Isomers of the perfluoroalkylacids PFOS and PFOA and their precursors in an environmental hotspot impacted by AFFFs

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Preface

This master thesis has been prolonged and written under challenging circumstances. Both due to troubles with the equipment which caused a lot of frustration and wasted a lot of time and private matters. The project has proven to be very difficult at times, often set aside for some time which made it even worse. It made me realize how little I knew and was able to do by myself which really underlined the importance of taking a master's degree. It was challenging both in practice and theory and I have learned a lot from it. I feel that it has already helped me in my career as since August I have been working full time as a chemist while writing this thesis and doing some of the last of my experiments.

The completion of this thesis would not be possible without the assistance of the great people working at the Marine Research Institute and University in Bergen.

I would like to thank you all individually;

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Abstract

Per- and polyfluoroalkyl substances (PFASs) are widely used manmade chemicals known to cause serious harm to the environment and human health. They are used in a wide range of products due to their unique properties to repeal water and oil, being resistant to heat and bacterial degradation. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic aicd (PFOS) are some of the most discussed and regulated PFAS currently. Isomers of PFOA and PFOS are the main focus of this thesis.

The objective of this study was to develop an analytical method for separation of PFOA and PFOS isomers. The method was then used on samples gathered from Flesland Airport, Bergen. A PFAS hotspot where PFAS was used extensively in aqueous film forming foams (AFFF) at their firefighting training spot. Samples of soil, biota and water were gathered for analysis, both from the inside and outside of the airport. The soil samples were gathered at different depths from the PFAS hotspot.

The data suggests that PFAS used at the training site originates from historical use of ECF AFFF and more recent use of telomerization AFFF as more linear PFOA and PFOS are being detected closer to the surface. Branched isomers were found increasingly enriched with the depth of the soil. Suggesting also a possible pattern of transport/leaching where the more water soluble branched isomers are being washed down faster with e.g. rain water.

TOPA has proven that targeted analyses of individual PFAS underestimate the level of contamination. The increases in concentration of individual PFOA isomers ranged from 314 to 4766 %. The data also suggest that the soil was largely contaminated with branched precursors and that TOPA is isomer specific, creating both linear and branched products.

PFAS contamination was also found in sea water and in small amounts in other aqueous samples from the airport. Additionally PFAS was found in biota samples, gathered just outside of the airport, in close proximity to popular fishing spots.

The results were validated by several quality parameters like selectivity, limit of detection and limit of quantification, precision and accuracy (trueness). To do so, a proficiency test of pork liver from the European Union Reference Laboratory was utilized.

Contents

Acronyms/Abbreviations

6:2 FTS	**Fluorotelomer sulfonic acid**
α	Selectivity factor
AABG	Advanced Active Beam Guide
AFFF	Aqueous film forming foams
AQT	Advanced Quadrupole Technology
B	Magnetic field
CO_2	Carbon dioxide
d.w.	Dry weight
ECF	Electrochemical fluorination
EFSA	European Food Safety Agency
EFV	Efavirenz
EOF	Extractable organofluoride
ESI	Electrospray ionization
EU	European Union
EURL	European Union Reference Laboratory
f	Frequency
HF	Hydrofluoric acid
HPLC	High performance liquid chromatography
IMR	Institute of Marine Research
ISTD	Internal standard
k	Retention factor
LC	Liquid chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
m	Mass of an ion
MS	Mass spectrometry
m/z	Mass to charge ratio
N	Efficiency factor
n	Number of observations
NaOH	Sodium Hydroxide
NOF	Non extractable organofluoride
NOx	Nitrogen oxides
OECD	Organization for Economic Co-operation and Development
P1MHpS	Perfluoro-1-methylheptanesulfonate
P35DMHxA	Perfluoro-3,5-dimethylhexanoic acid
P35DMHxS	Perfluoro-3,5-dimethylhexanesulfonate
P3MHpA	Perfluoro-3-methylheptanoic acid
P3MHpS	Perfluoro-3-methylheptanesulfonate
P45DMHxA	Perfluoro-4,5-dimethylhexanoic acid
P45DMHxS	Perfluoro-4,5-dimethylhexanesulfonate
P4MHpA	Perfluoro-4-methylheptanoic acid
P4MHpS	Perfluoro-4-methylheptanesulfonate
P55DMHxA	Perfluoro-5,5-dimethylhexanoic acid
P55DMHxS	Perfluoro-5,5-dimethylhexanesulfonate
P5MHpA	Perfluoro-5-methylheptanoic acid
P5MHpS	Perfluoro-5-methylheptanesulfonate

P6MHpA	Perfluoro-6-methylheptanoic acid
P6MHpS	Perfluoro-6-methylheptanesulfonate
PFAA	Perfluoroalkyl acid
PFAS	Per- and polyfluoroalkyl substances
PFCA	Perfluoroalkyl carboxylic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFSA	Perfluoroalkyl sulfonate acid
POPs	Persistent organic pollutants
PTFE	Polytetrafluoroethylene
q	Charge of an ion
R_s	Resolution
RSD	Relative standard deviation
SD	Standard deviation
TOPA	Total oxidizable precursor assay
UF_6	Uranium hexafluoride
UN	United Nations
w.w.	Wet weight
$\bar{x}$	Mean

1. Introduction

During the last few decades there have been increasingly more evidence for Per- and Polyfluoroalkyl Substances (PFASs in short) being persistent, bioaccumulative, very toxic, and ubiquitous contaminants in both humans and wildlife (4, 10, 11) as well as in commonly consumed foods (9). Some of the negative effects of PFAS on human health include thyroid disease, inflammatory bowel disease, liver diseases and cancer due to these being the target organ for long-chain PFAS storage (4, 8). Kidneys are also affected by PFAS exposure which may lead to disease and/or cancer. PFAS also impairs fertility and lead to hormonal changes and imbalances (increase in cholesterol, diabetes etc.) (4, 8).

In 2021 the Organization for Economic Co-operation and Development (OECD) revised the definition of PFAS (6). This resulted in an increase in compounds that fall under this new PFAS definition. PubChem, a popular database of chemicals and molecules which contains 116 million compounds now contains over 7 million PFAS due to this change (29). PFAS are used in a variety of everyday products like paint, Teflon pans, paper, clothing, electronical devices as well as in the industry in form of aqueous film forming foams (AFFF), storage units, mining equipment and so on (4, 7). The main exposure route of PFAS for most of both humans and probably for wildlife is through food and drinking water (5). PFAS are also classified as persistent organic pollutants (POPs) and often called the “forever chemicals” since their unique chemical structures provides them with incredible stability (4, 6). The strength of carbon fluoride bond is known to be one of the strongest in organic chemistry, with increasing strength as more fluorine atoms are attached to the carbon (35).

PFAS source identification and characterization is required for proper remediation approaches that reduce human exposure to PFAS trough dietary sources, such as fish and water. Different sources can have characteristic isomer specific profiles, resulting in specific chemical fingerprints, which has been proposed as an approach to elucidate the source of PFAS contamination (14).

1.1 Research questions and aim

This study aims at investigating the occurrence of unknown PFAS precursors at the airport impacted site using the total oxidizable precursors assay (TOPA) and investigate the isomer profile of the resulting acids, in particular perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic aicd (PFOS). This data can be later used to investigate the

contributions from the degradation of PFAS precursors to the burden of the perfluoroalkyl acids. This study is based on the following hypotheses:

I. The PFOA and PFOS isomer profiles in samples depends on the type of PFAS source and on the history of PFAS production.
II. The degradation of unknown PFAS precursors is isomer dependant, branched precursors will degrade at higher rates compared to linear ones.
III. Targeted analysis of perfluoroalkyl acids underestimate the PFAS contamination.

For this, analytical methods will be developed and to applied samples from the hotspot Flesland airport using liquid chromatography-high resolution mass spectrometry (Orbitrap).

2. Background, theory and methodology

2.1 History and applications

The development of fluoropolymer industry started as early as in the late 1930s with an important discovery for the future PFAS industry; Teflon (polytetrafluoroethylene, PTFE) which was made by Dr. Plunkett at DuPont in 1938. Dr. Plunkett found that the substance had incredible chemical and physical properties; low surface friction, high heat resistance and being chemically inert which led to further investigations (3). Today Teflon is known best for its application in non-stick cookware and have been applied in many products like lubricants, electrical equipment, containers, tunings and other (3, 7). The structure of PTFE defines it as a PFAS due to its carbon atoms being fully fluorinated (see figure 2.1 and chapter 2.2). Initially PFAS compounds didn't find no use for their applications up until the 1940s and the World War II. PFAS played an important role in the Manhattan project as they are generally chemically inert and were used for valves and gaskets that would resist chemical attack by very corrosive material uranium hexafluoride (UF6) used to separate Uranium-235 from Uranium-238 trough gaseous diffusion (3). After the war other PFAS, including PFOS and PFOA quickly became used in a variety of applications and commonly found products (7).

