N), and ACS-grade calcium chloride ($CaCl_2$) were purchased from Fisher Scientific (Ottawa, ON).

3.3.2 Soil Microcosm Setup

St-Bernard soil was collected from McGill University McDonald campus in Sainte-Anne-de-Bellevue, QC, at the same location where the soil used for a previous aerobic biotransformation study of perfluoroalkyl sulfonamide derivatives was collected (Mejia Avendaño and Liu 2015). The soil was sieved via a 2-mm sieve immediately upon collection and stored at -4 °C and used within three months. Part of the soil was rendered sterile via autoclaving. Three cycles of autoclaving were performed at 121 °C for 60 min per cycle, and the soil was incubated at room temperature for 24 h in between cycles. To further inhibit microbial or enzymatic activities, three antibiotics (kanamycin sulfate, chloramphenicol, and cycloheximide) were added to the autoclaved soil to reach a concentration of 100 mg/kg soil (Liu et al. 2010). The soil moisture content was adjusted with a sterile $CaCl_2$ solution to a gravimetric moisture content of 22%, which approximated 80 % of the field capacity of the soil.

The same semi-dynamics setup using 500-mL glass bottles as the one used by Mejia Avendaño and Liu (2015) was used to incubate ~110 g soil (oven dry weight, 103 °C). The airtight caps were fitted with two openings: one connected to an SPE C18 cartridge (Maxi-Clean™, Alltech) to trap volatile fluorinated compounds and to allow air exchange, and the second one kept closed during incubation and open only during headspace sampling. Three bottles were prepared for each treatment and each diPAP: (1) untreated (matrix) live soil spiked with 500 µL methanol; (2) 6:2 diPAP (or 8:2 diPAP) treated live soil with an initial concentration of 4.22 nmol/ml for 6:2 diPAP (or 3.37 nmol/ml for 8:2 diPAP); and (3) 6:2 diPAP (or 8:2 diPAP) treated sterile soil with the same initial concentrations as treatment (2).

3.3.3 Sampling and sample preparation

The diPAPs were incubated in the soil microcosm for 112 d and sampling was conducted at day 0, 3, 7, 14, 21, 28, 56, 84, and 112. At each sampling time point, the bottle headspace was first purged through an SPE C18 cartridge for 5 min at a flow rate of ~ 100 ml/min air, which was sterilized via a 0.3 μm air filter (HEPA-CAP 36, Whatman). The cartridge was removed and eluted with 4 mL ACN to extract volatile compounds. Then the bottle cap was removed to allow soil sampling. PAPs and FTOHs were extracted from 2.5 g soil using 5 mL of 4:1 v/v ACN/HAc (HAc = 1 M), which was chosen among six tested solvent mixtures as described in **Section 3.4.1**. The soil-solvent slurry was subject to sonication at 30 °C for 60 min, mixing at 250 rpm on a horizontal shaker for 60 min, and centrifugation at 4000 *g* for 20 min. Then the supernatant was transferred to a second polypropylene tube, and the extraction procedure was repeated. For the rest of degradation products, a similar sequential extraction procedure was used except that another 2.5 g of soil was extracted using 4.5 ml ACN and 0.5 ml of 0.25 M NaOH. The method was validated previously in FTOH biodegradation studies for satisfactory recoveries of FTOH degradation products (Liu et al. 2010, Wang et al. 2009). Solvent extracts were stored at -20 °C before analysis.

3.3.4 Evaluation of extraction methods

Prior to the soil biotransformation, six different solvents were tested for recovering 6:2 and 8:2 monoPAPs and diPAPs from the soil. About 2.0 g soil (dry weight) was weighed into a 15-mL polypropylene tube and spiked with PAPs in a MeOH solution to give an initial concentration of about 2 μg/g-soil for each PAP. Then soil was extracted immediately with one of the six solvents including: 9/1 (v/v) ACN/NaOH (NaOH = 0.25 M), MeOH containing

1% NH_4OH, EA, MTBE, 4/1 (v/v) MTBE/HAc (HAc = 1 M) and 4/1 (v/v) ACN/HAc (HAc = 1 M) at 1/2 (w/v) soil/solvent ratio. After sonication at 30 °C for 60 min, mixing at 250 rpm for 60 min, and centrifugation at 4000 *g* for 20 min, the supernatant was removed and transferred to a second polypropylene tube. The soil controls without PAPs and the solvent controls without soil were also conducted following the same procedure as the above for quality assurance.

3.3.5 Chemical analysis

All the chemicals of interests were analyzed using a Shimadzu Nexera ultra-high performance liquid chromatography interfaced with an AB Sciex Qtrap 5500 mass spectrometer (LC-MS/MS). PAPs, FTOHs, and volatile degradation products were quantified using the Method I (**Table S3.2** in the SI) under a basic mobile phase, and the rest of degradation products were quantified using the Method II (**Table S3.3** in the SI) under an acidic mobile phase. Instead of using GC-MS for FTOH analysis, we have developed the LC-MS/MS method (Method I) that is capable of separating and quantifying all the PAPs and four fluorinated alcohols within the same run. The presence of a volatile base (NH_4OH) has been found to allow efficient ionization of FTOHs by suppressing formation of FTOH adducts (Liu and Lee 2005). The same mobile phase has also been found to result in good peak shapes and the best signal strength for 6:2 and 8:2 monoPAPs (Riddell et al. 2011). Prior to chemical analysis, all the extract was centrifuged at 10,000 *g* for 10 min to remove ultrafine particles and then spiked with corresponding internal standard mixtures. The information on the internal standards and calibration methods can be found in **Table S3.1-3.3**.

3.3.6 Quality control measures

All setups were prepared and processed in triplicate, and all analytical results were reported as the average of the three values. Except for soil incubation vessels, plastics tubes and vials were used for the sample preparation and storage to minimize potential adsorption to solid surfaces (Riddell et al. 2011). The stability of PAPs during LC-MS/MS analysis was assessed by injecting each PAP separately to confirm that PAPs would not break down and release FTOHs. The potential matrix effect was evaluated by comparing the signal responses of the selected target compounds in soil extracts with those in a clean solvent (**Figure S3.4**).

3.3.7 Data analysis

The DT_{50} values (time required for 50% dissipation of the initial concentration) of 6:2 and 8:2 diPAPs were determined using the Kinetic Graphic User Interface (KinGUII) v2.1 (KINGUii), which is an R-based software package designed specifically for determining kinetic parameters from environmental fate studies (Boesten et al. 2005). Iteratively Reweighted

Least Squares (IRLS) method was used for simulation. The biotransformation of both compounds were tested with four kinetic models: single first-order kinetics model (SFO), double first-order in parallel model (DFOP), hockey-stick model, and first-order multiple compartment model (KINGUii). In the single first-order kinetics model, the DT_{50} values are also referred to as half-lives.

3.4 Results and discussion

3.4.1 Evaluation of extraction methods for PAPs

Six solvent mixtures were examined for their extraction efficiencies and the ability to suppress or enhance the hydrolysis of PAPs during soil-solvent extraction. As shown in **Figure 3.1** and **Table S3.5**, in the immediate spike-recovery test, none of the six solvents was able to extract 6:2 and 8:2 monoPAPs with satisfactory recoveries and without causing solvent-enhanced hydrolysis. The best recoveries of the monoPAPs were achieved with 9/1 ACN/NaOH, but they were only 24.3% and 8.78% for 6:2 and 8:2 monoPAPs, respectively. For the rest of solvents, the monoPAPs were largely recovered as 6:2 or 8:2 FTOHs, demonstrating that the extensive hydrolysis has occurred during the extraction processes. The challenge of achieving good recoveries of monoPAPs from activated sludge and soil samples has been previously documented though the cause was not identified. Lee et al. (2010) reported rapid decreases of 6:2 monoPAP even in sterile activated sludge controls. The researchers hypothesized that it was caused by strong binding of the monoPAP to biomass due to the dianionic phosphate center of the compound. The monoPAP data were omitted in another study on biotransformation of PAPs in a biosolids-applied soil due to poor recoveries (Lee et al. 2013). In this study, we have shown that low recoveries of monoPAPs can be attributed to the extensive solvent-enhanced hydrolysis of monoPAPs in all commonly used solvents. Jackson and Mabury (2012) previously showed that in the presence of intestinal alkaline phosphatase, monoPAPs would hydrolyze in about 100 s, demonstrating the unstable nature of monoPAPs. Though solvents of different properties could be further tested, the study showed the significant challenge of reliable measurement of monoPAPs in the environmental samples with microbial or enzymatic activities such as soil and activated sludge.

6:2 and 8:2 diPAPs have exhibited much higher stability and lower tendency to undergo solvent-enhanced hydrolysis (**Figure 3.1**). Satisfactory recoveries of 6:2 and 8:2 diPAPs were achieved with 4/1 MTBE/HAc (115% for 6:2 diPAP and 99.3% for 8:2 diPAP) and 4/1 ACN/HAc (113% for 6:2 diPAP and 99.9% for 8:2 diPAP) (**Table S3.5**). HAc seemed to play a more important role than the type of the solvents in achieving satisfactory recoveries and minimizing undesirable solvent-enhanced hydrolysis of diPAPs. It is notable that even for these two solvent mixtures wth HAc slight solvent-enhanced hydrolysis has occurred, as shown by the generation of 6:2 FTOH

(5.4 – 5.5%) and 8:2 FTOH (5.1 – 5.2%) above the impurity levels (< 1.0%) in the diPAP standards. Compared to the performance of other solvents, the slight hydrolysis occurring to 4/1 MTBE/HAc and 4/1 ACN/HAc were considered to have a negligible impact on the accurate measurement of the diPAPs. Between the two solvent mixtures, 4/1 ACN/HAc was chosen for PAP extraction because of better solvent compatibility with the LC-MS/MS system. One issue of using ACN was potential slow polymerization of monoPAPs into diPAPs (Personal communications, Riddell N., 2015) in the solvent. Therefore the samples were analyzed as soon as possible after they were prepared.

Dasu et al. (2010) have demonstrated that solvent with high dielectric constants such as ACN and MeOH significantly enhance hydrolysis of a fluorotelomer stearic acid ester, but not the less polar solvents such as MTBE and EA. We have found that MTBE and EA had low recoveries (< 25%) for diPAPs and were unable to break analyte-matrix interactions effectively. No hydrolysis occurred to EA, but up to 25.6% of diPAP was recovered as 8:2 FTOH after MTBE extraction. The basic solvents, 9/1 (v/v) ACN/NaOH and MeOH containing 1% NH_4OH, showed better recoveries than MTBE or EA. No solvent-enhanced hydrolysis of FTOHs was observed with 9/1 (v/v) ACN/NaOH; the recovery of 8:2 diPAP was close to 100%, but 6:2 diPAP only around 30%. More than 77% of 6:2 and 8:2 diPAPs was recovered by using MeOH containing 1% NH_4OH, but there was significant solvent-enhanced hydrolysis occurring to 8:2 diPAP.

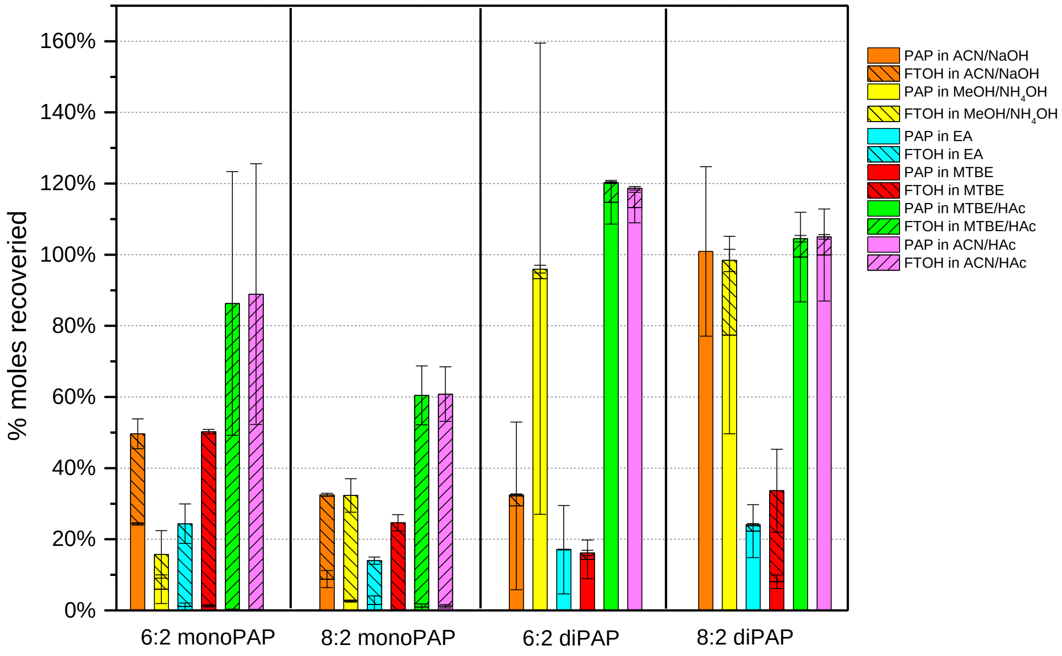

Figure 3.1: Total mole-based recoveries of PAPs and corresponding FTOHs in spike-recovery tests using live soil and six extraction solvents. All the FTOHs are plotted as the mole based equivalence of the PAPs.

It was hypothesized that the solvent-enhanced hydrolysis was caused by microbial and enzymatic activities that were not deactivated by organic solvents (Dasu et al. 2010). The hypothesis is consistent with the observation that solvent-enhanced hydrolysis of all of the PAPs was almost non-exist (**Table S3.4** in the SI) during extraction of the autoclaved soil, which had no microbial and enzymatic activities. However, we cannot entirely rule out chemical factors that might have contributed to the phenomenon. A perfluoroethyl chain adds to steric hindrance and reduces access to a phosphorus center to active enzymatic sites, potentially reducing hydrolysis rates. In the meantime, the electron-withdrawing nature of the perfluoroethyl chain can enhance nucleophilic attack and could subsequently increase hydrolysis rates. Rate of enzymatic hydrolysis of phosphate esters is also known to be affected by the presence of metal ions, mineral surfaces, and solution pH (Baldwin et al. 1995, Vincent et al. 1992). We suspect that such solvent-enhanced hydrolysis is not unique to the soil. Other types of samples with biological or enzymatic activities towards ester bonds could exhibit similar phenomena. Monitoring potential generation of FTOHs provides more sensitive and reliable assessment of the stability of PAPs in a matrix of interest than monitoring the levels of PAPs alone.