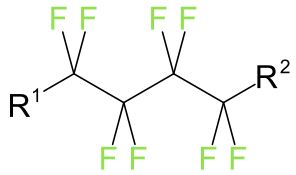


Figure 2.1. *Structure of PTFE, a fluoropolymer. The R^1 and R^2 are further continuations of the chain.*

PFAS like PFOA and PFOS were also one of the first PFAS compounds to enter mass production in the 1940s - 1950s by the 3M company, earlier called Minnesota Mining and Manufacturing Company. Both PFOA and PFOS has been widely used in electrical, automotive, architectural and construction industries in a variety of products. Initially PFOA was used as emulsion polymerization aid in the synthesis of PTFE but has later, together with PFOS, also been used in aqueous film forming foams, a major PFAS pollutant relevant to this study, used in fire extinguishers (7, 24).

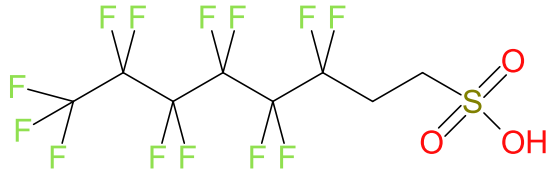
Many more PFAS like products and chemicals has been in production, some sources suggest that more than 4700 different PFAS has been manufactured since the 1940s and been on the global marked and 7 million PFAS compounds are already registered (6, 29). In late 1990s and early 2000s PFAS has gained a lot of attention due to the increasing concerns about PFAS bioaccumulation and potential toxicity (6, 24). In May 2000 the 3M company has announced

a voluntary phaseout of PFOS, PFOA, perfluorohexanesulfonic acid (PFHxS) and their related precursors (6). Since then, PFAS manufacture, and import has been continuously regulated at international level (see chapter 2.6).

2.2 Definition of per and polyfluoroalkyl substances

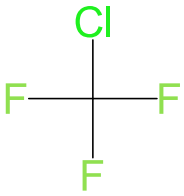
Historically the most accepted and widely used definition of PFAS was proposed and published in an article from 2011 (1). The aim of the article was to provide an overview of PFASs detected in the environment and provide a clear and specific, and descriptive terminology for PFASs. PFASs were defined as “the highly fluorinated aliphatic substances that contain 1 or more C atoms on which all the H substituents (present in the nonfluorinated analogues from which they are notionally derived) have been preplaced by F atoms, in such manner that they contain the perfluoroalkyl moiety $C_nF_{2n+1}^-$ “.

Recently in 2021 a new definition has been proposed by the Organisation for Economic Co-operation and Development (OECD) with an attempt to close in the gaps in the previous definition from 2011. This new definition defines PFASs as “fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e., with a few noted exceptions, any chemical with at least a perfluoroinated methyl group ($-CF_3$) or a perfluoroinated methylene group ($-CF_2-$) is a PFAS." (6). Examples of some simple PFASs and non-PFASs fluorinated compounds, according to the OECD definition, can be seen in the figure below (figure 2.2). Fluorotelomer sulfonic acid (6:2 FTS) is a PFAS found in several biota and water samples gathered across Germany (21). Chlorotrifluoromethane is an example of a non PFAS compound showcased by the OECD (6). One of the compounds; Efavirenz (EFV) is a medicine used for treatment of HIV/AIDS which is a PFAS compound according to the definition.

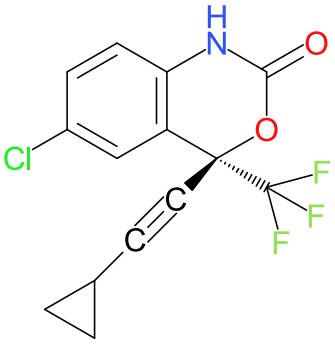


Fluorotelomer sulfonic acid

(6:2 FTS)



Chlorotrifluoromethane



Efavirenz (EFV)

Figure 2.2. *Example of a PFAS (6:2 FTS) and Efavirenz (EFV) vs a non-PFAS fluorinated compound (chlorotrifluoromethane) in accordance to the OCED definition of PFAS.*

Extensive usage of acronyms is often necessary in communicating PFAS due to long names. All acronyms will be spelled once as they are first being mentioned but can also be found in the list of abbreviation's at the start of this thesis. Figure 2.3 below illustrates an example where the acronyms are explained in relation to the name of its corresponding isomers.

PFOA	P4MHpA
Perfluorooctanoic acid	Perfluoro-4-methylheptanoic acid

Figure 2.3. *Names and their corresponding acronyms of compounds relevant to this study.*

2.3 PFAS of interest

The two main PFAS of interest are PFOA and PFOS which occur in various structural isomers. There are potentially 89 possible structural isomers of linear PFOS but only 10 of these are being found in environmental samples (2, 10, 17). Likewise, there are 38 possible structural isomers of PFOA but not all have been detected in the environment. Linear structures of PFOA and PFOS of interest can be seen in the figure 2.4 below. Branched structures of targeted PFOS and PFOA can be seen in tables 2.1 and 2.2 respectively.

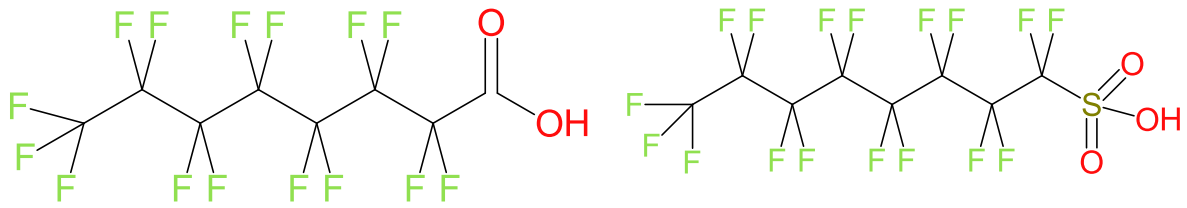


Figure 2.4. *Linear structures of PFOS and PFOA.*

Table 2.1. *Structures, names and acronyms of targeted PFOS isomers.*

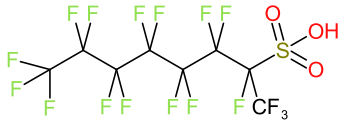
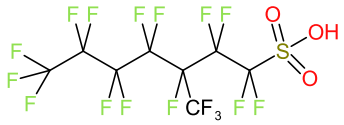
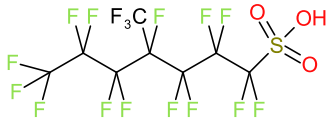
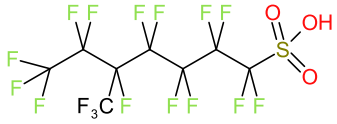
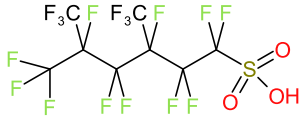
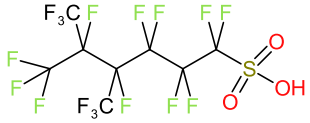
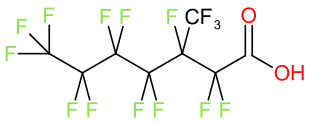
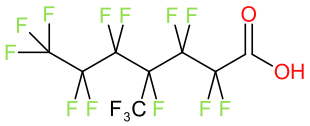
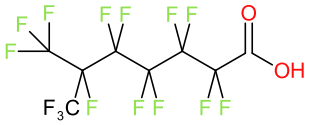
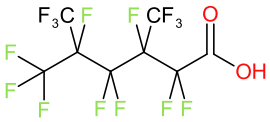
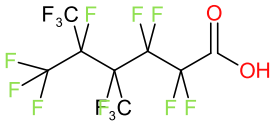
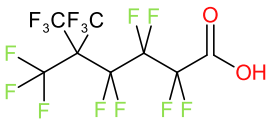
Acronym	Name	Chemical structure
P1MHpS	Perfluoro-1-methylheptanesulfonate	
P3MHpS	Perfluoro-3-methylheptanesulfonate	
P4MHpS	Perfluoro-4-methylheptanesulfonate	
P5MHpS	Perfluoro-5-methylheptanesulfonate	
P6MHpS	Perfluoro-6-methylheptanesulfonate	
P35DMHxS	Perfluoro-3,5-dimethylhexanesulfonate	
P45DMHxS	Perfluoro-4,5-dimethylhexanesulfonate	
P55DMHxS	Perfluoro-5,5-dimethylhexanesulfonate	

Table 2.2. Structures, names and acronyms of targeted PFOA isomers.

Acronym	Name	Chemical structure
P3MHpA	Perfluoro-3-methylheptanoic acid	
P4MHpA	Perfluoro-4-methylheptanoic acid	
P5MHpA	Perfluoro-5-methylheptanoic acid	
P6MHpA	Perfluoro-6-methylheptanoic acid	
P35DMHxA	Perfluoro-3,5-dimethylhexanoic acid	
P45DMHxA	Perfluoro-4,5-dimethylhexanoic acid	
P55DMHxA	Perfluoro-5,5-dimethylhexanoic acid	

2.4 Chemical properties

PFAS are known for their unique chemical and physical properties which are widely wanted and applied in chemical, electrical, automotive, architectural and construction industries (3, 7). PFAS products are known for their excellent water and oil repellent attributes, they are also known to be resistant to friction, and degradation by other chemicals, bacteria, or heat. Those properties lead to their wide application and consequently ubiquitous occurrence throughout the environment and bioaccumulation in biota (4). The use of PFAS compounds like PFOA and PFOS in AFFF are due to their hydrophobic tails and hydrophilic heads which make them function as surfactants, effectively reducing the surface tension in water. This reduction helps the water to soak into a material easier, increasing the effectiveness of cooling the burning object or substance (34).

The properties of PFAS are due to the carbon-fluorine bond which is known to be one of the strongest bonds in organic chemistry. The bond dissociation energy (energy to break one mol of bonds) energy is around 105,4 kcal/mol, around 7 % more than in the case of carbon-hydrogen bonding (around 98,8 kcal/mol). It also increases as more fluoride atoms are being attached to the carbon atom. The C-F bond is also relatively intermediate in length at around 1,35 Å, which decreases as more fluoride atoms are attached to the carbon atom which also contributes to bonds stability. Due to the electronegative nature of fluoride, it also introduces strong polarization of this bond, further stabilizing it (35). It is possible to degrade PFAS under laboratory controlled conditions, like with the TOPA but in nature such harsh conditions are rare and PFAS tend to resist most natural processes (20).

The Danish Ministry of Environment reported PFOS (519 mg/L at 20 ± 0.5 ºC) to be significantly less water soluble then PFOA (4.1 g/L at 22 ºC) (46). It was also reported that PFOA is a weaker Brønsted acid (pKa 2.5) compared to PFOS (pKa -3) (47).

Branched and linear PFOS/PFOA can differ in their chemical properties due to differences in chemical structure. Variations in structure might influence the stability of the compound, meaning that some might degrade and behave differently in the environment. Different isomers might also exhibit different toxicological profiles, they may also vary in bioaccumulation and transformation (32). Their environmental distribution and mobility might also be affected, some isomers might be more retained by e.g., soil, aquatic environments then others (17, 18). A study from 2019 have shown that total PFOS increases in concentration by depth in soil affected by AFFF, the highest PFOS concentrations were found at depths from 0 to 1 meters (18).

A study previously done at Flesland Airport show that linear isomers of PFOS and PFOA in soil varies with distance from the centre of the hotspot (17). Branched isomers have shown to be more water soluble and are found enriched in the water at sites contaminated with AFFF (17). An overview of the data from this study can be seen in table 2.3 below. In addition to the data shown in table 2.3, the composition of the branched isomer profiles was made by the study. Dimethyl PFOS and PFOA isomers were detected at very at low concentrations. P3/P4/P5 PFOS, measured as one peak in the study, was the most dominant branched PFOS isomer. Followed by P1 and P2 PFOS. For PFOA only the P5 and P3/P4/P6 (measured as one) PFOA were found to be the most dominant (17).

Table 2.3. Overview of the data found in a previous study done at Flesland Airport.

	Soil at centre	Soil 100 meters from centre	Water	Fish liver
Linear PFOS	63 %	85 %	58 - 61 %	87 - 90 %
Branched PFOS	37 %	15 %	39 - 42 %	7 - 10 %
Linear PFOA	80 %	100 %	75 %	ND
Branched PFOA	20 %	0 %	15 %	ND

2.5 PFOS and PFOA synthesis

Two primary methods of synthesis have been used for manufacture of PFOS and PFOA; electrochemical fluorination (EFC) and telomerization. Both differ in synthesis mechanisms which also results in different isomer composition of the product.

ECF has been the main method for PFOS synthesis science the 1940s until its phase-out from 2002. It was first developed for a large scale manufacture by todays 3M company. This method yields 70 % ± 1.1 % of L-PFOS and the remaining 30 % ± 0.8 % is made up of branched PFOS isomers. As for ECF-PFOA yields are around 78 % ± 1.2 % linear and 22 % ± 1.2 % branched isomers (14). The process of synthesis entails electrolysis of a solutions containing organic compounds and anhydrous hydrofluoric acid (HF) (14, 23).

After the phase-out of ECF in 2002, telomerization has become the main synthesis method for production of PFOA and PFOS. The synthesis involves a reaction of a telogen (short chain molecule) with a nucleophile in the presence of a catalyst. The reaction selectively produces compounds with specific chain lengths (23). Telomerization retains the structure of the starting material which is typically linear resulting in isometrically pure product of ~ 100 % linear PFOS and PFOA (6, 14). An overview of the differences between ECF and telomerization can be seen in table 2.4. Other methods of PFAS synthesis also exists but there is no evidence of these to be used at a large industrial scale.

Table 2.4. Summary of differences in isomeric composition of PFOA and PFOS produced by ECF and telomerization used for source elucidation.

	L-PFOS	Branched PFOS	L-PFOA	Branched PFOA
ECF	~ 70 %	~ 30 %	~ 78 %	~ 22 %
Telomerization	~ 100 %	~ 0 %	~ 100 %	~ 0 %

Based on this knowledge PFAS of different origin can be differentiated. However, this can be complicated by the fact that isomer patterns can be significantly influenced by differences in sorption and by the differential uptake and elimination of branched isomers compared to linear ones (32).

2.6 Regulation

The regulation of PFAS started in the early 2000s as the awareness of potentially harmful effects of PFAS on the environment and human health grew in the literature and scientific communities. As the discussions continued large PFAS manufactures began to voluntarily phase out some of the discussed long-chain PFAS (3, 6). Some countries have implemented their own regulations to combat PFAS contamination in the environment in addition to international treaties throughout the last few decades. In pop-culture PFAS itself and PFAS industry has been highlighted in a negative light in movies such as “Dark Waters” and “The Devil We Know”. PFAS has also gathered a lot of negative media attention in articles and stories of people negatively affected by PFAS contamination and legal battles against PFAS manufactures throughout the years.

At the international level, The Stockholm Convention on Persistent Organic Pollutants (POPs) which is a United Nations (UN) treaty is probably the most cited and well-known piece of collaborative policy regarding environmental health and safety and tagged PFAS as “forever chemicals”. The convention was first adopted in 2001 and became an international law later in 2004 (36). Firstly in 2009 the signatories of the agreement agreed to restrict the production and use of PFOS (with a few exceptions) and adding it to the list of POPs. Later in 2019 the members agreed to ban the use of AFFFs containing PFOA and removed the exceptions for PFOS use. Currently 152 countries have signed the Stockholm Convention, including both developed and developing countries across the globe. Many member countries are still to follow-up with the restrictions. China for example, also a signatory to the Stockholm Convention, is one of the largest producers and consumers of PFAS in the world and still allows the use of AFFFs (36).

In 2006 the European Union (EU) acknowledged that “PFOS fulfils the criteria for classification as a very persistent, very bioaccumulative and toxic” chemical. The European Commission estimates 100,000 sites are emitting PFAS as of 2020. Furthermore, the same year, the European Food Safety Agency (EFSA) reported and identified fish meat, eggs, raw

and fruit products as common sources of dietary exposure of PFOA, PFHxS, PFOS and perfluorononanoic acid (PFNA) and reviewed human health risk related to PFASs. The commission established a tolerable weekly intake (TWI) of 4,4 ng kg^{-1} body weight (bw) for these four compounds (33, 36). Norway as well as several other European countries (Germany, Sweden etc.) in accordance with the recent recommendations all EU members should monitor the amount of PFOS, PFOA, PFNA, PFHxS in their foods up-until the year of 2025. If possible, also test for the amount of similar compounds but which have different alkyl chain length and other less similar compounds. Further investigation and steps are to be taken if concentrations of these compounds exceed a given limit. All member states should also provide the monitoring data to the EFSA (15).