3.4.2 Biotransformation of 6:2 diPAP

The decreasing 6:2 diPAP and the simultaneous generation of a range of degradation intermediates and end products indicate that 6:2 diPAP was being transformed. The relatively constant 6:2 diPAP concentrations and the lack of any degradation products in the sterile treatment (**Figure. 3.3**) verified that 6:2 diPAP transformation was predominantly controlled by microbial processes in the soil. The DT_{50} value of 6:2 diPAP in the live soil was estimated to be 11.5 days using the DFOP model (**Figure 3.2a**), and 14.5

days using the SFO mode. DFOP was found to well describe the fast initial decrease in 6:2 diPAP concentrations and followed by a slower decline with a lower chi^2 error (2.855) whereas the SFO model provided poor fit for later data points with a high chi^2 error (14.14). Detailed regression data can be found in the SI (**S3.1**).

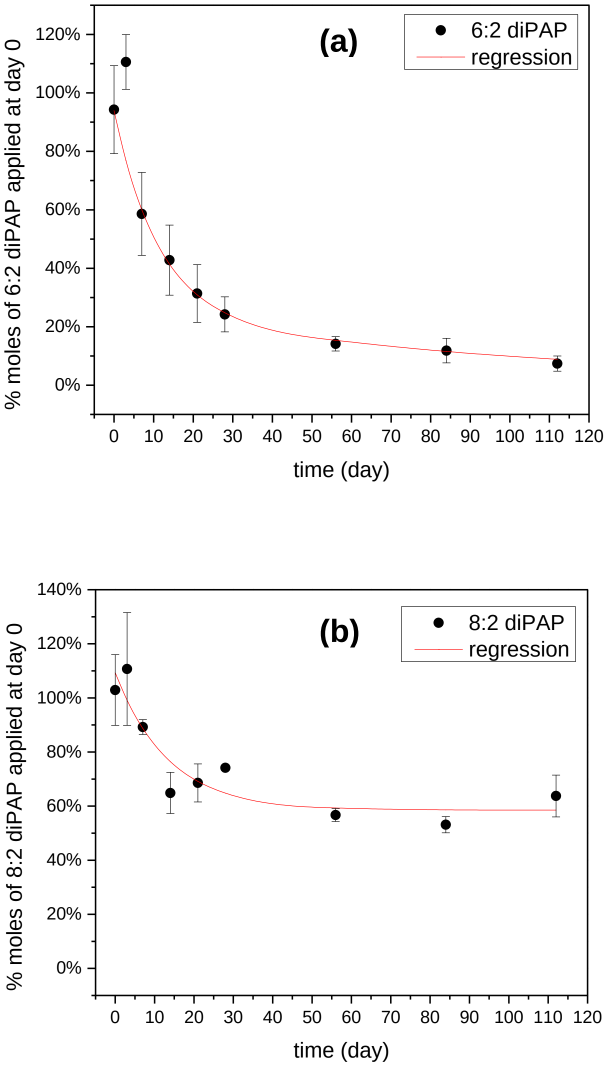

Figure 3.2: Time profiles of (a) 6:2 diPAP and (b) 8:2 diPAP in the live soil. The solid lines are fitted curves of a double first-order in parallel (DFOP) model.

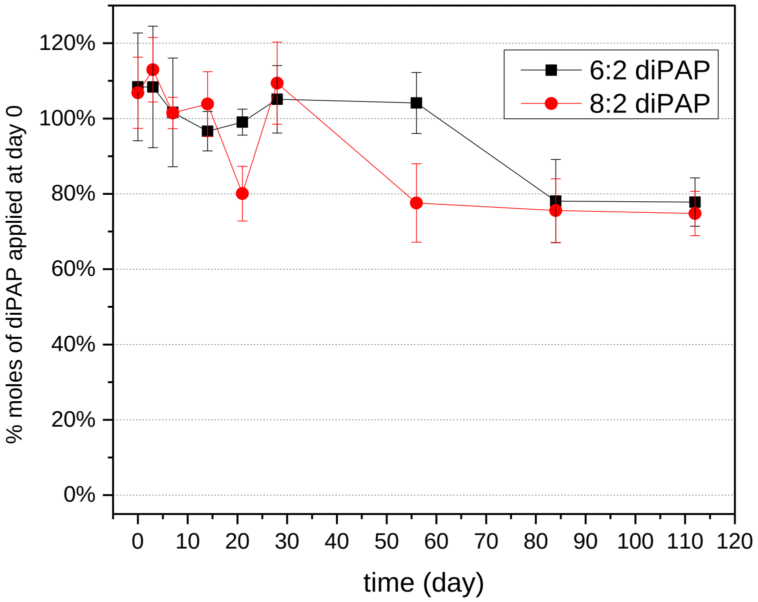

Figure 3.3: Time profiles of 6:2 diPAP and 8:2 diPAP in the sterile soil.

The time profiles of the degradation products of 6:2 diPAP are shown in **Figure 3.4**. According to the biotransformation pathways of 6:2 diPAP proposed previously (Lee et al. 2010, Liu et al. 2010), we would expect the following degradation products: 6:2 monoPAP, 6:2 FTOH, 6:2 FTCA, 6:2 FTUA, 5:2 sFTOH, 5:2 ketone, 5:3 acid, PFBA, PFPeA and PFHxA. Due to the solvent-enhanced hydrolysis, concentrations of 6:2 monoPAP and 6:2 FTOH could not be reliably measured in the soil and thus not reported. 5:2 sFTOH, another major degradation intermediate, increased initially as it was being generated, reached the highest concentration of 1.64% on day 14, and declined afterward as it was being degraded to downstream compounds

(**Figure 3.4**). The percentages of volatile products, including 6:2 and 8:2 FTOHs, and 5:2 and 7:2 sFTOHs, recovered in the headspace relative to the total mass recovered were shown in **Figure 3.5.**

For the terminal degradation products of PFBA, PFPeA, and PFHxA, their concentrations showed consistent increases over time. By day 112, they reached 7.26%, 6.42%, and 5.99%, respectively, of the 6:2 diPAP applied on day 0. 5:3 Acid can slowly degrade in activated sludge, but its biotransformation in soil is inhibited by sorption to soil particles (Li et al. 2012). As we did not detect any potential degradation products of 5:3 acid, 5:3 acid was considered as a terminal product in the study. The formation yield of 5:3 acid was 9.34% by the end of incubation.

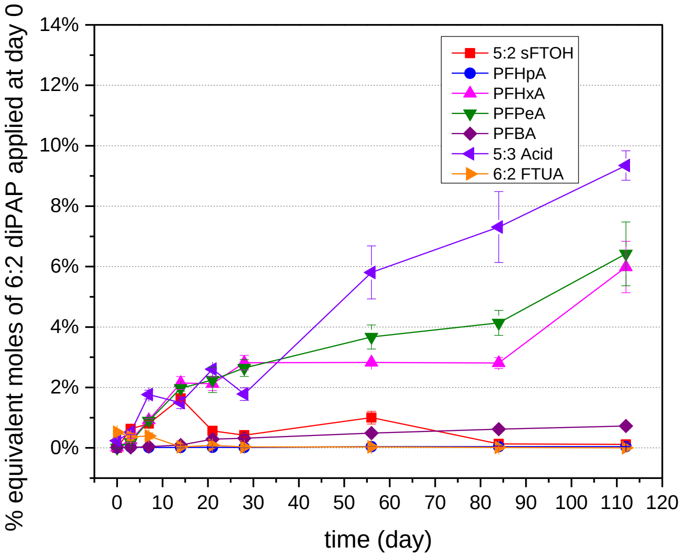

Figure 3.4: Individual degradation products of 6:2 diPAP detected in the live soil. Some error bars are invisible since they are smaller than the symbol height.

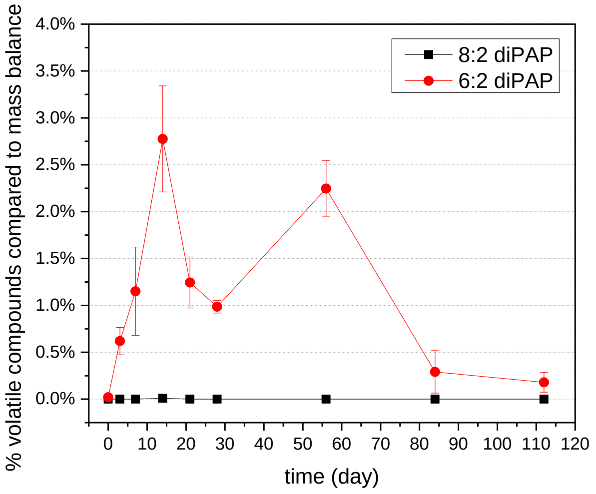

Figure 3.5: Total volatile compounds detected in the headspace. For 6:2 diPAP, the compounds detected in the headspace were 6:2 FTOH and 5:2 sFTOH; for 8:2 diPAP, they were 8:2 FTOH and 7:2 sFTOH. Some error bars are invisible since they are smaller than the symbol height.

3.4.3 Biotransformation of 8:2 diPAP

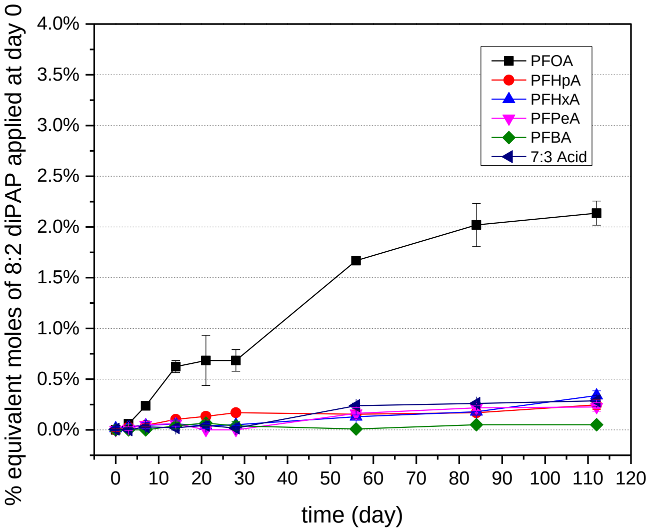

Figure 3.6: Time profiles of degradation products of 8:2 diPAP in the live soil.

The biotransformation of 8:2 diPAP in soil proceeded much slower than that of 6:2 diPAP as shown in **Figure 3.2b**. The DT_{50} value of 8:2 diPAP in soil was larger than 1000 days using the DFOP model (**Table S3.5**), and 114 days using the SFO. Similar to 6:2 diPAP, the DFOP model was able to describe the declining trend more precisely (chi^2 error = 8.475) than the SFO model (chi^2 error = 13.4). The longer half-life is attributed to its longer perfluoroethyl chain, larger molecular size, and lower bioavailability when compared to 6:2 diPAP. The degradation products of 8:2 diPAP were similar to those of 6:2 diPAP, except that their perfluoroethyl chains were two carbons longer. However, due to the solvent enhanced hydrolysis, the concentration of 8:2 monoPAP and 8:2 FTOH in the soil may be unreliable and therefore not reported. The major metabolite, PFOA, reached 2.14% at day 112. Due to the slow degradation rate of 8:2 diPAP, there was no significant generation of the primary terminal degradation products of PFHxA, 7:3 acid and PFHpA,. At day 112, each only accounted for 0.34%, 0.29% and 0.25%. respectively, of the total mass applied (**Figure. 3.6)**. The proposed intermediate metabolites, 8:2 FTCA, 8:2 FTUA, and 7:2 sFTOH were not observed over the whole period of study probably because of their low concentrations.

3.4.4 Matrix effect, extraction efficiencies in soil, and mass balance

Potential matrix effects were examined by comparing LC-MS/MS response ratios of spiked target analytes in soil extracts with clean solvents as illustrated in **Figure S3.4**. The ratios for target PAPs and FTOHs were found in the range of 1.19 to 0.92 at the concentration of 2.5 ng/ml. This suggested the matrix effects were insignificant for PAPs and FTOHs in this study. Previously, the potential matrix effects for the degradation products were examined by Liu et al. (2010), and it was found insignificant either using the same extract solvent as the one in the current study. The immediate recoveries of all the parent compounds and degradation products were illustrated in **Figure S3.5**. The target compounds were extracted from soil immediately by the two different extraction methods as described earlier. PAPs and FTOHs were extracted by 4:1 ACN/HAc (HAc 1 M) and the other degradation products were extracted by 9:1 ACN/NaOH (NaOH 0.25 M). Except for 6:2 and 8:2 monoPAPs, the immediate recoveries for the other target analytes were found satisfactory ranging from 72.9% to 120%.

The recoveries of all the target compounds in each sequential extraction step were investigated at each sampling time point in the live soil (**Figure S3.5**). The results were expressed as the percentage of mole concentration of each compound in each extraction sequence over the total mole concentrations in the two extractions. As shown in **Figure S3.5**, most compounds can be efficiently extracted in the first extraction with the exception of 5:3 acid, which means two sequential extractions were sufficient for extracting majority of the target compounds from the live soil. The cause of low recoveries with 5:3 acid is unclear.

The mass balance was calculated as the sum of equivalent mole fractions of all the quantifiable parent compounds (6:2 diPAP and 8:2 diPAP) and degradation products (including 8:2 monoPAP, 8:2 FTOH, 7:2 sFTOH, PFOA, PFHpA, PFHxA, 7:3 Acid and 8:2 FTUA for 8:2 diPAP study; 6:2 monoPAP, 6:2 FTOH, 5:2 sFTOH, PFHxA, PFPeA, PFBA and 5:3 Acid for 6:2 diPAP study) in both soil extracts and headspace. As shown in **Figure 3.7**, the total recoverable mass decreased over time. 6:2 diPAP-treated live soil showed declining mass balance from 108% to 40.3% over 112 days while 8:2 diPAP-treated soil from 124% to 68.7%. In PFAS soil biotransformation studies, the time-dependent decrease of the total mass balance, or the total solvent extractable fraction, has been noted in multiple studies irrespective of chemical type or soil type (Liu and Avendaño 2013). Declining mass balance over time, in general, can be attributed to unidentifiable or non-quantifiable degradation products as well as the formation of soil bound residues (Liu and Avendaño 2013). Based on the overall low yield of identifiable degradation products, it is unlikely that unidentifiable or non-quantifiable degradation products (i.e., 5:2 and 7:2 ketone, degradation products of 5:3 and 7:3 acids) would account for a significant fraction of the missing mass. Instead, Liu et al. (2010) and Wang et al. (2009) suggested that formation of soil bound residues, which is mainly catalyzed by microbial processes, is the primary cause of low mass balance. Thus, faster and more extensive biotransformation is linked to lower mass balance, which is consistent with the findings of the current study that fast-degrading 6:2 diPAP showed the less recoverable total mass.