Previously mentioned medication used to treat malaria and AIDS/HIV falls under the definition of PFAS and therefore prone to the same restrictions proposed by the EU. If not organised, reckless banning of PFAS compounds might led to shortage of vital medicines.

Despite the already well established regulation and evidence of risks associated with PFAS exposure no international restrictions differentiate between the linear and branched isomers of PFOA, PFOS and other PFAS. The restrictions usually refer to all PFOA and PFOS isomers as one, as well as some of their derivates (salts etc.).

2.7 Transport and exposure

Sites with high concentrations of PFAS, by production or extensive use are called PFAS “hotspots”. Water near such site can become contaminated which also helps to spread water soluble PFAS across long distances. Soil can become contaminated, PFAS can leach trough the soil into the ground water deposits which are often used for drinking or industry. Volatile PFAS can become airborne and travel long distances, PFAS has even been detected at mount Everest (44). It is speculated that such volatile PFAS are the reason for those findings. Biological factors, bacteria, wildlife can consume PFAS from the contaminated sites. The consumed PFAS can then biomagnify up the food chain, meaning that animals and humans higher up in the food chain are increasingly more exposed (17, 18, 19).

PFAS exposure in humans come mainly from production and dietary intake. PFAS accumulate in various foods, especially fish from contaminated bodies of water. Drinking contaminated water and using contaminated water in plant irrigation also leads to exposure.

PFAS can also be found in indoor dust and air due to their presence in many consumer products (5).

2.8 High Performance Liquid chromatography

In this study, high performance liquid chromatography (HPLC) using reversed phase column chemistry is utilized for separation of target analytes. In HPLC the solvent, called the mobile phase is pumped through the chromatographic column which contains the stationary phase. In traditional liquid chromatography (LC), the mobile phase travels through the column by the force of gravity. The use of a pump in HPLC reduces the time of separation and helps to overcome the drop in pressure in the packed column. A HPLC instrument consists of pump, injection system, column, and a detector (mass spectrometer in this case). A schematic overview of a HPLC system can be seen in figure 2.5 below (37).

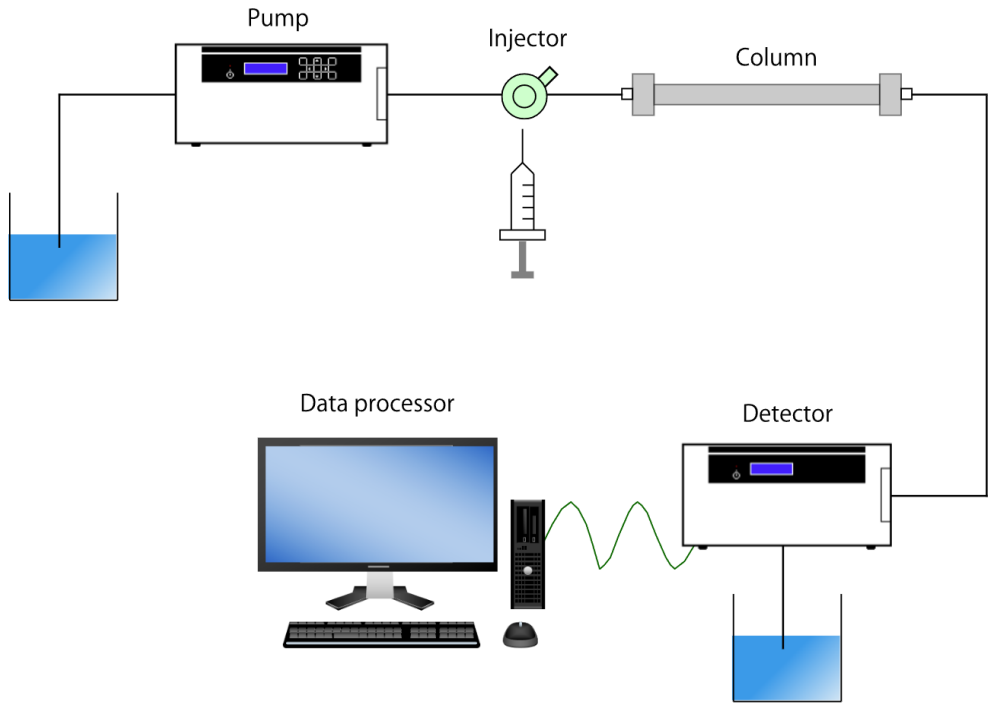


Figure 2.5. *General and simplified scheme of a HPLC instrument setup (https://www.jasco-global.com/principle/principles-of-hplc-2-hplc-system-configuration/).*

The principle of reversed phase chromatography is based on hydrophobic interactions between the stationary phase and the analyte. In reversed phase chromatography, the stationary phase is made of a non-polar material, in the case of this study the C18 column contains octadecylsilane chains and the F5 column pentafluorophenylpropyl chains. When a sample is injected into a chromatographic column, analytes with higher affinity for the non-polar stationary phase will be retained by it and stay longer inside of the column. On the other

hand, analytes with lower affinity for the non-polar stationary phase and higher affinity for the polar mobile phase will be less retained. This results in different molecules (or analytes) to be separated and leave the column at different times. The choice of mobile and stationary phases is required for optimal analyte separation. Other factors that also affect the analyte separation and can be adjusted are e.g. temperature, flow rate, gradient. The efficiency of peak separation is described by the resolution function (R_s, formula 2.1):

Formula 2.1. Resolution function for a chromatographic system.

$$R_s = \frac{\sqrt{N}}{4}\frac{(\alpha - 1)}{\alpha}\frac{k}{(k+1)}$$

The formula takes into consideration efficiency ($\frac{\sqrt{N}}{4}$ term), retention ($\frac{k}{(k+1)}$ term) and selectivity ($\frac{(\alpha-1)}{\alpha}$ term) all of which are important and affect the separation (41). As the R_s value increases the separation is better.

The retention factor k, used in the retention term, describes how long the analyte in question resides in the mobile phase relative to the time its spends in the stationary phase, it increases with retention time. The higher the k, the higher the retention term which positively affects the separation (R_s). This suggests that better retention of target analytes helps with better separation. This can be done by increasing column length (not possible if only one column is available), decreasing the flow rate or decreasing the amount or/and strength of the mobile phase (41).

The selectivity term defines the ratio of retention between two peaks; α is calculated by dividing the retention term k of the second peak by the retention term k of the first peak. To increase the selectivity term one must increase α and to do this the k term of the second peak must be as high as possible compared to the k term of the first peak. This can be affected by changing the mobile phase or temperature but also the previously mentioned column length (41).

The efficiency term compares the performance of different columns. It is calculated with the N term called efficiency and is based on a relationship between retention time and peak width

at base. The higher the N value the narrower the peak width which leads to easier separation of nearby compounds and overall better resolution (R_s) (41).

Separation of linear, monomethyl and dimethyl isomers of PFOA and PFOS was previously done successfully in different matrices. A study from 2010 showed that in both PFOA and PFOS separation the linear isomer elutes last, which tends to be the case for longer compounds (14). This is due to shorter compounds being more efficiently retained by the stationary phase. The smaller molecules can diffuse more efficiently into the stationary phase, spending more time in it. This might also be due to branched compounds being more soluble compared to the linear (14, 17).

2.9 Mass spectrometry

Mass spectrometry (MS) is a widely used analytical technique often used in hyphenation with chromatography systems. In MS, the sample is first ionized to prepare it for MS analysis and separation. In this work the ionization of the sample is done by the electrospray ionization (ESI) technique. First, a liquid sample is sprayed into a fine mist with the help of a gas, often nitrogen, which helps to carry the sample. The sprayed sample travels through a high voltage magnetic field which helps to convert the sample spray into fine aerosol; a mixture of air and fine, charged sample particles (37). The sample is now ionized due with the help of the magnetic field and can now enter the mass spectrometer. In the mass spectrometer the ions are being separated based on their mass to charge ratio (m/z) in the ion trap. The ion trap works by trapping the ions in a oscillating motion around its round shape, the frequency of these oscillations is directly proportional to their m/z. These frequencies create a frequency spectrum which can then be transformed into a mass spectrum by the Fourier Transport. Based on predefined data and criteria, the system automatically selects a subset of ions for further analysis and fragmentation, in this study by higher energy collision dissociation. The fragments are then analyzed which can be used to identify the structure of the precursor ion. When used with HPLC the mass spectrum acquirees an additional dimension: retention time. This means that the analytes eluting at their distinct retention times also get their own distinct mass spectrum (37, 38).

The process of analysis in HPLC-MS can be broken down into four points:

1. Chromatographic separation of the analytes in the sample.
2. Continuous ionization of the analytes.

3. Trapping and detection of ions in the ion trap.
4. Mass analysis and separation. The generated ions are separated and/or fragmented based on their m/z.

The Orbitrap is a type of mass analyzer known for its high mass accuracy (< 5 ppm) and high resolution (resolving power of 100000 or more, full width at half maximum)(38). This is due to its unique function, combining both an outer ion trap and Orbitrap analyzer. Ions are first trapped and accumulated in the outer ion trap, improving sensitivity, and helping to detect trace amounts of molecules. Then the ions are then released into the Orbitrap analyzer where molecules oscillate along the axis of a central cylindrical electrode. The frequency (f) of oscillations is inversely proportional to the square root of the ion's m/z and a mass spectrum can be made (see formula 2.2) (37, 38). A schematic picture of an Orbitrap can be seen in figure 2.6 below.

Formula 2.2. The relationship between frequency and m/z. Where B is the magnetic field, m is the mass of the ion, q is the charge of the ion and m/z is the mass to charge ratio.

$$f = \frac{qB}{2\pi m} = \frac{B}{2\pi}\frac{1}{m/z} \rightarrow f \sim \frac{1}{\sqrt{m/z}}$$

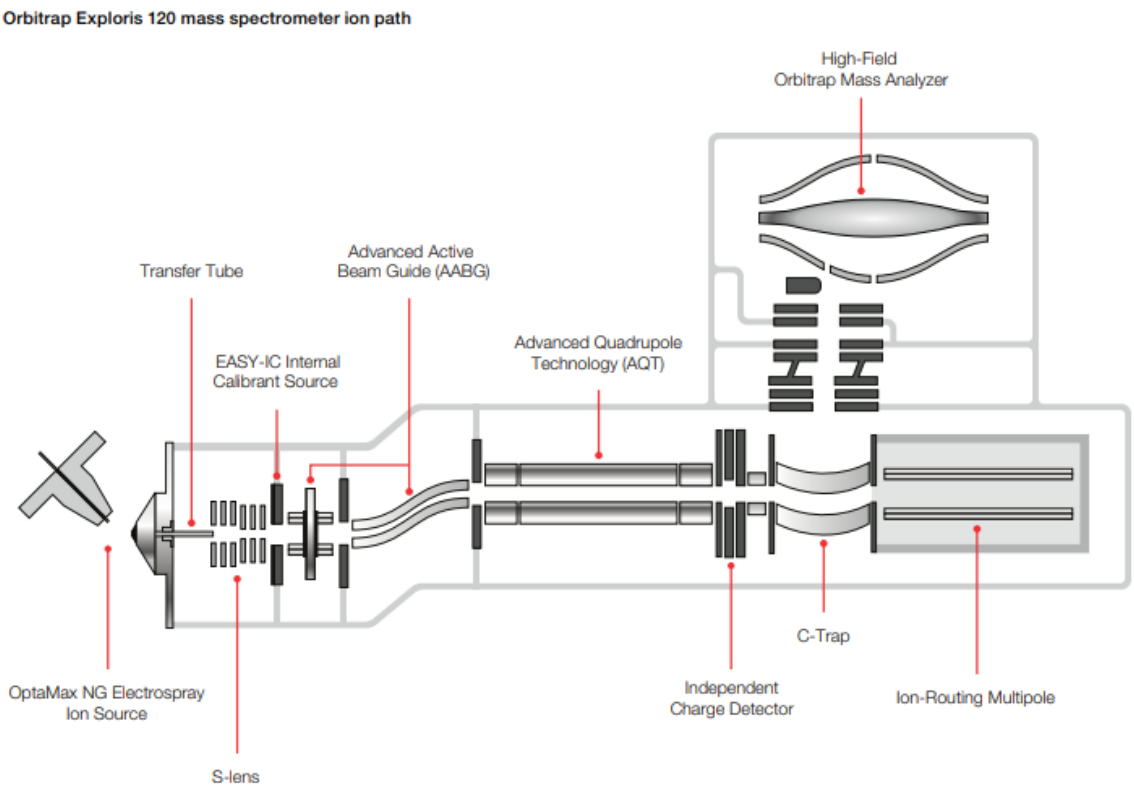


Figure 2.6. *A schematic overview of the Orbitrap instrument. Found and copied from the instrument manual (Orbitrap Exploris 120, Thermo Scientific).*

2.10 Previous studies

Several previous studies on PFAS contaminated soil due to extended use of AFFF on airports has been made throughout the years all around the world (17, 18, 19). For this thesis the site of interest was a firefighting training site located at Flesland, Bergen Airport located in Western Norway. A similar study was conducted at this site back in 2011, focusing on the distribution and levels on structural isomers of perfluoroalkyl acids (PFAAs) at the site (17). The findings from this study were previously discussed in chapter 2.4. Many of the previous studies focus solely on legacy PFAS with limited data on potential precursors which can overtime transform into legacy PFAS (20). Newer studies (18, 21, 22) tend to include the potential precursors as part of the analysis (standards, targeted etc.) or/and use methods to calculate or estimate the amount of unknown PFAS. The most used methods for precursor and unknown PFAS estimation currently is fluorine mass balance (or extractable organofluoride, EOF) and the TOPA. Both of which have their limitations and advantages. The total extractable organic fluorine are the fluorine compounds that can be successfully extracted for analysis. Targeted analyses often times underestimate the total PFAS contamination due to

limited access to standards, time and other resources. Many of the PFAS might be still unknown and tools like TOPA help to estimate the total burden of contamination. Targeted analyses and TOPA in relation to EOF is shown in figure 2.7 below.

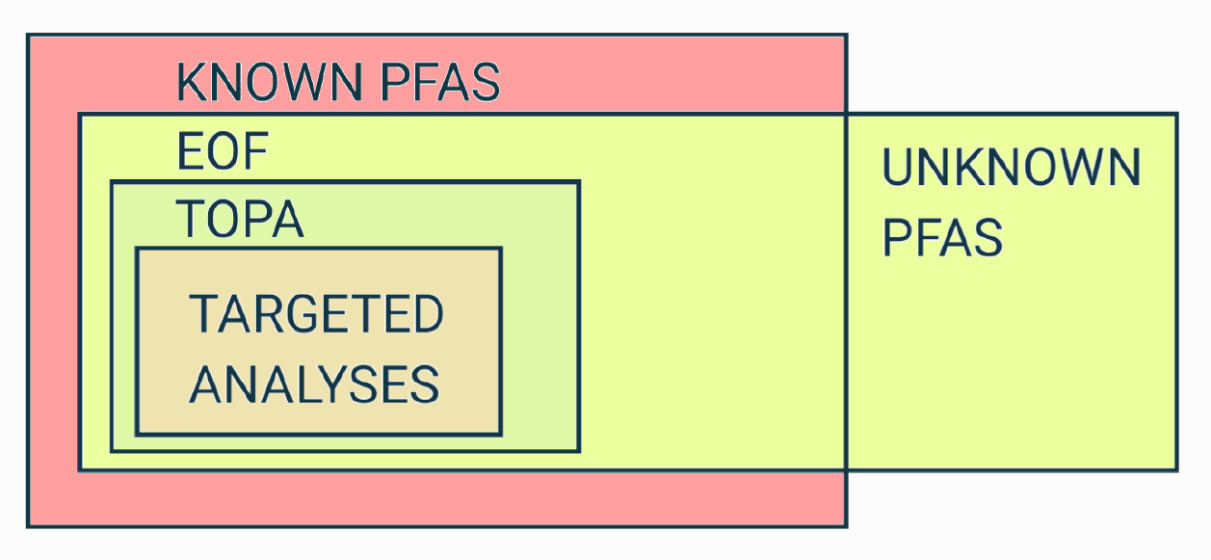


Figure 2.7. *An overview of targeted analyses vs TOPA and OEF.*

2.11 TOPA

TOPA is a method developed to indirectly measure unknown precursors of perfluoroalkyl carboxylic (PFCA) and sulfonic (PFSA) acids. It was first used and published by Houtz and Sedlak in 2012 (20) and have since seen many alternations in its procedures and been applied in many studies. In this study it was decided to increase the dosage of the oxidants up to 200 mM sodium hydroxide (NaOH) and 75 mM potassium persulfate compared to the first study made by Houtz and Sedlak (20). This was done in hope to oxidize more of the potential precursors.