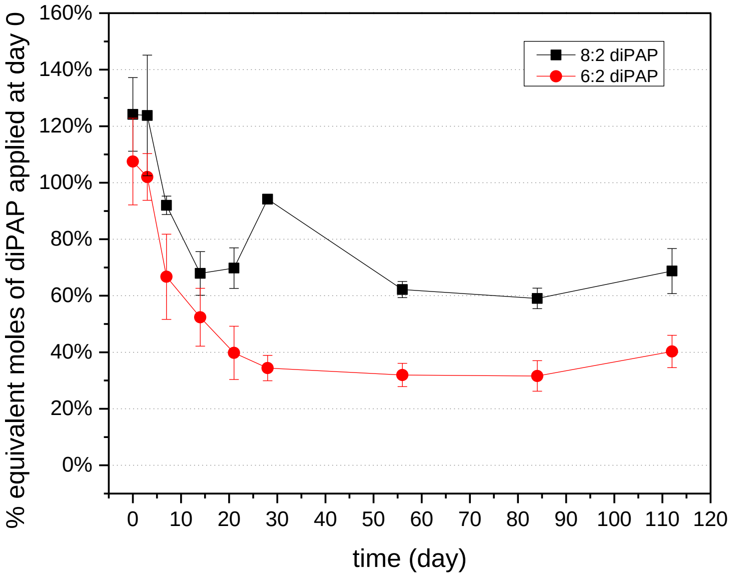

Figure 3.7: Mass balance (mole-based) of 6:2 diPAP and 8:2 diPAP in the live soil.

3.5 Environmental Implications

It has been of great interest to understand the recalcitrance of PFAS surfactants and polymers with perfluoroalkyl chains, and what key factors impact their biodegradability and degradation rates in the natural environment. As of today, biotransformation in aerobic soils of nine low-molecular-weight fluorotelomer derivatives has been investigated under similar experimental conditions (Liu and Avendaño 2013). They are 8:2 FTOH, 6:2 FTOH, 8:2 fluorotelomer stearate ester (8:2 FTSE), 8:2 fluorotelomer citrate ester (8:2 TBC), 8:2 fluorotelomer acrylate (8:2 FTAC), 8:2 fluorotelomer methacrylate (8:2 FTMAC), hexamethylene 1,6-di-8:2 fluorotelomer urethane (HMU), 6:2 and 8:2 diPAPs. Biotransformation of *N*-Ethyl perfluorooctane sulfonamidoethanol (EtFOSE) and *N*-Ethyl perfluorooctane sulfonamide (EtFOSA), which are used in similar applications as fluorotelomer derivatives but synthesized through electrochemical fluorination processes, has also been recently studied (Mejia Avendaño and Liu 2015). As the half-lives of the other PFAS were all determined using the SFO, we also plotted the half-lives of diPAPs in **Figure 3.8** to facilitate the comparison of their stability. As shown in the figure, there is a rough linear relationship between the molecular weights of the nine compounds and their half-lives in aerobic soils. It has been recognized that the stability of non-fluorinated polyaromatic hydrocarbons (PAHs) is associated with their molecular weights and the octanol-water partition coefficient ($\log K_{ow}$). With increasing molecular weight and $\log K_{ow}$, Cerniglia (1992) has observed that the recalcitrance of PAHs increased correspondingly. The Figure 3.8 demonstrates a similar trend with PFAS if the same kinetics model is applied. The difference with PFAS is that reliable measurement of $\log K_{ow}$ for PFAS is often not achievable; therefore the molecular weight is probably the only acceptable indicator of the general stability of PFAS-based compounds.

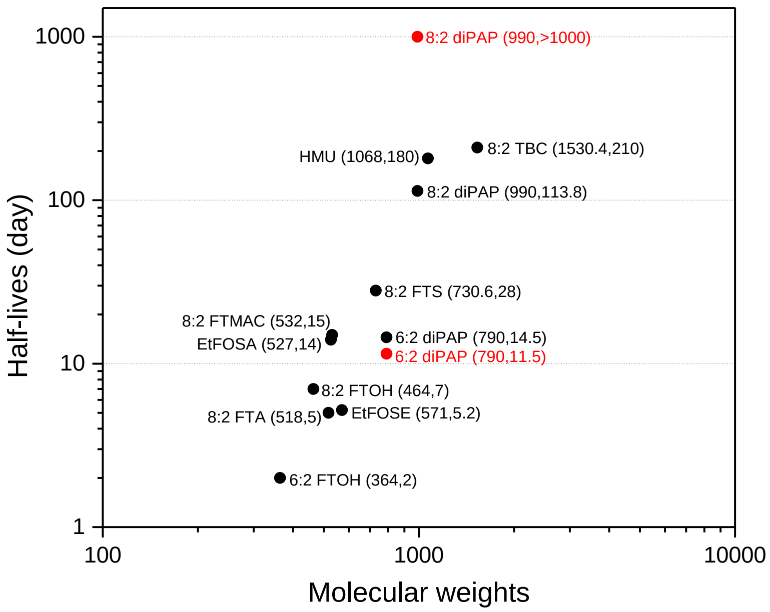

Figure 3.8: The relationship of molecular weights of eleven PFAS substances and their half-lives in aerobic soils. The first number on each label is the molecular weight and the second number the half-life. The half-lives shown as black symbols were estimated using SFO model. The half-lives shown as red symbols were estimated using DFOP model.

However, when DT_{50} values determined using the DFOP model, which was found to have a high statistical significance in describing the experimental data, were plotted (**Figure 3.8**, red symbols), 8:2 diPAP deviates from the general trend. We have found that just as the SFO model was unable to describe the slow declining trend for the diPAPs, other studies have also noticed a similar deficiency with the model, but did not test alternatives. Therefore we think it would be prudent to examine whether it would be appropriate to apply SFO for the sake of simplicity while sacrificing accuracy in predicting the microbial stability of PFAS. Alternative models have been well established in predicting the stability of pesticides in soils, and could be adapted with little modifications (Boesten et al. 2005).

Another caveat of the rough linear relationship shown in **Figure 3.8** is that it cannot be extrapolated to higher molecular weight range, where no reliable experimental data are yet available. A few studies have predicted environmental half-lives of fluorotelomer-based polymers and oligomers of high molecular weights (Russell et al. 2010, Russell et al. 2008OS, Washington et al. 2009). However, the accuracy of those predictions is questionable due to various experimental challenges associated with PFAS-based polymer substances. Though the linear relationship shown in **Figure 3.8** is probably an oversimplification of the factors that control the recalcitrance of low-molecular-weight PFAS, it has practical utilities of allowing for quick prediction of many other similar PFAS that have not been subject to experimental evaluation.

3.6 Acknowledgement

This project is supported by NSERC Discovery Grant, and Fonds de recherche du Québec - Nature et technologies (FRQNT). We thank Professor Scott Mabury at University of Toronto for providing 6:2 and 8:2 monoPAP standards.

3.7 Supplementary information for Chapter III.

Table S3.1: Standards of Perfluoroalkyl and polyfluoroalkyl substances

Acronym	Accurate Mass	Chemical Formula	CAS	Source	Purity
6:2 diPAP	789.982326	(O)P(OH)(OCH2CH2C6F13)2	57677-95-9	Toronto Research Chemicals, Canada	97%
8:2 diPAP	989.969549	(O)P(OH)(OCH2CH2C8F17)2	678-41-1	Toronto Research Chemicals, Canada	97%
6:2 monoPAP	443.979612	(O)P(OH)2(OCH2CH2C6F13)	57678-01-0	University of Toronto, Canada	97%
8:2 monoPAP	543.973224	(O)P(OH)2(OCH2CH2C8F17)	57678-03-2	University of Toronto, Canada	97%
6:2 FTOH	364.013279	C6F13CH2CH2OH	647-42-7	Sigma-Aldrich, USA	97%
8:2 FTOH	464.006891	C8F17CH2CH2OH	678-39-7	Synquest, USA	97%
5:2 sFTOH	314.016473	C5F11CH(OH)CH3	914637-05-1	DuPont, USA	99%
7:2 sFTOH	414.010085	C7F15CH(OH)CH3	24015-83-7	DuPont, USA	99%
PFBA	218.286476	C3F7COOH	375-22-4	Sigma-Aldrich, USA	98%
PFPeA	263.983282	C4F9COOH	2706-90-3	Sigma-Aldrich, USA	97%
PFHxA	318.280088	C5F11COOH	307-24-4	Sigma-Aldrich, USA	>97%
PFHpA	363.976894	C6F13COOH	375-85-9	Sigma-Aldrich, USA	99%
PFOA	418.273700	C7F15COOH	335-67-1	Sigma-Aldrich, USA	96%
8:2 FTUA	457.979928	C7F15CF=CHCOOH	70887-84-2	DuPont, USA	99%
5:3 Acid	342.011388	C5F11CH2CH2COOH	914637-49-3	DuPont USA	99%
7:3 Acid	442.005000	C7F15CH2CH2COOH	812-70-4	DuPont USA	99%
Isotopically labeled internal standards					
[M+4] 6:2 diPAP	Sodium bis(1H,1H,2H,2H-[1, 2-$^{13}C_2$]perfluorooctyl)phosphate			Wellington Laboratories, Canada	>99%
[M+4] 8:2 diPAP	Sodium bis(1H,1H,2H,2H-[1, 2-$^{13}C_2$]perfluorodecyl)phosphate			Wellington Laboratories, Canada	>99%
[M+2] 6:2 monoPAP	Sodium 1H,1H,2H,2H-[1, 2-$^{13}C_2$]perfluorooctylphosphate			Wellington Laboratories, Canada	>99%
[M+2] 8:2 monoPAP	Sodium 1H,1H,2H,2H-[1, 2-$^{13}C_2$]perfluorodecylphosphate			Wellington Laboratories, Canada	>99%
[M+5] 6:2 FTOH	[1,1,2,2-D; 3-13C] 6-2 Fluorotelomer alcohol			DuPont USA	>97%
[M+5] 8:2 FTOH	[1,1,2,2-D; 3-13C] 8-2 Fluorotelomer alcohol			DuPont USA	>97%
[M+4] PFBA *	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid			Wellington Laboratories, Canada	>99%
[M+2] PFHxA *	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid			Wellington Laboratories, Canada	>99%
[M+4] PFOA *	Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid			Wellington Laboratories, Canada	>99%

* the internal standards were obtained from the supplier in a mixture named MPFCA-MXA.

Table S3.2: Instrumental method parameters for analysis of **PAPs and FTOHs** by LC-MS/MS (Method I)

<table>
<tr><td>Instrument:</td><td>Shimadzu ultra-high performance liquid chromatography (LC-30AD) interfaced with an AB Sciex Qtrap 5500 mass spectrometer. Operated in the negative ion multiple reaction monitoring mode.</td></tr>
<tr><td>Analytical Column:</td><td>Waters ACQUITY BEH C8 column (1.7 um, 100 mm x 2.1 mm)</td></tr>
<tr><td>Delay Column:</td><td>Kinetex EVO C18 (50 mm x 3 mm, 5 µm particle size)</td></tr>
<tr><td>Column Temperature:</td><td>40°C</td></tr>
<tr><td>Mobile Phases:</td><td>A: 0.1% ammonium hydroxide in water
B: methanol</td></tr>
<tr><td>Gradient Profile:</td><td>
<table>
<tr><th>Time (min)</th><th>Percentage A</th><th>Flow Rate (mL/min)</th></tr>
<tr><td>0.0</td><td>80</td><td>0.200</td></tr>
<tr><td>0.5</td><td>80</td><td>0.200</td></tr>
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<tr><td>6.0</td><td>0</td><td>0.200</td></tr>
<tr><td>8.0</td><td>0</td><td>0.200</td></tr>
<tr><td>8.1</td><td>80</td><td>0.200</td></tr>
<tr><td>9.0</td><td>80</td><td>0.200</td></tr>
</table>
</td></tr>
<tr><td>Injection Volume:</td><td>20 µL</td></tr>
<tr><td>Monitored Ion Transitions:</td><td>
<table>
<tr><th>Analytes</th><th>Ion Transitions</th><th>DP</th><th>Collision Energy</th><th>CXP</th></tr>
<tr><td>8:2 diPAP</td><td>989 > 97</td><td>-5</td><td>-112</td><td>-5</td></tr>
<tr><td>[M+4] 8:2 diPAP</td><td>993> 97</td><td>-5</td><td>-112</td><td>-5</td></tr>
<tr><td>6:2 diPAP</td><td>789 > 443</td><td>-55</td><td>-30</td><td>-19</td></tr>
<tr><td>[M+4] 6:2 diPAP</td><td>793> 445</td><td>-55</td><td>-30</td><td>-19</td></tr>
<tr><td>8:2 monoPAP</td><td>543 > 97</td><td>-70</td><td>-50</td><td>-15</td></tr>
<tr><td>[M+2] 8:2 monoPAP</td><td>545 > 97</td><td>-70</td><td>-50</td><td>-15</td></tr>
<tr><td>6:2 monoPAP</td><td>443 > 97</td><td>-40</td><td>-22</td><td>-5</td></tr>
<tr><td>[M+2] 6:2 monoPAP</td><td>445 > 97</td><td>-40</td><td>-22</td><td>-5</td></tr>
<tr><td>6:2 FTOH</td><td>363 > 303</td><td>-119</td><td>-15</td><td>-13</td></tr>
<tr><td>8:2 FTOH</td><td>463 > 403</td><td>-128</td><td>-15</td><td>-17</td></tr>
<tr><td>7:2 sFTOH</td><td>39 3> 219</td><td>-71</td><td>-21</td><td>-9</td></tr>
<tr><td>5:2 sFTOH</td><td>293 > 119</td><td>-33</td><td>-13</td><td>-12</td></tr>
<tr><td>[M+5] 8:2 FTOH</td><td>426 > 405</td><td>-128</td><td>-15</td><td>-17</td></tr>
</table>
</td></tr>
<tr><td>LC/MS/MS Analog Parameters:</td><td>Curtain Gas (CUR) = 30 Collision Gas (CAD) = 10
Ion Spray Voltage = -4500 Source Temperature (°C) = 350
Ion Source Gas 1 (GS1) = 55 Ion Source Gas 2 (GS2) = 50</td></tr>
<tr><td>Calibrations</td><td>Quantitation for PAPs was achieved with a 9-point linear regressed calibration curve (1/x weighted) spanning 0.01 to 50 ng/ml.