The idea of the procedure is to convert unknown and known precursors of PFCAs and PFSAs by exposing them to hydroxyl radicals (figure 2.8) generated by thermolysis of persulfates under alkaline condition into known, measurable and stable PFCAs. These can then be measured to estimate the precursor concentration in a sample, see figure 2.9. Not all precursors can be oxidized in this way, but the method can check if there are at least some unknown PFAS in the sample and estimate the degree of contamination (20, 21). Another limitation is that it is hard to known if all of the oxidizable precursors have been oxidized without conducting additional experiments (20).

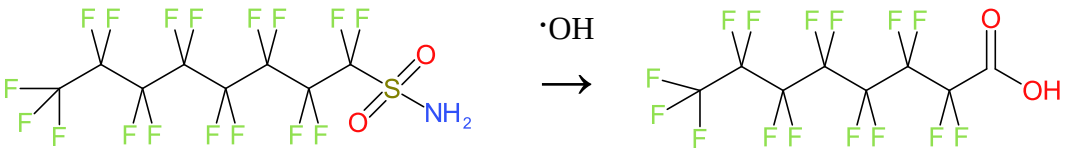


Figure 2.8. *Example of using TOPA to convert perfluorooctane sulfonamide (left molecule) into a stable end product and target analyte, POFA.*

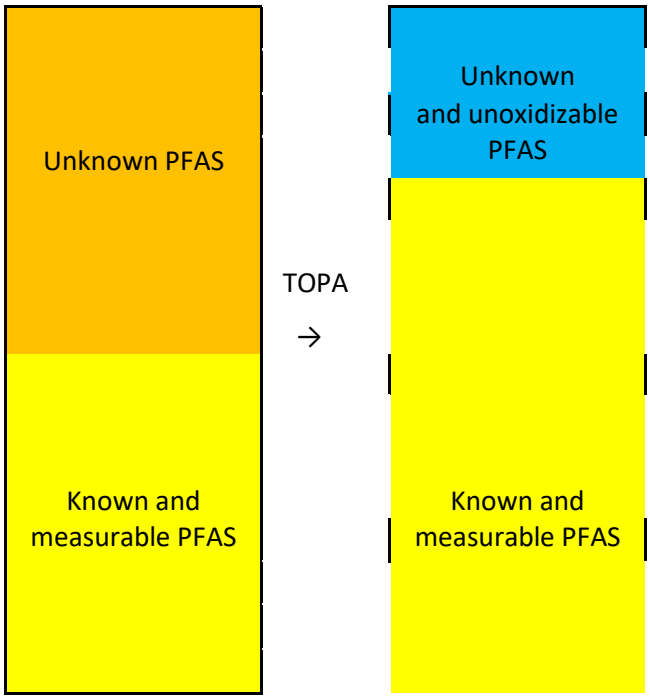


Figure 2.9. *Visual representation of unknown PFAS being oxidized into known and measurable products.*

2.12 Study site

Flesland Airport is located on the Western coast of Norway, approximately 12 kilometres southwest from Bergen's city centre. It is the second largest airport in Norway. As a major airport, is it associated with a wide range of activities that can impact the environment, some of which are the release of greenhouse gases such as carbon dioxide (CO_2), pollutants like nitrogen oxides (NOx), noise pollution and the analytes of interest, forever chemicals; PFAS.

As mentioned previously PFAS are used in a wide variety of products, one of which are AFFF used in firefighting foams. Those have been used extensively at the airport both as a necessity and in training. At the airport, AFFF has been used in training at a designated training site. This training site is considered to be a PFAS "hotspot" and suspected to be highly

contaminated. Historically, several sites have been used, cleaned up by removing the soil from the airport. The airport and the training site are close to several various bodies of water, including ponds, lakes, rivers, and fjords. Sampling in these areas, especially rivers going out of the airport into the sea can reveal potential pollutants. The airport is also near civilian housings which as previously reported in the media is struggling with PFAS contamination in their drinking water (42).

Soil and water samples has been collected both inside and outside of the Airport area. Marked locations can be seen in figure 2.10.

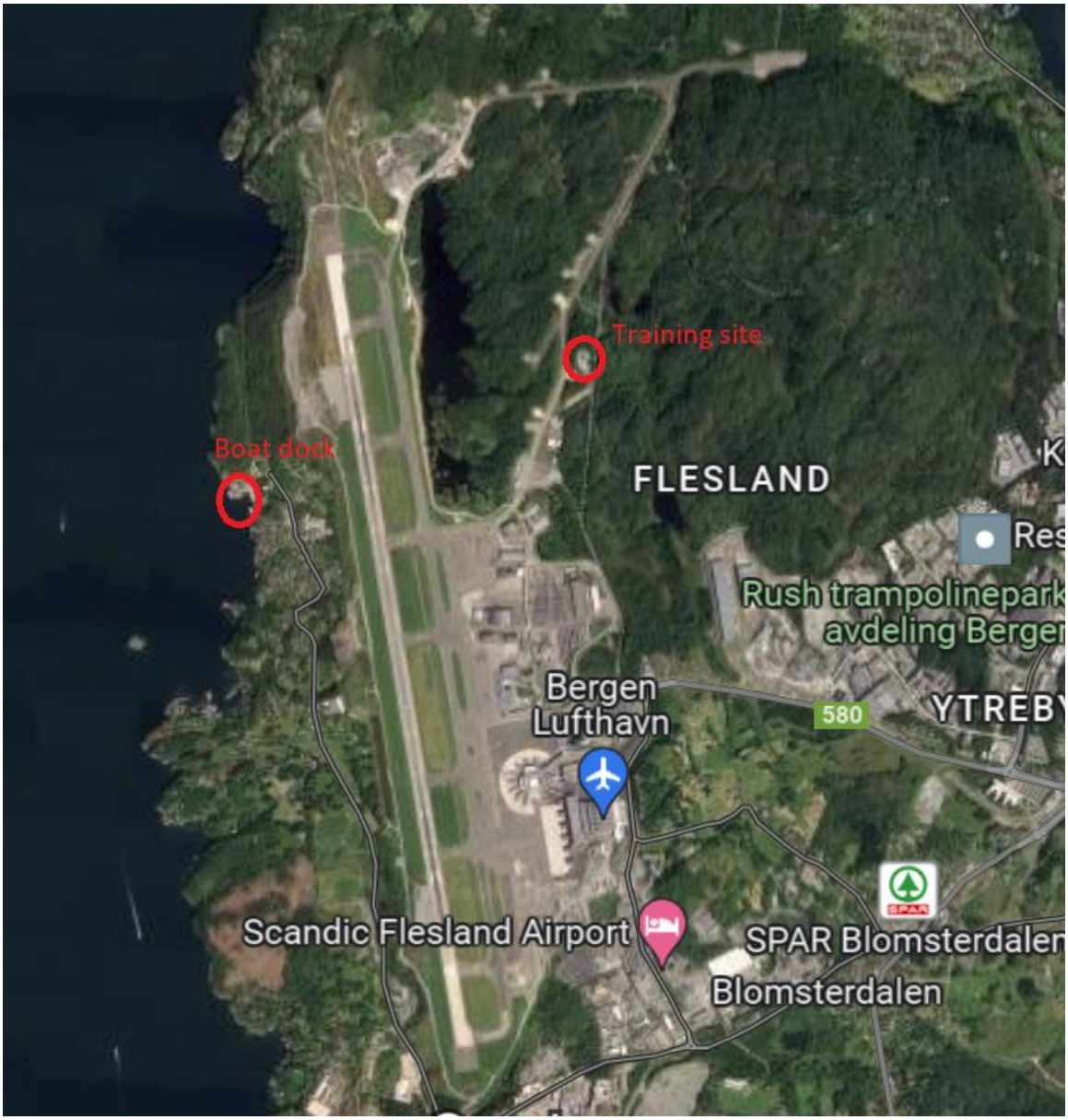


Figure 2.10. *Overview of selected sampling sites in Flesland Airport, Bergen. Taken from google maps in February 2022.*

3. Methods and procedures

3.1 Chemicals and equipment

An overview of used chemicals can be seen in table 3.1, standards in table 3.2, and equipment in table 3.3 below.

Table 3.1. *Overview of all the chemicals used in the project.*

Chemicals	Tag	Supplier	Note
MilliQ water	1.1	Lab made	18.2 MΩ cm^{-1}
Methanol	1.2	Honeywell	CHROMASOLV™ LC-MS Ultra. Tested for UHPLC ≥ 99.9%.
Nitrogen gas	1.3	-	-
25 % Ammonium hydroxide solution	1.4	Merck	Emsure.
Formic Acid	1.5	Merck	98 – 100 %, Emsure.
Acetic acid (glacial)	1.6	Merck	100 %, Emsure.
Hydrochloric acid	1.7	Merck	37 %, Supelco.
Potassium persulfate	1.8	Sigma-Aldrich	≥ 99.0 %
Sodium hydroxide	1.9	Merck	Emsure
Ammonium formate	1.10	Merck	97 %, reagent grade.
Ammonium acetate	1.11	Sigma-Aldrich	≥ 98 %
Sand, white quartz.	1.12	-	SiO_2

Table 3.2. *Overview of all the standards used in this project.*

Standard (supplier name)	Tag	Supplier	Note
MPFAC-C-ES	2.1	Wellington Lab/Greyhound Chromatography	Internal standard (ISTD) used in calibration standards and spiking of biota and water samples.
M8PFOS	2.2	Wellington Lab/Greyhound Chromatography	Internal standard (ISTD) used for spiking of soil samples.
PFAC-MXB	2.3	Wellington Lab/Greyhound Chromatography	Native mix of linear PFOA and PFOS used in calibration solutions and quality control sample.
P1MHpS	2.4	Wellington Lab/Greyhound Chromatography	Native P1MHpS standard. Used in calibration standards.
P3MHpS	2.5	Wellington Lab/Greyhound Chromatography	Native mix of P3MHpS and P3MHpA. Used in calibration standards.
P4MHpS	2.6	Wellington Lab/Greyhound Chromatography	Native mix of P4MHpS and P4MHpA. Used in calibration standards.

P5MHpS	2.7	Wellington Lab/Greyhound Chromatography	Native mix of P5MHpS and P5MHpA. Used in calibration standards.
P6MHpS	2.8	Wellington Lab/Greyhound Chromatography	Native mix of P6MHpS and P6MHpA. Used in calibration standards.
P55DMHxS	2.9	Wellington Lab/Greyhound Chromatography	Native mix of P55DMHxS and P55DMHxA. Used in calibration standards.
P45DMHxS	2.10	Wellington Lab/Greyhound Chromatography	Native mix of P45DMHxS, P35DMHxS, P45DMHxA and P35DMHxA. Used in calibration standards.

Table 3.3. *Overview of all the equipment and instruments used in the project.*

Equipment	**Tag**	**Description**	**Producer**
Analytical balance	3.1	0.1 mg accuracy	-
Ultrasonic bath	3.2	-	-
Centrifuge	3.3	Model 5810	Eppendorf
Water purification system	3.4	18.2 MΩ cm^{-1}	-
15 mL centrifuge tubes	3.5	PP	-
50 mL centrifuge tubes	3.6	PP	-
250 mL wide mouth container	3.7	PP	Nalgene
1000 mL wide mouth bottle	3.8	PP	Nalgene
20 mL reagent tubes ASPEC	3.9	PS	VWR
5 mL reagent tubes ASPEC	3.10	PS	NUNC
Oasis® WAX 3cc 60mg 30 µm	3.11	SPE column	Waters
5 mL syringes	3.12	Plastic	Terumo
0.45 µm syringe filter	3.13	Nylon	Millipore
LC vials	3.14	PP	Waters
Screw corks for LC vials	3.15	PTFE septum	Waters
Vanquish™ HPLC with autosampler, column oven and pump module.	3.16		Thermo Fischer
Orbitrap 120 Exploris system	3.17	-	Thermo Fischer
Acquity UPLC® BEH C18 column	3.18	130Å, 1.7 µm, 2.1 mm x 50 mm	Waters
Ascentis® Express F5 column	3.19	2.7 µm, 4.6 mm x 100 mm	Sigma-Alrich
Guard column with Ascentis® Express F5 guard cartridge	3.20	2.7 µm	Sigma-Alrich
ASPEC GX−274	3.21	Automated SPE Cartridge system	NerliensMeszansky
Vortex mixer	3.22	For single tube	-
FreeStyle	3.23	Software	Thermo Fisher

TraceFinder	3.24	Software	Thermo Fisher
Microsoft package	3.25	Version 2310	Microsoft
Heated water bath	3.26	-	-
Peristaltic pump 12 Vdc standard	3.27	Envirotecnics	Pump for ground water extraction. Provided by AVINOR.
Aluminium traces	3.28	-	-
Kitchen knife	3.29	-	-
Kitchen cutting board	3.30	Plastic	-
Scalpels	3.31	-	-
Kitchen blender	3.32	-	-
Kitchen stand mixer	3.33	-	-
Large boxes for transport	3.34	Plastic	-
50 mL containers for fish liver	3.35	PS	-
100 mL containers for fish muscle	3.36	PS	-
Plastic bags food grade	3.37	Plastic	-
Ball Mill machine	3.38	Steel	-
PP SPE reservoir columns	3.39	PP	Chromabond®
Manual SPE setup including pump	3.40	-	-
Multirotor	3.41	-	VWR
1 litre LC bottles with screw on cork	3.42	Glass and plastic	MicroSolv

3.2 Preparation of standards, solutions and reagents:

The reagent solutions used in this project were prepared as follows:

2 mM ammonium acetate solution was prepared by weighing approximately 154 mg of ammonium acetate into a 1 liter volumetric flask. About 500 mL of MilliQ was added and the flask was shaken until the solid were dissolved. Then MilliQ was then added to the mark and the flaks was shaken again.

20 mM ammonium formate/20 mM formic acid solution was prepared by weighting approximately 1.26 grams of ammonium acetate into 1 liter LC bottle. Afterwards, 1 liter volumetric flask was filled to the mark with MilliQ water, 847 µL of MilliQ was then replaced by 847 µL of formic acid. The flask was shaken before the acidified water was transferred into the LC bottle with ammonium acetate. The bottle was then shaken and stirred.

200 mM sodium hydroxide, 75 mM potassium persulfate solution was prepared by weighing approximately 20 grams of potassium persulfate and 8 grams of sodium hydroxide and transferring it to a 1 liter LC bottle. Afterwards, 1 liter of MilliQ water was measured in a 1 liter volumetric flask and transferred to the LC bottle. The bottle was then carefully stirred and shaken until the solids were dissolved.

0.5 % (v/v) ammonium hydroxide in methanol solution was prepared by filling a 500 mL volumetric flask with methanol to the mark and pipetting 10 mL out of it. Afterwards 10 mL of 25 % ammonium hydroxide solution was added to the volumetric flask. The flask was then stirred and shaken.

1 % (v/v) ammonium hydroxide in methanol solution was prepared by filling a 500 mL volumetric flask with methanol to the mark and pipetting 20 mL out of it. Afterwards 20 mL of 25 % ammonium hydroxide solution was added to the volumetric flask. The flask was then stirred and shaken.

0.5 % (v/v) acetic acid in methanol was prepared by filling a 500 mL volumetric flask with methanol to the mark and pipetting 2.5 mL out of it. Afterwards 2.5 mL of acetic acid was added to the volumetric flask. The flask was then stirred and shaken.

2 % (v/v) formic acid solution was prepared by filling a 500 mL volumetric flash with MilliQ water to the mark and pipetting 10 mL out of it. Afterwards 10 mL of acetic acid was added to the volumetric flask. The flask was then stirred and shaken.

1:9 (v/v) MilliQ water in methanol was prepared by adding 27 mL of methanol and 3 mL of MilliQ water to a 50 mL centrifuge tube. The tube was then shaken and stirred.

Quality control sample used for quality control and accuracy check:

Quality control sample was prepared by adding 9 ng of native PFOA and PFOS mix (PFAC-MXB) and 9 ng of internal standard (ISTD) (MPFAC-C-ES) to a LC vial. The sample was then analysed together with the other samples.