Quantitation for FTOHs was achieved with an 8-point linear regressed calibration curve (1/x weighted) spanning 2 to 80 ng/ml.</td></tr>
</table>

Table S3.3: Instrumental method parameters for analysis of fluorinated carboxylates by LC-MS/MS (Method II)

<table>
<tr><td>Instrument:</td><td>Shimadzu ultra-high performance liquid chromatography (LC-30AD) interfaced with an AB Sciex Qtrap 5500 mass spectrometer. Operated in the negative ion multiple reaction monitoring mode.</td></tr>
<tr><td>Analytical Column:</td><td>Agilent Zorbax RX-C8 (150 mm x 2.1 mm, 5 µm particle size)</td></tr>
<tr><td>Delay Column:</td><td>Kinetex EVO C18 (50 mm x 3 mm, 5 µm particle size)</td></tr>
<tr><td>Column Temperature:</td><td>40°C</td></tr>
<tr><td>Mobile Phases:</td><td>A: 0.15% acetic acid in water
B: 0.15% acetic acid in acetonitrile</td></tr>
<tr><td>Gradient Profile:</td><td><table>
<tr><th>Time (min)</th><th>Percentage A</th><th>Flow Rate (mL/min)</th></tr>
<tr><td>0.0</td><td>90</td><td>0.400</td></tr>
<tr><td>0.2</td><td>90</td><td>0.400</td></tr>
<tr><td>4.5</td><td>5</td><td>0.400</td></tr>
<tr><td>7.0</td><td>5</td><td>0.400</td></tr>
<tr><td>7.1</td><td>90</td><td>0.400</td></tr>
<tr><td>7.5</td><td>90</td><td>0.400</td></tr>
</table></td></tr>
<tr><td>Injection Volume:</td><td>10 µL</td></tr>
<tr><td>Monitored Ion Transitions:</td><td><table>
<tr><th>Analytes</th><th>Ion Transitions</th><th>DP</th><th>CE</th><th>CXP</th></tr>
<tr><td>PFBA</td><td>213 > 169</td><td>-20</td><td>-14</td><td>-7</td></tr>
<tr><td>[M+4] PFBA</td><td>217> 172</td><td>-20</td><td>-14</td><td>-7</td></tr>
<tr><td>PFPeA</td><td>263 > 219</td><td>-25</td><td>-12</td><td>-9</td></tr>
<tr><td>PFHxA</td><td>313 > 269</td><td>-45</td><td>-14</td><td>-10</td></tr>
<tr><td>[M+2] PFHxA</td><td>315 > 270</td><td>-45</td><td>-14</td><td>-10</td></tr>
<tr><td>PFHpA</td><td>363 > 319</td><td>-45</td><td>-14</td><td>-13</td></tr>
<tr><td>PFOA</td><td>413 > 369</td><td>-45</td><td>-16</td><td>-15</td></tr>
<tr><td>[M+4] PFOA</td><td>417 > 372</td><td>-45</td><td>-16</td><td>-15</td></tr>
<tr><td>8:2 FTUA</td><td>357 > 393</td><td>-60</td><td>-30</td><td>-17</td></tr>
<tr><td>5:3 Acid</td><td>341 > 237</td><td>-64</td><td>-19</td><td>-10</td></tr>
<tr><td>7:3 Acid</td><td>441 > 337</td><td>-35</td><td>-18</td><td>-15</td></tr>
</table></td></tr>
<tr><td>LC/MS/MS Analog Parameters:</td><td>Curtain Gas (CUR) = 30 Collision Gas (CAD) = 6
Ion Spray Voltage = -4500 Source Temperature (°C) = 550
Ion Source Gas 1 (GS1) = 50 Ion Source Gas 2 (GS2) = 50</td></tr>
<tr><td>Calibration</td><td>Quantitation was achieved with an 8-point linear regressed calibration curve (1/x weighted) spanning 0.1 to 20 ng/ml.</td></tr>
</table>

Table S3.4: Formation of 6:2 and 8:2 FTOH during solvent-enhanced hydrolysis of PAPs in live soil and autoclaved soil

	6:2 FTOH/ 6:2 monoPAP	8:2 FTOH/ 8:2 monoPAP	6:2 FTOH/ 6:2 diPAP	8:2 FTOH/ 8:2 diPAP
Live soil	88.7 ± 36.7%	59.6 ± 7.7%	5.43 ± 0.47%	5.09 ± 0.63%
Autoclaved soil	0.854 ± 0.479%	1.98 ± 0.26%	0 ± 0%	0 ± 0%

Table S3.5: Detection limits of target analytes

Analyte	LOD (ng/ml)	Analytes	LOD (ng/ml)
6:2 diPAP	0.001	PFBA	0.094
8:2 diPAP	0.005	PFPeA	0.042
6:2 monoPAP	0.005	PFHxA	0.039
8:2 monoPAP	0.004	PFHpA	0.019
6:2 FTOH	0.408	PFOA	0.045
8:2 FTOH	0.221	8:2 FTUA	0.007
5:2 sFTOH	0.078	5:3 Acid	1.034
7:2 sFTOH	0.007	7:3 Acid	0.313

Table S3.6: Immediate recoveries and hydrolysis of PAPs in different extraction solvents

Extraction solvent	**Immediate recoveries (%)**				**Hydrolysis of PAPs (% of PAP theoretical concentration)**							
					In soil extract (%)				**In spike solvent control (%)**			
	6:2 monoPAP	8:2 monoPAP	6:2 diPAP	8:2 diPAP	6:2 FTOH/ 6:2 monoPAP	8:2 FTOH/ 8:2 monoPAP	6:2 FTOH/ 6:2 diPAP	8:2 FTOH/ 8:2 diPAP	6:2 FTOH/ 6:2 monoPAP	8:2 FTOH/ 8:2 monoPAP	6:2 FTOH/ 6:2 diPAP	8:2 FTOH/ 8:2 diPAP
9:1 ACN/NaOH	24.3 ± 0.338	8.78 ± 2.39	29.4 ± 23.6	101 ± 23.8	25.4 ± 4.18	23.7 ± 0.456	3.05 ± 0.300	0	0	0	0	0
MeOH with 1% NH_4OH	5.97 ± 4.03	2.61 ± 0.29	93.3 ± 66.3	77.4 ± 27.7	9.8 ± 6.70	29.7 ± 4.70	2.69 ± 1.07	20.9 ± 3.15	1.57 ± 0.481	7.42 ± 0.848	0	5.20 ± 0.375
EA	1.08 ± 0.969	1.67 ± 2.44	17.1 ± 12.4	22.3 ± 7.5	23.3 ± 5.54	12.3 ± 1.02	0.530 ± 0.091	1.80 ± 0.299	1.75 ± 0.650	5.00 ± 0.528	0	0.781 ± 0.0530
MTBE	1.31 ± 0.342	0.0860 ± 0.149	14.4 ± 5.43	8.07 ± 1.89	48.9 ± 0.647	24.5 ± 2.30	1.82 ± 0.734	25.6 ± 11.7	3.83 ± 0.596	11.0 ± 5.57	0	12.9 ± 5.02
4:1 MTBE/HAc	0.291 ± 0.0750	0.943 ± 0.891	115± 6.14	99.3 ± 12.6	86.0 ± 37.1	59.5 ± 8.29	5.49 ± 0.252	5.17 ± 0.902	2.78 ± 0.473	3.32 ± 0.534	0	0.302 ± 0.0770
4:1 ACN/HAc	0.171 ± 0.104	1.16 ± 0.454	113 ± 4.27	99.9 ± 12.9	88.7 ± 36.7	59.6 ± 7.67	5.43 ± 0.473	5.09 ± 0.629	2.89 ± 0.458	3.23 ± 0.0450	0	0.0570 ± 0.0550

S3.1: Calculation of half-lives of 6:2 diPAP and 8:2 diPAP in the live soil

Assuming double first order in parallel (DFOP) degradation kinetics, then the regression equation is:

$$y = M0 \times g^{-tk_1} + M0 \times (1-g)^{-tk_2}$$

t: time (day)

y: % moles of 6:2 diPAP (or 8:2 diPAP) applied at day t

M0: % moles of 6:2 diPAP (or 8:2 diPAP) applied at day 0

k_1, k_2: rate constants

g: fraction of degradation occurring under rate constant k_1

	M0		k_1		k_2		g	
	Value	Standard error	Value	Standard error	Value	Standard error	Value	Standard error
6:2 diPAP	9.39e+01	1.54	9.06e-02	5.76e-04	9.30e-03	1.72e-02	7.33e-01	5.82e-02
8:2 diPAP	1.09e+02	8.24	7.46e-02	6.01e-02	2.34e-14	4.26e-03	4.65e-01	1.96e-01

DT_{50} calculations:

For 6:2 diPAP, when y = 0.5, t = 11.5

For 8:2 diPAP, when y = 0.5, t > 1000

Assuming simple first order (SFO) degradation kinetics, then the regression equation is:

$$y = M0 \cdot e^{-kt}$$

t: time (day)

y: % moles of 6:2 diPAP (or 8:2 diPAP) applied at day t

M0: % moles of 6:2 diPAP (or 8:2 diPAP) applied at day 0

k: rate constant

	M0		k_1	
	Value	Standard error	Value	Standard error
6:2 diPAP	8.94e+01	6.56	4.79e-02	2.77e-04
8:2 diPAP	9.22e+01	7.35	6.09e-03	2.18e-03

DT_{50} calculations:

For 6:2 diPAP, when y = 0.5, t = 14.5

For 8:2 diPAP, when y = 0.5, t =113.8

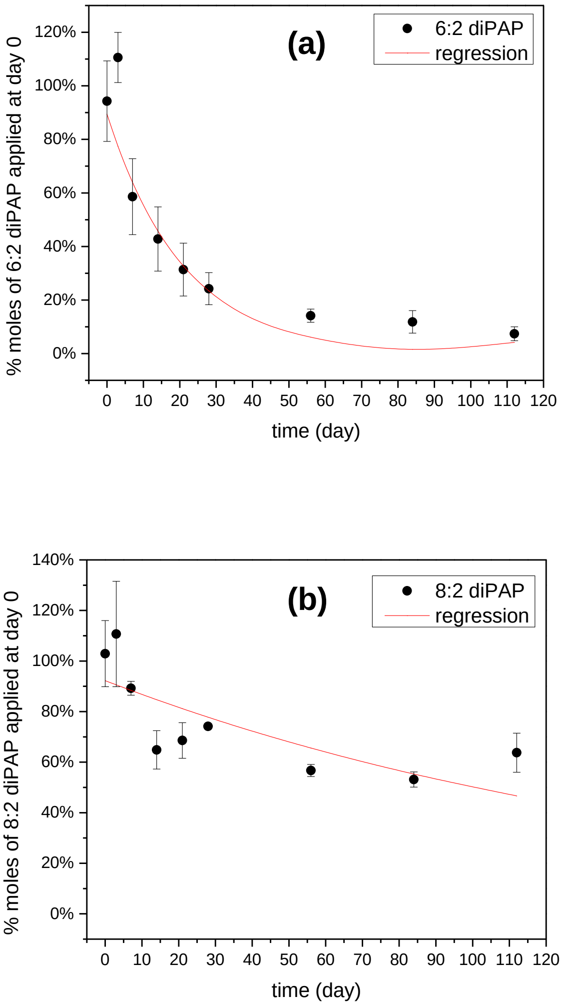


Figure S3.1: Time profiles of (a) 6:2 diPAP and (b) 8:2 diPAP in the live soil.

The solid lines are fitted curves of a simple first order (SFO) model.

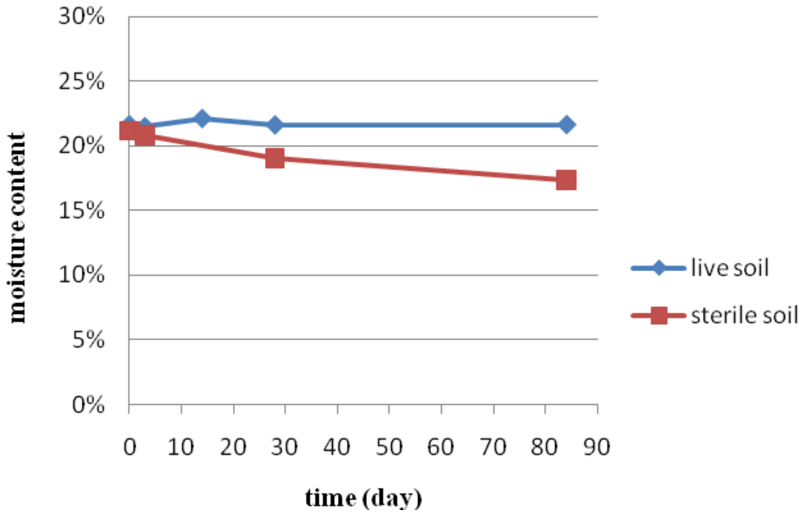


Figure S3.2: Moisture content of live soil and sterile soil throughout the incubation [calculated as (wet weight - dry weight)/(wet weight)×100%]. Some error bars are invisible since they are smaller than the symbol height.

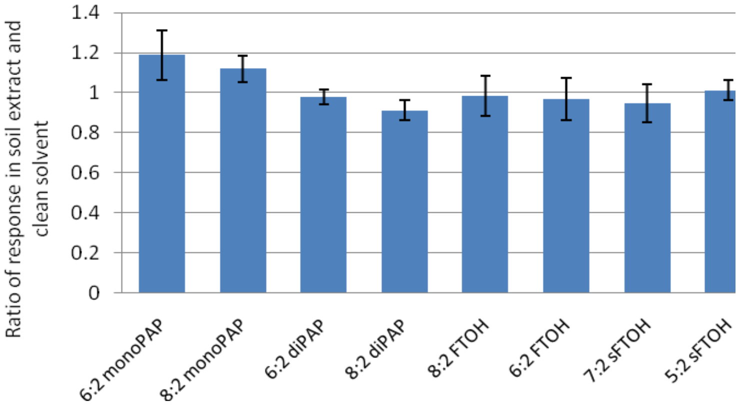


Figure S3.3: Response ratio of selected target compounds in soil extracts with respect to clean solvent (4:1 ACN/HAc) at a concentration of 2.5 ng/mL. A ratio of 1 represents a complete lack of matrix effect.

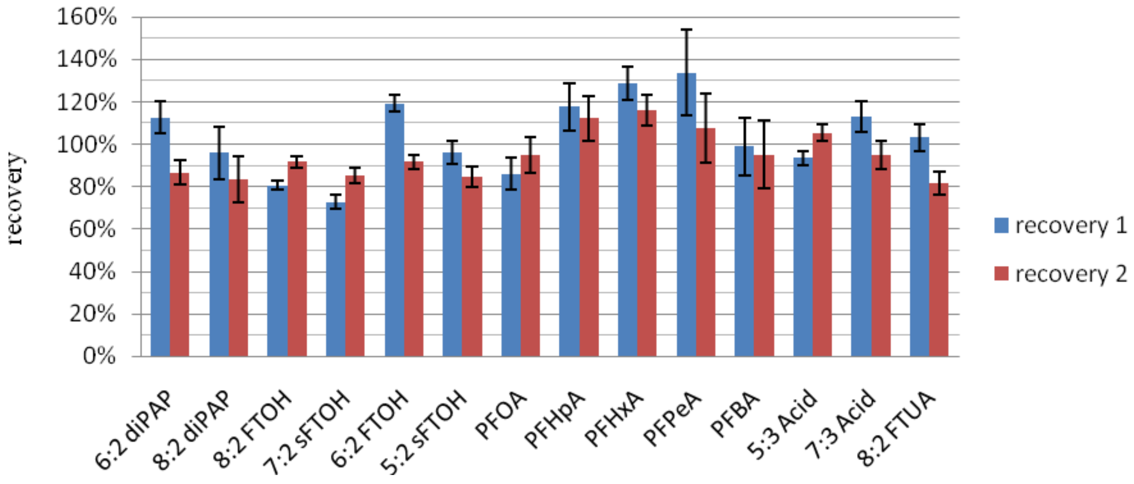
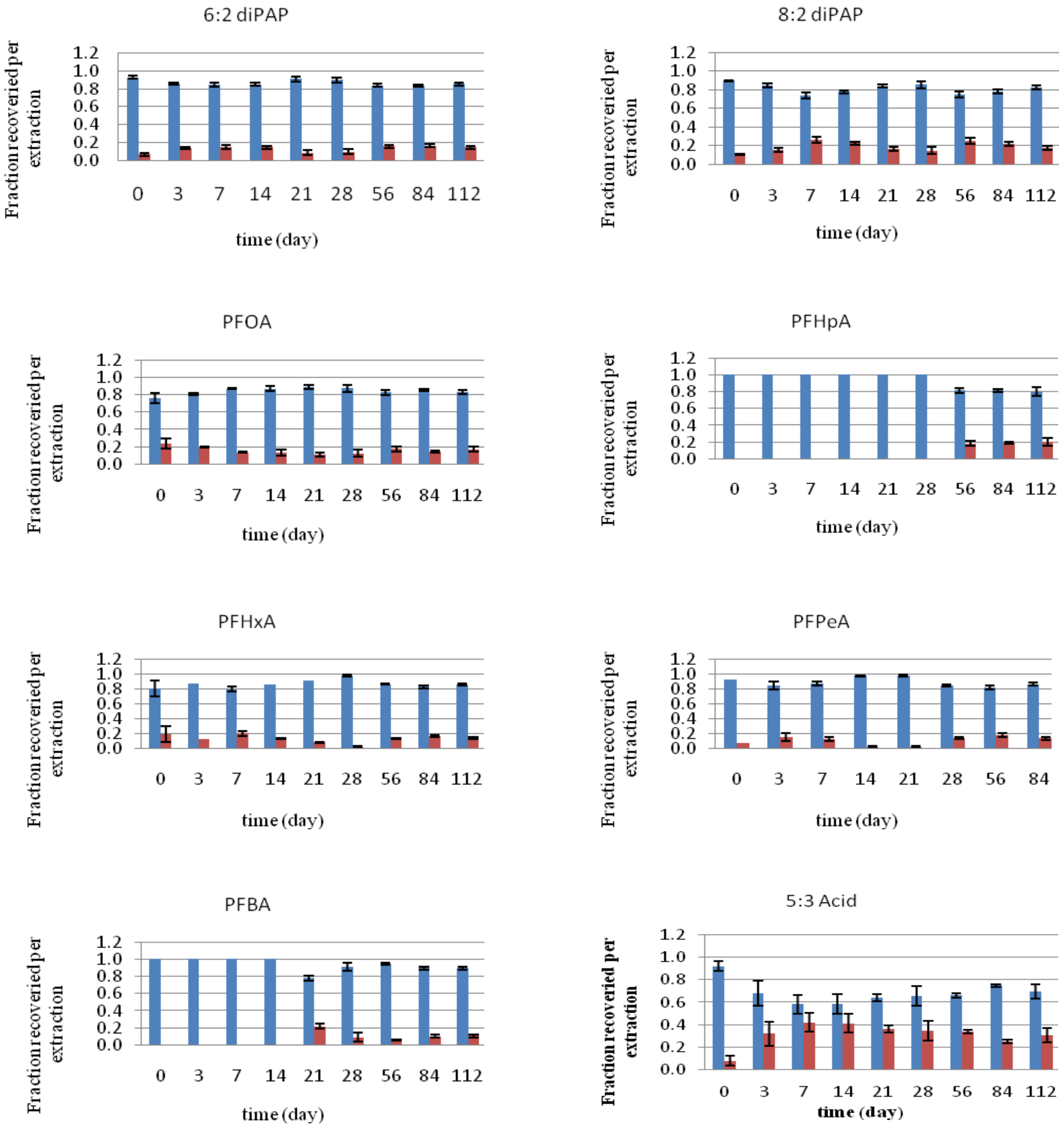


Figure S3.4: Immediate recoveries of parent compounds and degradation products. PAPs and FTOHs were extracted by 4:1 ACN/HAc. The other compounds were extracted by 9:1 ACN/NaOH. Recovery 1 was calculated as the measured concentration over the theoretical spiked concentration. Recovery 2 was calculated as the measured concentration over the measured concentration in spiked control.



6:2 diPAP

8:2 diPAP

PFOA

PFHpA

PFHxA

PFPeA

PFBA

5:3 Acid

Figure S3.5: Results of extraction efficiencies in each sequential extraction step. Blue bars represent the fraction recovered from the first extraction. Red bars present the fraction recovered from the second extraction. Some error bars are invisible.

CHAPTER IV. INVESTIGATION OF KNOWN AND UNKNOWN PERFLUOROALKYL AND POLYFLUOROALKYL SUBSTANCES (PFASs) IN MUNICIPAL BIOSOLIDS IN CANADA

4.1 Abstract

Land application of municipal biosolids is suggested to be one of the routes through which perfluoroalkyl and polyfluoroalkyl substances (PFASs) in the waste stream is released into the environment. However, there is little data available about the levels of PFASs in biosolids in Canada. In the present study, 12 biosolids samples were collected from four provinces in Canada to assess the levels of identifiable and unidentified PFASs. The identifiable PFASs included perfluoroalkyl carboxylic acids (PFCAs, C4-C14, 5:3 and 7:3 acids), perfluoroalkyl sulfonic acids (PFSAs, C4, C6, C8, and C10), fluorotelomer sulfonic acids (4:2 FTS, 6:2 FTS, and 8:2 FTS) fluorotelomer alcohols (8:2 FTOH, 6:2 FTOH, 7:2 sFTOH, and 5:2 sFTOH) and polyfluoroalkyl phosphate esters (8:2 diPAP, 6:2 diPAP, 8:2 monoPAP, and 6:2 monoPAP). The predominant PFASs in most of the samples were PFBS (4.66 - 48.1%), 5:3 acid (0 - 42.9%), 6:2 diPAP (2.21 – 26.5%), 8:2 FTOH (7.73 – 37.8%) and 6:2 FTOH (0 – 20.9%). The results suggested that the long-chain PFASs such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acids (PFOS) were no longer the most dominant PFASs in biosolids,

which might result from the phase-out of and restriction actions for these compounds. A persulfate oxidation based assay was applied to assess the levels of unidentified PFASs. Further optimization is required before the assay can be successfully applied.

4.2 Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) with unique properties have been widely used for over half a century in industrial applications and consumer products such as water-repellent coatings, stain-repellent fabrics, food contact papers, aqueous film-forming foams (AFFFs), lubricants, leveling agents, etc. (Moody and Field 2000, Rao and Baker 1994b, Banks et al. 2013). Recent years, PFASs have been found to be globally distributed in outdoor/indoor air and dust (Paul et al. 2008), various water bodies (Paul et al. 2008, Ding et al. 2012, Esparza et al. 2011), soil (Yoo et al. 2010), sediment (Benskin et al. 2012, Esparza et al. 2011, Higgins et al. 2005), biosolids (Sepulvado et al. 2011), wildlife (Houde et al. 2011), food products and humans (D'eon et al. 2009, Kubwabo et al. 2013). Many PFAS are environmentally persistent or can undergo limited transformations only to form to more persistent perfluoroalkyl acids (PFAAs). The PFASs containing long perfluoroalkyl chains are found to be bioaccumulative and toxic to animals in laboratories (U.S. EPA 2006b, UNEP 2006). Levels of PFAS in wastewater treatment plant (WWTP), effluents, and biosolids have been closely examined to obtain the information on the amount and types of PFAS used in household and industry. Such data are also crucial to understanding the input of PFASs into the downstream environment (rivers, land, and landfills) (Becker et al. 2008, Allred 2015, Myers et al. 2012, So et al. 2007, Lin et al. 2010). Almost every type of PFAS that has been targeted can be found in WWTP, effluents, and biosolids (Kunacheva et al. 2011, Chen et al. 2012, Venkatesan and Halden 2013, Zhang et al. 2013).

Biosolids refer to the solid portion of municipal wastewater that has been stabilized and reduced in volume. The use and disposal of wastewater biosolids in Canada include land application as fertilizers or supplements, incineration, and landfilling (U.S. EPA 1999). Land application is the preferred method as it returns abundant nutrients and organic materials to land at low costs. Although the use and disposal of wastewater biosolids are regulated in Canada, there is no regulation with respect to the levels of organic pollutants such as PFASs or other emerging contaminants. Therefore, disposal of biosolids through land application represents a probably major route by which PFASs are released to the environment. As plant bioavailability of PFASs has been demonstrated (Blaine et al. 2013, Blaine et al. 2014), the practice could lead to PFAS transfer to plants and eventually to plant consumers such as cattle and humans.

Although the levels of PFAS in biosolids in the USA have been examined in several studies (Sepulvado et al. 2011, Venkatesan and Halden

2013, Lindstrom et al. 2011, Long et al. 2013), the biosolids in Canada has been little examined (Gottschall et al. 2010). In addition, no study has been conducted to examine the levels of unidentified PFAS in biosolids. Recent studies have shown that there is significance presence of unknown PFASs in PFAS-containing products and environmental samples such as sediments and urban runoff. Weiner et al. (2013) found that only part of the total organic fluorine content in AFFFs can be attributed to identifiable PFASs (e.g., PFAAs) while a large portion remained unidentified. Yeung et al. (2013a) measured the concentrations of fourteen PFASs and total organic fluorine in Lake Ontario sediment samples. It was found that the identifiable PFAAs only accounted for 2% to 44% of the total organic fluorine in sediment core samples. In addition, Houtz and Sedlak (2012) found that urban runoff contained a high percentage of unidentified PFAS. Furthermore, it is known that non-fluorinated parts of many PFAS molecules can break down in WWTP and produce a range of degradation products, which are not often included in environmental monitoring studies (Liu and Avendaño 2013).

Therefore, the objective of this study was to quantify 28 selected PFASs and estimate the levels of the unidentified PFASs in the Canadian biosolids samples collected from four provinces. The data will contribute to understanding whether biosolids represent a significant source of PFAS to the environment in Canada.

4.3 Materials and methods

4.3.1 Chemicals

The following PFAS chemical standards were obtained and their names, molecular weights, CAS numbers, sources, and purities are listed in **Table S4.1**: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrA, PFTeA, 5:3 Acid, 7:3 Acid, PFBS, PFHxS, PFOS, PFDS, 4:2 FTS, 6:2 FTS, 8:2 FTS, 8:2 FTOH, 6:2 FTOH, 7:2 sFTOH, 5:2 sFTOH, 8:2 diPAP, 6:2 diPAP, 8:2 monoPAP and 6:2 monoPAP. The isotopically labeled internal standards used for chemical analysis are detailed in **Table S4.2**. HPLC-grade solvents including methanol (MeOH), acetonitrile (ACN), methyl tert-butyl ether (MTBE), tetrabutylammonium (TBA, 98%), certified sodium carbonate solution (1 N), and ammonium hydroxide (NH_4OH) were purchased from Fisher Scientific (Ottawa, ON).

4.3.2 Sample collection

The biosolids were collected from 12 WWTPs in four Canadian provinces in 2014 and 2015. The details of the biosolids were listed in **Table S4.3**. All of the samples were shipped on ice and stored at -20 °C until sample processing. The water content was measured based on moisture loss after oven dry at 103°C for 24 h. The water content was calculated as (wet weight - dry weight)/(wet weight) x 100%. Throughout the study, the weight of biosolids referred to the oven dry weight unless otherwise specified.

4.3.3 Biosolids extraction

Before sample processing, the biosolids samples were thawed at room temperature and then homogenized through manual or mechanical shaking to achieve consistency by visual inspection. The biosolids were extracted by an ion pairing method which was modified from the method described by Hansen et al. (2001). Briefly, about 5 g of biosolids sample was added into a 50-mL polypropylene centrifuge tube, and the following solutions were added in sequence: 1 mL of 0.5 M TBA solution (an ion-pairing agent), 2 mL of 0.25 M sodium carbonate buffer solution, and 10 mL of MTBE. Then the tube was subject to sonication at 40 °C for 30 min and mechanical shaking at 250 rpm for 30 min on a horizontal shaker. After the tube had been centrifuged at 4000 *g* for 20 min, the MTBE layer accumulating on the top of the solution was removed and transferred into a second 50 mL polypropylene tube. The extraction procedure was repeated two more times except that only MTBE was added. All three extracts were combined in the new polypropylene tube and evaporated to around 500 µL under a gentle nitrogen stream. The concentrated extract was then cleaned up using Envi-Carb black carbon. Briefly, 25 mg of Envi-Carb power was first rinsed with 1 mL of MeOH. The concentrated extract was added to the Envi-Carb in a 2-mL microcentrifuge tube, and then the slurry was vortexed for 60 s. After centrifugation at 10,000 *g* for 10 min, the supernatant was transferred to a new vial. The Envi-Carb was then rinsed with 0.5 mL of MeOH. The extract and rinsing solvent were combined and filtered through a 0.2 μm spin filter. Finally, the extract was diluted 10 times in MeOH and stored at -20 °C before chemical analysis.

4.3.4 TOP Assay

A total oxidizable precursor (TOP) assay developed by Houtz and Sedlak (2012) was applied to the biosolid extracts to evaluate the potential presence of unidentified PFASs. A similar procedure as the one described by Houtz et al. (2013) was used with minor modifications. In brief, the above-diluted extract was added to 2 mL microcentrifuge tube and evaporated to dryness under a gentle stream of nitrogen gas. The removal of the organic solvent is critical for allowing sufficient amount of the oxidant (persulfate) to react with PFASs. Then, the following solutions were added to the tube: 1.2 mL of de-ionized water, 656 μL of 175 mM potassium persulfate solution, and 57.4 μL of 5 M NaOH solution. The tube was then vortexed briefly and kept in a hot water bath at 85 °C for 6 hours. After the conclusion of the reactions, the samples were stored at -20 °C before chemical analysis. Before analysis, all samples were neutralized with 10 μL of 6 M HCl.

4.3.5 Analytical Methods

Twenty-eight PFASs were identified and quantified using the same LC-MS/MS system and analytical methods as in the previous study (Chapter III). PAPs and FTOHs were analyzed using the Method I (**Table S3.2** in the SI of

Chapter III) under a basic mobile phase, and other polyfluoroalkyl and perfluoroalkyl acids were quantified using the Method II (**Table S4.3** in the SI) under an acidic mobile phase. Compared to the Method II employed in Chapter III, more analytes were included for the analysis of biosolids, and the detailed parameters for these analytes can be found in **Table S4.3.** Prior to chemical analysis, all the samples were centrifuged at 10,000 *g* for 10 min to remove ultrafine particles and then spiked with corresponding internal standard mixtures. The information on the internal standards and calibration methods can be found in **Table S3.1-3.3** in the SI.

4.3.6 Quality Assurance

An immediate spike recovery test was performed using the biosolid C-BIOS to examine the effectiveness of the ion pairing extraction method. A mixture of target analytes in a MeOH stock solution was added into the C-BIOS slurry. The spiked concentrations of target analytes in C-BIOS ranged from 0.002 to1.59 nmol/g, which were at least one order of magnitude greater than those of background level in the biosolid. The same extraction procedures as described in **Section 4.3.3** were used. The biosolid without the addition of target analytes was also extracted to allow quantitation of the background PFAS levels. All samples were processed in triplicate, and all analytical results were reported as the average of the three values.

4.4 Results and discussion

4.4.1 Evaluation of extraction methods

Ideally, a spike recovery test would be conducted using a sample with no background interferences. Since all biosolid samples contain detectable levels of PFASs, the test was performed by spiking at the levels at least one order of magnitude higher than those of the background. The immediate recoveries were calculated as (concentration in spiked extract – background concentration) / (theoretical spiked concentration). The ion pairing method showed satisfactory recoveries (70 – 130%) for 21 out 28 target analytes as listed in **Table S4.4**. Three analytes had recoveries slightly outside the range: PFPeA at 155%, 8:2 FTOH at 133%, and 7:3 acid at 138%. The overall low recoveries for the PAPs, which ranged from 0.46 ± 0.2% (8:2 monoPAP) to 48.6 ± 4.87% (6:2 diPAP), were not unexpected. In our previous study (Chapter III), we have showed PAPs would undergo solvent-enhanced hydrolysis during solvent extraction of soil with microbial and/or enzymatic activities. For monoPAPs, none of the commonly used solvents could achieve satisfactory recoveries and most would lead to hydrolysis of 6:2 and 8:2 monoPAPs (Chapter III, Figure 3.1). For selected diPAP (e.g., such as 8:2 diPAP) limited hydrolysis would occur in MTBE solvent (Chapter III). Though biosolids are different from soil in chemical and biological compositions, low recoveries due to solvent-enhanced hydrolysis were highly likely given significant microbial activities in biosolids.

ACN and MeOH are the solvents that have been reported in the literature for the use of soil or biosolids extraction of PFASs. Sepulvado et al. (2011) used 99/1 (v/v) MeOH/ammonium hydroxide for biosolid extraction and reported satisfactory recoveries for 16 PFASs. We tested the method and found the method would not work for any biosolid samples we have obtained. The solvent mixture would extract a large amount of fatty and proteinaceous content out of biosolids. Those substances would pose as a sticky, oily mixture with crystalline materials forming at lower than the room temperature and did not allow further sample preparation steps including sample concentration and Envi-carb cleanup. ACN was also tested, but it posed the same problem. The ion pairing method that was originally developed for extracting PFAS from blood samples (Hansen et al. 2001) was shown in the current study be suitable for the extraction of biosolids. Nevertheless, the current study has also suggested it is unlikely to have a single method that would work for every PFAS compound because of the wide range of PFAS chemical structures and properties. If the goal were to recovery PAPs from biosolids, other extraction methods should be applied to prevent solvent-enhanced hydrolysis.

4.4.2 Concentrations of target PFASs in biosolids

In the 12 WWTP biosolids collected in Canada, the most abundant PFAS was PFBS, accounting for 48.1%, 39.8%, 45.9%, 35.8%, 42.6% and

36.7%, respectively, in N-BIOS, U-BIOS, Q-BIOS, F-BIOS, V-BIOS, and R-BIOS (**Figure 4.1 and 4.2)**. The concentrations of PFOS in the samples ranged from 0.001 to 0.006 nmol/g **(Figure 4.2)**, accounted for 0.43% to 4.49% of the total. The values were much lower than the previously reported PFOS levels in municipal biosolids in the USA, 80 – 219 ng/g (or 0.16 – 0.44 nmol/g) (Sepulvado et al. 2011). The significant difference in PFOS concentrations in biosolids may be due to the phase-out and regulatory actions towards long-chain PFAS including PFOS. In 2000 to 2002, 3M phased out the production of PFOS and perfluorooctane sulfonyl fluoride (POSF)-based chemicals and replaced with perfluorobutane sulfonyl fluoride (PBSF)-based ones, which would lead to PFBS. Moreover, in 2009, PFOS and its related chemicals were added to Annex B of the *Stockholm Convention on Persistent Organic Pollutants* (UNEP 2009). In the study by Sepulvado et al. (2011), the biosolids samples were collected in 2004 to 2007, which was almost ten years before the present study. It has been reported that levels of PFOS in many environmental compartments have been in decline since 2002 (Liu et al. 2014, Toms et al. 2014). In the meantime, the dominant PFASs existed in the environment have been found to transition from the long-chain to the short-chain compounds, like PFBS, which was among the most abundant PFAS in the study. In addition, the proportions of all the detected perfluoroalkyl carboxylic acids (PFCAs, C4-C14) in the 12 biosolids samples were only in the range of 3.05% (N-BIOS) to 20.3% (P-

BIOS) of the total, and the concentrations ranged from 0.008 to 0.016 nmol/g. The findings suggested that PFCAs were not the predominant PFAS components in the biosolids samples.

5:3 Acid was another primary PFAS found in L-BIOS (36.8%), S-BIOS (42.9%), and C-BIOS (26.1%). 5:3 Acid has been little targeted in environmental monitoring studies. The only recognized source is from biotransformation of 6:2 FTOH and other fluorotelomer-based compounds with 6:2 perfluoroethyl chain (such as 6:2 diPAP). In fact, the significant presence of fluorotelomer-based compounds can be seen in **Figure 4.1 and 4.2**. Total fluorotelomer-based compounds and their characteristics degradation products (except for PFCAs and PFSAs) accounted for more than 50% of PFAS in multiple biosolid samples, such as CO-BIOS, L-BIOS, S-BIOS, and C-BIOS. In CO-BIOS and P-BIOS 8:2 FTOH was found to be the most abundant PFAS, constituting 24.8% and 14.5%, respectively, of the total. Similarly, the dominant PFAS in E-BIOS was 6:2 FTOH, accounting for 20.9% of the total. FTOHs are not directly used in consumer products, but they could appear as impurities (Dinglasan-Panlilio and Mabury 2006). Very importantly, FTOHs are biotransformation intermediates of many fluorotelomer-based compounds with hydrolysable functional groups, such as PAPs (Lee et al. 2010, Lee et al. 2013, D'eon and Mabury 2007), fluorotelomer stearate esters (Dasu et al. 2012), and even fluorotelomer-based polymers (Russell et al. 2008). 6:2 and 8:2 diPAPs were also found to account for high proportions

(6.98% - 36.3%) in all the samples compared to the other analytes. Since the method recoveries of PAPs were much lower than 100%, the actual concentrations of PAPs in the biosolids are likely to be at much higher than the measured concentrations. Other fluorotelomer-based compounds were less abundant than the above compounds. 5:2 sFTOH was below detection in all the samples, which can be attributed to its volatility. 4:2 FTS was not observed in any of these samples while the concentrations of 6:2 FTS and 8:2 FTS were very low, less than 1.10% of the total PFAS. FTS are common components in fluorotelomer-based AFFFs and have been found to occur at high levels in AFFF-impacted ground waters (Schultz et al. 2004).

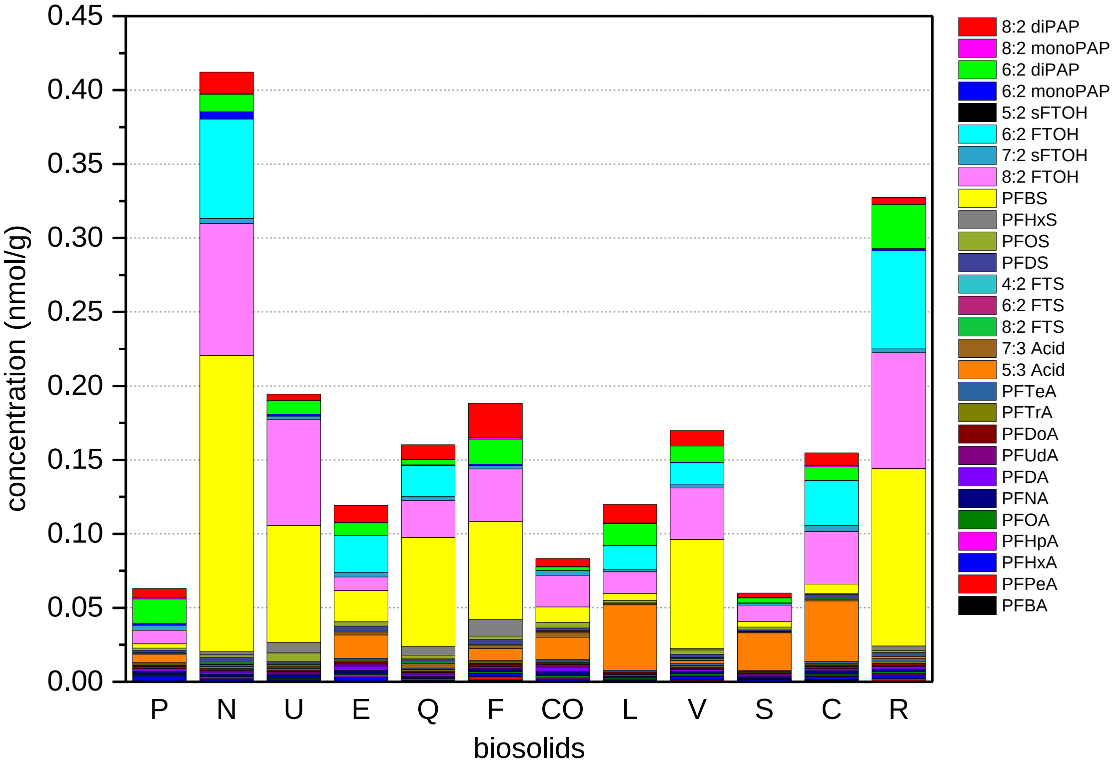


Figure 4.1: Compositions of PFASs in different biosolids

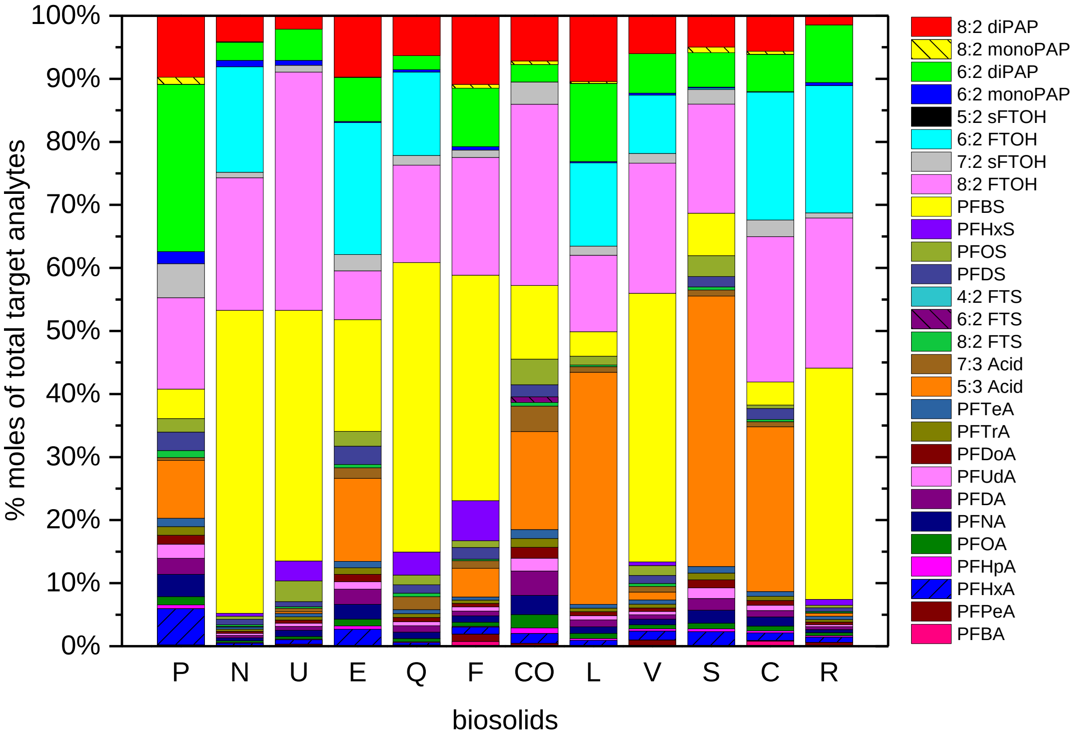


Figure 4.2: The relative abundance (mole-based) of each PFAS in 12 biosolid samples. The percentages were calculated as the concentration of certain PFAS over the total concentration of all the detected PFASs.

4.4.2 Unidentified PFASs in biosolids

The results of oxidative treatment of biosolid extract performed under the TOP assay were shown in **Figure 4.3**. The results did not meet the expectation that higher levels of PFCAs would occur after the oxidative treatment. As illustrated in **Figure 4.3**, only the concentrations of PFBA increased in all the samples tested. In C-BIOS, P-BIOS, L-BIOS, and V-BIOS, the concentration of PFPeA also increased slightly after the oxidation. In P-

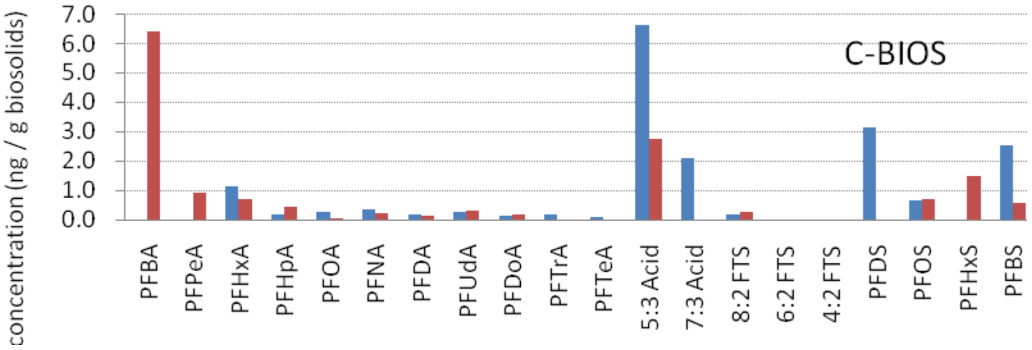
BIOS and R-BIOS, no PFPeA was observed either before or after the oxidative treatment. Concentrations of longer chained PFCAs generally decreased after the oxidation rather than increased. It was also not expected that the concentration 5:3 acid even increased after oxidative treatment in a few biosolids. 5:3 Acid contains three non-fluorinated carbon and is hypothesized to be oxidizable by persulfate. We suspected that two causes contributed to the higher 5:3 acid levels: (1) 5:3 acid was not efficiently oxidized during the assay, and (2) the high pH condition led to dissociation of "5:3 acid + organic materials complex" to increase detectable 5:3 acid as observed in the past study (Liu et al. 2010).

The TOP assay relies on the oxidative power of hydroxyl and sulfate radicals (Houtz and Sedlak 2012). Under basic pH and high temperature, persulfate ($S_2O_8^{2-}$) would undergo thermolysis first to produce sulfate radicals as shown by the Reaction 1. At pH values above 12, the sulfate radicals are quickly converted to hydroxyl radicals ($^{\bullet}OH$) according to Reaction 2. Hydroxyl radicals were believed to the major oxidant (Houtz and Sedlak 2012) though the role of sulfate radicals cannot be entirely dismissed.

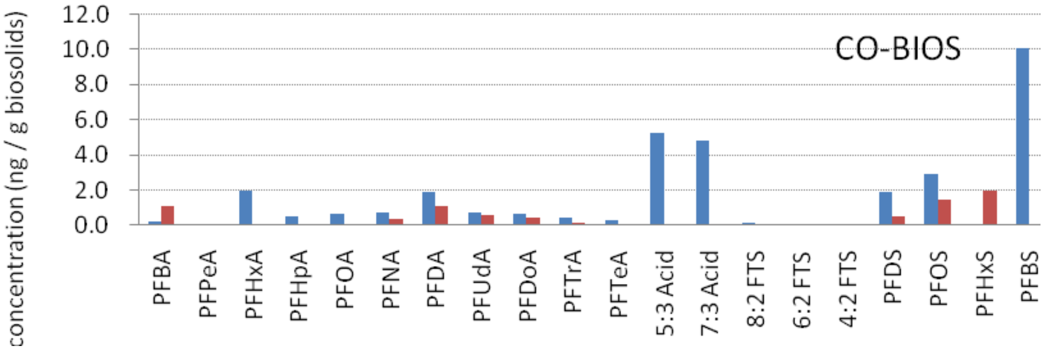
$$S_2O_8^{2-} + \text{heat} \rightarrow 2SO_4^{-\bullet}$$ **(Reaction 1)**

$$SO_4^{-\bullet} + OH^- \rightarrow SO_4^{2-} + {}^{\bullet}OH$$ **(Reaction 2)**

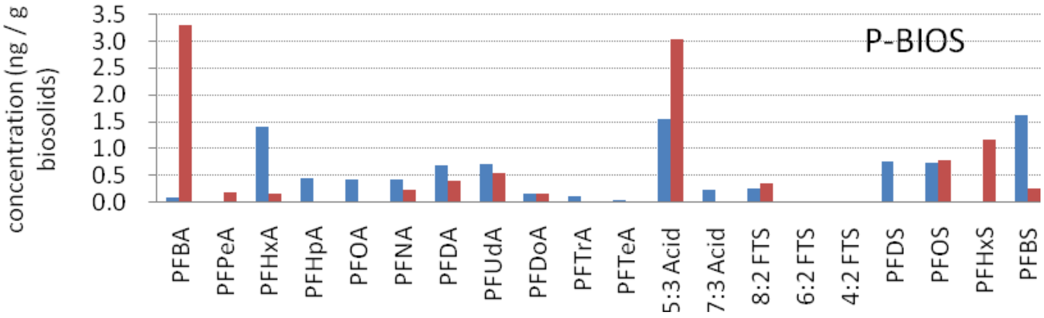
(a)


concentration (ng / g biosolids)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
C-BIOS
PFBA
PFPeA
PFHxA
PFHpA
PFOA
PFNA
PFDA
PFUdA
PFDoA
PFTrA
PFTeA
5:3 Acid
7:3 Acid
8:2 FTS
6:2 FTS
4:2 FTS
PFDS
PFOS
PFHxS
PFBS

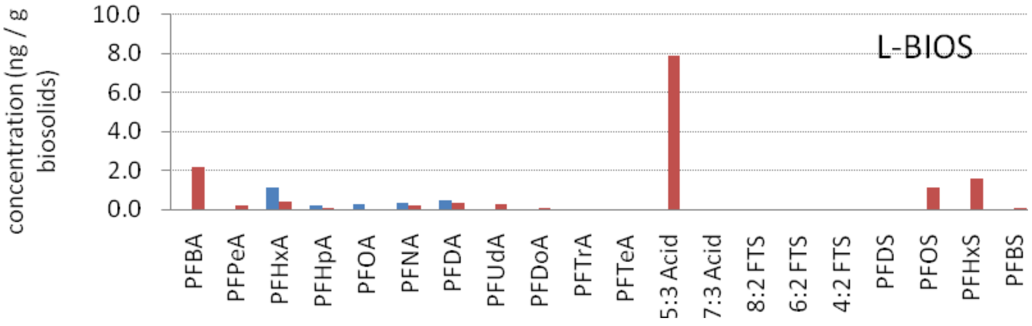
(b)


concentration (ng / g biosolids)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
CO-BIOS
PFBA
PFPeA
PFHxA
PFHpA
PFOA
PFNA
PFDA
PFUdA
PFDoA
PFTrA
PFTeA
5:3 Acid
7:3 Acid
8:2 FTS
6:2 FTS
4:2 FTS
PFDS
PFOS
PFHxS
PFBS

(c)


concentration (ng / g biosolids)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P-BIOS
PFBA
PFPeA
PFHxA
PFHpA
PFOA
PFNA
PFDA
PFUdA
PFDoA
PFTrA
PFTeA
5:3 Acid
7:3 Acid
8:2 FTS
6:2 FTS
4:2 FTS
PFDS
PFOS
PFHxS
PFBS

(d)


concentration (ng / g biosolids)
10.0
8.0
6.0
4.0
2.0
0.0
L-BIOS
PFBA
PFPeA
PFHxA
PFHpA
PFOA
PFNA
PFDA
PFUdA
PFDoA
PFTrA
PFTeA
5:3 Acid
7:3 Acid
8:2 FTS
6:2 FTS
4:2 FTS
PFDS
PFOS
PFHxS
PFBS

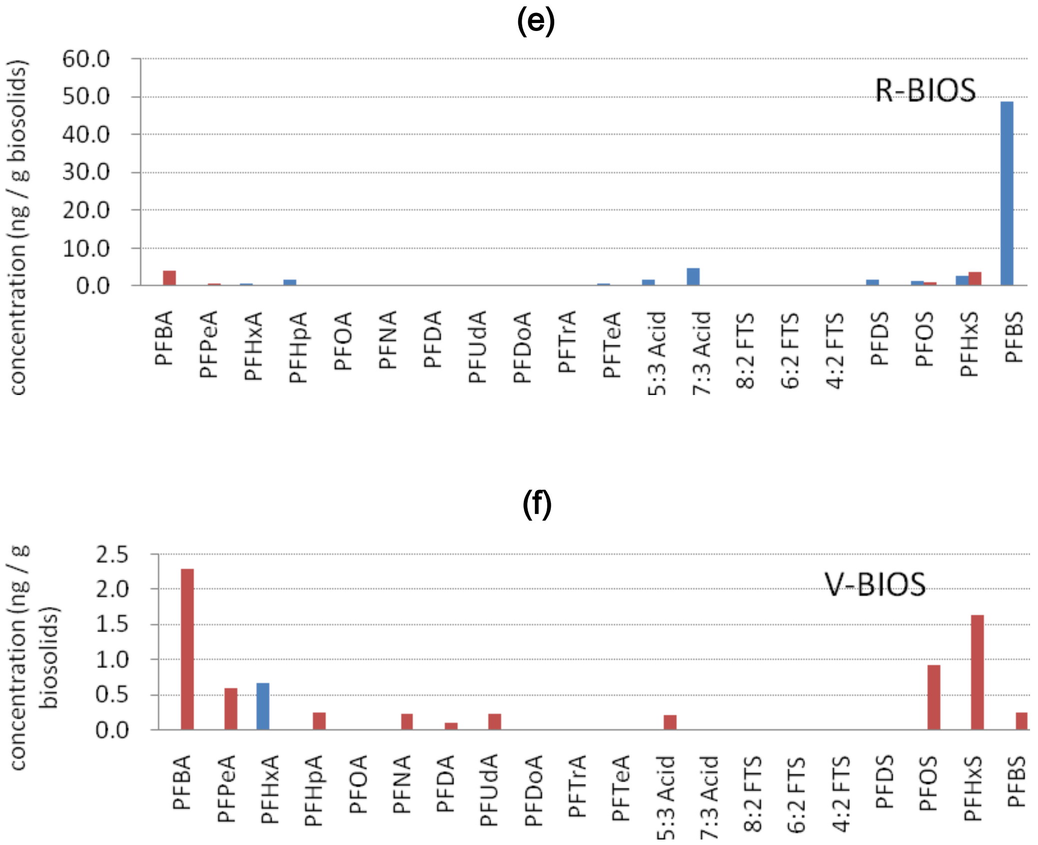


Figure 4.3 (a-f): Concentration of detected PFASs in biosolids before and after oxidative treatment. Blue bars represent the levels of PFASs before the oxidation, and red bars represent levels of PFASs after oxidation.

In our assay, after the samples were kept at 85 °C for 6 hours, the samples were cooled down and later on stored at -20 °C without HCl neutralization. It is likely during the times when samples were not frozen, the radicals still have residual oxidative powers at the elevated pH and still were attacking PFAS. Though at the optimized conditions (pH 12, 85 °C, 6 hours), perfluoroalkyl chains were largely stable against such oxidation, prolonged exposure of perfluoroalkyl chains to the oxidants can lead to their significant breakdown. For instance, persulfate oxidation at 85 °C for 30 h was found to result in decomposition of PFOA and 43.6% of its mineralization (Liu et al. 2012). Therefore, in future studies the assay procedures will be modified by conducting HCl neutralization at the end of the 6-hour reaction. In addition, samples will be analyzed as soon as the TOP assay is terminated to minimize potential decay of PFASs.

4.5 Future study

This study has provided preliminary data regarding the concentrations of PFASs in Canadian biosolids. The predominant PFASs in most of the samples were PFBS and fluorotelomer-based compounds rather than PFOS, which was the primary compound reported in a previous study on the biosolids in the U.S. (Sepulvado et al. 2011). The trend of increasing PFBS and decreasing PFOS may be caused by the phase-out and regulatory actions towards the long chain PFASs. It might also suggest that different PFAS-containing products are used in Canada from those in the U.S.

As for the unidentified PFAS, an improved TOP assay or other alternative method, such as measurement of total organic fluorine content should be tested (Yeung et al. 2013b). The environmental implications of unidentified PFASs are yet to be understood.

4.6 Supplementary information for Chapter IV

Table S4.1: Perfluoroalkyl and poly fluoroalkyl substances used in this study

Acronym	Accurate	Chemical Formula	CAS	Source	Purity
6:2 diPAP	789.982326	$(O)P(OH)(OCH_2CH_2C_6F_{13})_2$	57677-95-9	Toronto Research Chemicals, Canada	97%
8:2 diPAP	989.969549	$(O)P(OH)(OCH_2CH_2C_8F_{17})_2$	678-41-1		97%
6:2 monoPAP	443.979612	$(O)P(OH)_2(OCH_2CH_2C_6F_{13})$	57678-01-0	University of Toronto, Canada	97%
8:2 monoPAP	543.973224	$(O)P(OH)_2(OCH_2CH_2C_8F_{17})$	57678-03-2		97%
6:2 FTOH	364.013279	$C_6F_{13}CH_2CH_2OH$	647-42-7	Sigma-Aldrich, USA	97%
8:2 FTOH	464.006891	$C_8F_{17}CH_2CH_2OH$	678-39-7	Synquest, USA	97%
5:2 sFTOH	314.016473	$C_5F_{11}CH(OH)CH_3$	914637-05-1	DuPont, USA	99%
7:2 sFTOH	414.010085	$C_7F_{15}CH(OH)CH_3$	24015-83-7		99%
5:3 Acid	342.011388	$C_5F_{11}CH_2CH_2COOH$	914637-49-3		99%
7:3 Acid	442.005000	$C_7F_{15}CH_2CH_2COOH$	812-70-4		99%
PFBA *	218.286476	C_3F_7COOH	375-22-4	Wellington Laboratories, Canada	99%
PFPeA *	263.983282	C_4F_9COOH	2706-90-3		99%
PFHxA *	318.280088	$C_5F_{11}COOH$	307-24-4		99%
PFHpA *	363.976894	$C_6F_{13}COOH$	375-85-9		99%
PFOA *	418.273700	$C_7F_{15}COOH$	335-67-1		99%
PFNA *	463.970506	$C_8F_{17}COOH$	375-95-1		99%
PFDA *	518.267312	$C_9F_{19}COOH$	335-76-2		99%
PFUdA *	563.964118	$C_{10}F_{21}COOH$	2058-94-8		99%
PFDoA*	618.260924	$C_{11}F_{23}COOH$	307-55-1		99%
PFTrA *	663.957730	$C_{12}F_{25}COOH$	72629-94-8		99%
PFTeA *	718.254536	$C_{13}F_{27}COOH$	376-06-7		99%
PFBS *	299.950269	$C_4F_9SO_3H$	375-73-5		99%
PFHxS *	399.943881	$C_6F_{13}SO_3H$	355-46-4		99%
PFOS *	499.937493	$C_8F_{17}SO_3H$	1763-23-1		99%
PFDS *	599.931105	$C_{10}F_{21}SO_3H$	335-77-3		99%
4:2 FTS	327.981569	$C_4F_9CH_2CH_2SO_3H$	757124-72-4		99%
6:2 FTS	427.975181	$C_6F_{13}CH_2CH_2SO_3H$	27619-97-2		99%
8:2 FTS	527.968793	$C_8F_{17}CH_2CH_2SO_3H$	39108-34-4		99%

* Obtained from the supplier in a mixture named PFCA-MXB.

Table S4.2: Isotopically labeled internal standards used in this study

Acronym	Chemical Name	Source	Purity
[M+4] 6:2 diPAP	Sodium bis(1H,1H,2H,2H-[1, 2-$^{13}C_2$]perfluorooctyl)phosphate	Wellington Laboratories, Canada	>99%
[M+4] 8:2 diPAP	Sodium bis(1H,1H,2H,2H-[1, 2-$^{13}C_2$]perfluorodecyl)phosphate		>99%
[M+2] 6:2 monoPAP	Sodium 1H,1H,2H,2H-[1, 2-$^{13}C_2$]perfluorooctylphosphate		>99%
[M+2] 8:2 monoPAP	Sodium 1H,1H,2H,2H-[1, 2-$^{13}C_2$]perfluorodecylphosphate		>99%
[M+5] 6:2 FTOH	[1,1,2,2-D; 3-13C] 6-2 Fluorotelomer alcohol	DuPont USA	>97%
[M+5] 8:2 FTOH	[1,1,2,2-D; 3-13C] 8-2 Fluorotelomer alcohol		>97%
[M+4] PFBA *	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	Wellington Laboratories, Canada	>99%
[M+2] PFHxA *	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid		>99%
[M+4] PFOA *	Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid		>99%
[M+5] PFNA *	Perfluoro-n-[1,2,3,4,5-$^{13}C5_2$]nonanoic acid		>99%
[M+2] PFDA *	Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid		>99%
[M+2] PFUdA *	Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid		>99%
[M+2] PFDoA *	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid		>99%
[M+4] PFHxS *	Sodium perfluoro-1-[$^{18}O_2$]hexanoic acid		>99%
[M+4] PFOS *	Sodium perfluoro-1-[[1,2,3,4-$^{13}C_4$]octanesulfonate		>99%

* the internal standards were obtained from the supplier in a mixture named MPFCA-MXA.

Table S4.3: Details of WWTP biosolids samples

Sample ID	WWTP Location	Year of Collection	Water Content
E-BIOS	British Columbia, Canada	2015	83.9% ± 0.5%
N-BIOS	British Columbia, Canada	2015	72.4% ± 0.9%
Q-BIOS	Ontario, Canada	2015	72.7% ± 0.2%
F-BIOS	New Brunswick, Canada	2015	70.1% ± 0.3%
U-BIOS	Quebec, Canada	2015	69.6% ± 1.1%
P-BIOS	Quebec, Canada	2014	51.2% ± 0.6%
CO-BIOS	Quebec, Canada	2014	74.7% ± 0.1%
L-BIOS	Quebec, Canada	2014	79.9% ± 0.2%
V-BIOS	Quebec, Canada	2014	65.9% ± 0.1%
S-BIOS	Quebec, Canada	2014	82.4% ± 0.7%
C-BIOS	Quebec, Canada	2014	75.5% ± 1.2%
R-BIOS	Quebec, Canada	2014	51.3% ± 0.0%

Table S4.4: Instrumental method parameters for analysis of polyfluoroalkyl and perfluoroalkyl acids by LC-MS/MS **(Method II)**

<table>
<tr><td>Instrument:</td><td>Shimadzu ultra-high performance liquid chromatography (LC-30AD) interfaced with an AB Sciex Qtrap 5500 mass spectrometer. Operated in the negative ion multiple reaction monitoring mode.</td></tr>
<tr><td>Analytical Column:</td><td>Agilent Zorbax RX-C8 (150 mm x 2.1 mm, 5 µm particle size)</td></tr>
<tr><td>Delay Column:</td><td>Kinetex EVO C18 (50 mm x 3 mm, 5 µm particle size)</td></tr>
<tr><td>Column Temperature:</td><td>40°C</td></tr>
<tr><td>Mobile Phases:</td><td>A: 0.15% acetic acid in water
B: 0.15% acetic acid in acetonitrile</td></tr>
<tr><td>Gradient Profile:</td><td>
<table>
<tr><th>Time (min)</th><th>Percentage A</th><th>Flow Rate(mL/min)</th></tr>
<tr><td>0.0</td><td>90</td><td>0.400</td></tr>
<tr><td>0.2</td><td>90</td><td>0.400</td></tr>
<tr><td>4.5</td><td>5</td><td>0.400</td></tr>
<tr><td>7.0</td><td>5</td><td>0.400</td></tr>
<tr><td>7.1</td><td>90</td><td>0.400</td></tr>
<tr><td>7.5</td><td>90</td><td>0.400</td></tr>
</table>
</td></tr>
<tr><td>Injection Volume:</td><td>10 µL</td></tr>
<tr><td>Monitored Ion Transitions:</td><td>
<table>
<tr><th>Analytes</th><th>Ion Transitions</th><th>DP</th><th>CE</th><th>CXP</th><th>Internal Standard</th></tr>
<tr><td>PFBA</td><td>213 > 169</td><td>-20</td><td>-14</td><td>-7</td><td>[M+4] PFBA</td></tr>
<tr><td>PFPeA</td><td>263 > 219</td><td>-25</td><td>-12</td><td>-9</td><td>[M+2] PFHxA</td></tr>
<tr><td>PFHxA</td><td>313 > 269</td><td>-45</td><td>-14</td><td>-10</td><td>[M+2] PFHxA</td></tr>
<tr><td>PFHpA</td><td>363 > 319</td><td>-45</td><td>-14</td><td>-13</td><td>[M+4] PFOA</td></tr>
<tr><td>5:3 Acid</td><td>341 > 237</td><td>-64</td><td>-19</td><td>-10</td><td>[M+4] PFOA</td></tr>
<tr><td>PFOA</td><td>413 > 369</td><td>-45</td><td>-16</td><td>-15</td><td>[M+4] PFOA</td></tr>
<tr><td>8:2 FTUA</td><td>357 > 393</td><td>-60</td><td>-30</td><td>-17</td><td>[M+4] PFOA</td></tr>
<tr><td>7:3 Acid</td><td>441 > 337</td><td>-35</td><td>-18</td><td>-15</td><td>[M+4] PFOA</td></tr>
<tr><td>PFNA</td><td>463 > 419</td><td>-30</td><td>-16</td><td>-17</td><td>[M+2] PFDA</td></tr>
<tr><td>PFDA</td><td>513 > 469</td><td>-60</td><td>-16</td><td>-19</td><td>[M+2] PFDA</td></tr>
<tr><td>PFUdA</td><td>563 > 519</td><td>-55</td><td>-18</td><td>-21</td><td>[M+2] PFUdA</td></tr>
<tr><td>PFDoA</td><td>613 > 569</td><td>-80</td><td>-18</td><td>-25</td><td>[M+2] PFDoA</td></tr>
<tr><td>PFTrA</td><td>663 > 619</td><td>-75</td><td>-18</td><td>-27</td><td>[M+2] PFDoA</td></tr>
<tr><td>PFTeA</td><td>713 > 669</td><td>-75</td><td>-22</td><td>-29</td><td>[M+2] PFDoA</td></tr>
<tr><td>PFBS</td><td>299 > 80</td><td>-90</td><td>-70</td><td>-5</td><td>[M+4] PFHxS</td></tr>
<tr><td>PFHxS</td><td>399 > 80</td><td>-45</td><td>-65</td><td>-13</td><td>[M+4] PFHxS</td></tr>
<tr><td>PFOS</td><td>499 > 80</td><td>-55</td><td>-98</td><td>-13</td><td>[M+4] PFOS</td></tr>
<tr><td>PFDS</td><td>599 > 80</td><td>-60</td><td>-100</td><td>-13</td><td>[M+4] PFOS</td></tr>
<tr><td>4:2 FTS</td><td>327 > 307</td><td>-62</td><td>-27</td><td>-13</td><td>[M+4] PFHxS</td></tr>
<tr><td>6:2 FTS</td><td>427 > 407</td><td>-62</td><td>-35</td><td>-17</td><td>[M+4] PFOS</td></tr>
<tr><td>8:2 FTS</td><td>527 > 507</td><td>-120</td><td>-39</td><td>-22</td><td>[M+4] PFOS</td></tr>
</table>
</td></tr>
<tr><td>LC/MS/MS Analog Parameters:</td><td>Curtain Gas (CUR) = 30
Collision Gas (CAD) = 6
Ion Spray Voltage = -4500
Source Temperature (°C) = 550
Ion Source Gas 1 (GS1) = 50
Ion Source Gas 2 (GS2) = 50</td></tr>
<tr><td>Calibration</td><td>Quantitation was achieved with an 8-point linear regressed calibration curve (1/x weighted) spanning 0.1 to 20 ng/ml.</td></tr>
</table>

Table S4.5: Immediate recoveries of target analytes using C-BIOS

Analyte	Recovery	Standard Deviation	Analyte	Recovery	Standard Deviation
PFBA *	75.4%	11.4%	4:2 FTS	147%	18.5%
PFPeA *	155%	48.8%	6:2 FTS	94.4%	15.1%
PFHxA *	125%	14.9%	8:2 FTS	103%	9.15%
PFHpA *	78.8%	24.3%	6:2 FTOH	114%	9.57%
PFOA *	94.7%	2.53%	8:2 FTOH	133%	15.6%
PFNA *	97.5%	11.3%	5:2 sFTOH	118%	9.38%
PFDA *	98.8%	10.5%	7:2 sFTOH	84.5%	13.0%
PFUdA *	88.0%	9.62%	5:3 Acid	79.6%	6.67%
PFDoA*	90.2%	10.4%	7:3 Acid	138%	13.6%
PFTrA *	153%	15.1%	6:2 diPAP	48.6%	4.87%
PFTeA *	124%	31.3%	8:2 diPAP	7.27%	1.41%
PFBS *	151%	35.8%	6:2 monoPAP	1.83%	0.41%
PFHxS *	73.9%	22.0%	8:2 monoPAP	0.46%	0.20%
PFOS *	76.4%	6.67%			
PFDS *	90.8%	9.28%			

Table S4.6: Detection limits of target analytes

Analyte	LOD (ng/mL)	Analyte	LOD (ng/mL)
6:2 diPAP	0.001	PFBA	0.094
8:2 diPAP	0.005	PFPeA	0.042
6:2 monoPAP	0.005	PFHxA	0.040
8:2 monoPAP	0.004	PFHpA	0.019
6:2 FTOH	0.408	PFOA	0.045
8:2 FTOH	0.221	PFNA	0.054
5:2 sFTOH	0.078	PFDA	0.028
7:2 sFTOH	0.007	PFUdA	0.030
5:3 Acid	1.034	PFDoA	0.035
7:3 Acid	0.313	PFTrA	0.118
PFBS	0.008	PFTeA	0.004
PFHxS	0.005	4:2 FTS	0.059
PFOS	0.006	6:2 FTS	0.124
PFDS	0.005	8:2 FTS	0.172

CHAPTER V. SUMMARY AND CONCLUSIONS

The thesis aimed to elucidate the environmental fate of an important class of PFAS compounds, namely PAPs, in aerobic soil and to estimate the potential input of PFAS into soil from municipal biosolids.

In Chapter III, a more efficient extraction method of diPAP from soil was developed and the solvent-induced hydrolysis of PAPs was investigated. The newly developed extraction method was applied to estimate the half-lives of 6:2 diPAP and 8:2 diPAP in soil and to assess the significance of PAPs as precursors of PFCAs. It was evident in this study that:

- Extensive hydrolysis of monoPAPs would occur no matter what solvent was used for soil extraction of these compounds, which meant it was of a great challenge to reliably measure the levels of monoPAPs in soil or other types of environmental samples with microbial or enzymatic activities.

- This is the first study to report the half-lives of PAPs in soil environment, which revealed the recalcitrance or the lack of it for PAPs in soil.

- An approximate linear relationship was found between the molecular weights of eleven PFAS compounds and their half-lives in soil. Thus, molecular weights may be a good indicator to predict the persistence of low-molecular-weight PFASs if they are biodegradable.

In Chapter VI, a modified MTBE ion pairing method was applied to measure the concentrations of 28 selected PFASs in municipal biosolids collected in Canada. It was demonstrated that:

- The predominant PFASs in most of the biosolids sample were PFBS, 5:3 acid, diPAPs and 8:2 FTOHs rather than PFOS reported in previous studies, which indicated that the phase-out actions of PFOS and related chemicals has a direct impact of PFAS compositions in environmental media.
- To further understand the environmental significance of PFASs originated from biosolids, the unidentified PFASs should be studied in the future as well as their environmental implications.

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