3.3 Sample selection, locations and sampling method

Samples of soil, water and biota were taken at locations both inside and outside of the Flesland airport, previously described in chapter 2.12. Inside of the airport, soil and ground water samples were gathered from around the “hotspot” (firefighting training site, see figure

2.10, chapter 2.12). Biota samples, water and foam samples were also gathered right outside of the airport, close to a small river going into the sea. The spot is also just south of a popular fishing spot, marked on google maps (coordinates; 60.30411, 5.20612). The fishing spot is 1.35 km away from the hotspot and around 680 meters away from the dockyard where the samples were gathered.

The sampling equipment which included stainless steel spoons and shovels, was all washed with methanol at the laboratory and cleaned with MilliQ and paper towels before each sampling to prevent cross contamination. The 250 and 1000 mL PP containers used for sampling were also washed with methanol and air dried in a fume hood the day before sampling.

The soil was collected from a hole made by a nearby excavator near the training site (see figure 3.1, yellow point also S3). About 500 grams of soil was sampled from three levels; surface level, about 0.5 meter depth (mid-level) and about 1 meter depth (bottom-level), one replicate each. It must be mentioned that previously, the surface of the training site has been covered in gravel and sand due to the high levels of PFAS contamination due to historical use of AFFF.

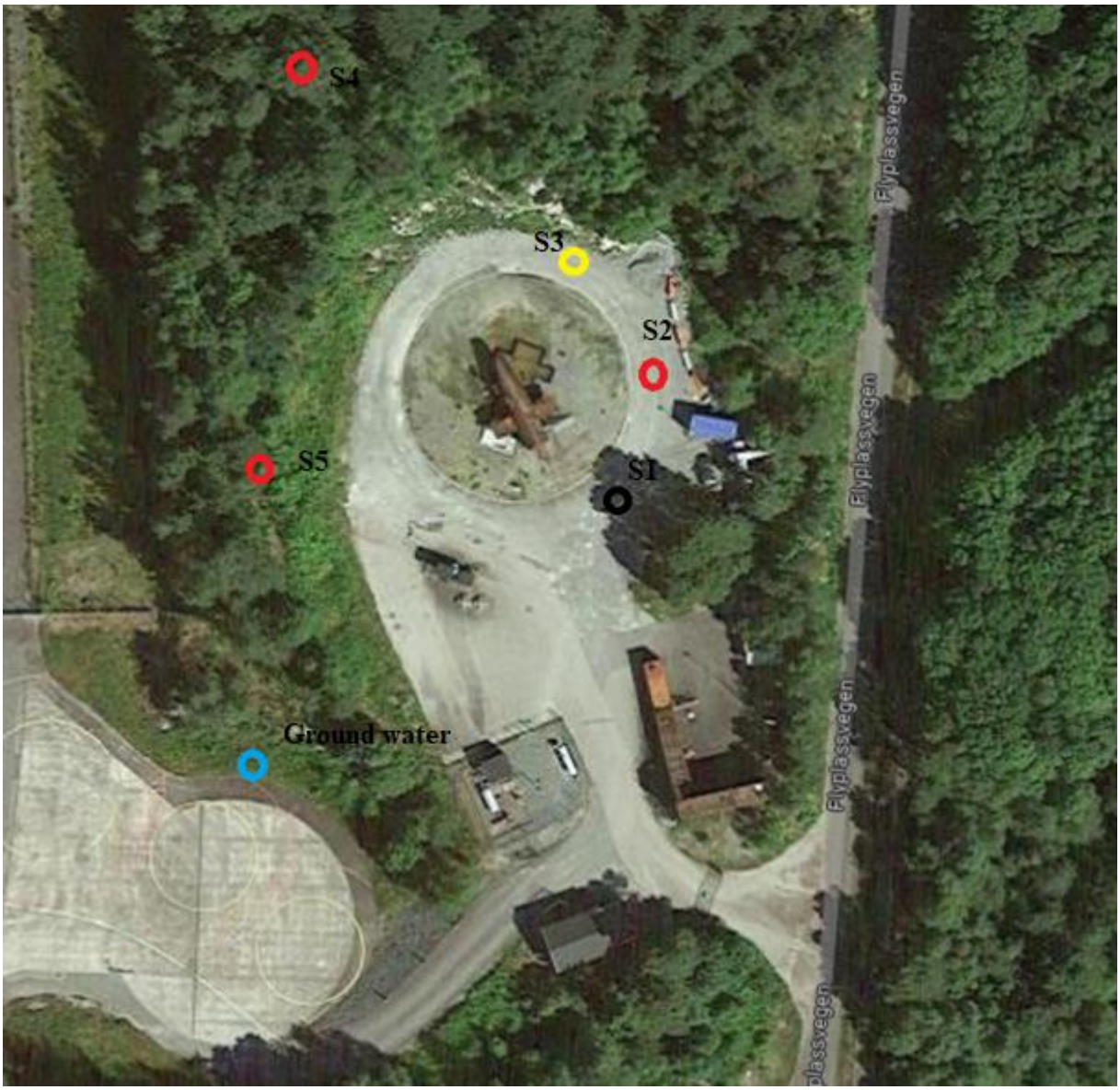


Figure 3.1. *A map showing the area of study, the firefighting training site.*

Biota samples were collected by fishing from the coast/dockyard right outside of the airport to the west (see figure 2.10 in chapter 2.12). In total five Atlantic mackerel (Scomber scombrus), one pollock (Pollachius pollachius) and one goldsinny wrasse (Ctenolabrus rupestris) were sampled using a fishing rod. It should be mentioned that mackerels are a migrating species in contrast to the two other species of fish caught that day, but they were taken for analysis regardless. Due to the fishing equipment being in full contact with sea water it was not washed beforehand or at the site. The fish were wrapped in plastic bags to prevent any cross contamination.

Ground water sample from the vicinity of the training site was collected (see figure 3.1, blue mark). Samples of sea water and water from a nearby stream that flows into the sea, at the

same dockyard that was previously used for fishing has also collected in addition to a sample of foam from the same stream. The ground water sample was provided on site by one of the workers using their own pumping equipment. Tubing's and hoses on the pumping equipment are only used at this specific site so there was little to no risk of cross contamination. It was pumped directly into the sampling bottle. Stream and sea water samples was gathered by submerging the 1000 mL wide mouth bottles in the water and filling them up to about 80 – 90 % of volume since the samples were to be frozen. The foam was gathered in the same type of bottle by scooping up the foam from the surface of the water, trying not to get any stream water inside.

Originally, soil samples were gathered from five different spots at different depths all around and near the training site (see figure 3.1, red points also S1, S2, S4, S3 and S5). Also, additional water samples from lakes and rivers around and inside the airport have also been sampled. Due to time constraints caused by issues with the initial LC column proposed in this project (see chapter 4.1) only the handful of samples mentioned above were taken for analysis (table 3.4) in the final method on a new column.

***Table 3.4**. Overview of the samples gathered for the project.*

	Analysis	**Details**	**Location**
Site 1 soil	Used for qualitative screening experiment, the results can be found in chapter 4.1.	A mix of soil dug up by the excavator. The soil depth ranging from surface up to one meter.	Hotspot, S1 in figure 3.1.
Site 2 soil	Homogenized, not analyzed.	Replicates of surface, mid-level (around 50 cm) and bottom level (around one meter) soil. Dug up by the excavator.	Hotspot, S2 in figure 3.2.
Site 3 soil	Analyzed and reported in the project. Used in TOPA.	Replicates of surface, mid-level (around 50 cm) and bottom level (around one meter) soil. Dug up by the excavator.	Hotspot, S3 in figure 3.3.
Site 4 soil	Homogenized, not analyzed.	A mix of soil ranging from the surface to around 10 cm in depth. Forest soil, gathered at a higher elevation then the hotspot.	Hotspot, S4 in figure 3.1.
Site 5 soil	Homogenized, not analyzed.	Replicates of surface soil and of soil at around 30 cm in depth. Forrest soil,	Hotspot, S5 in figure 3.1.

		gathered slightly below of the hotspots elevation.	
Five mackerels	Analyzed and reported	Different sizes, the exact data on it was lost.	Boat dock in figure 2.9.
One Goldshiny wrassle	Analyzed and reported	Small size, around 15 cm in length.	Boat dock in figure 2.9.
One pollock	Analyzed and reported	Small size, between 15 and 20 cm in length.	Boat dock in figure 2.9.
Ground water	Analyzed and reported		Hotspot, blue mark or "Ground water" in figure 3.3.
River water	Analyzed and reported		Boat dock in figure 2.9.
Foam sample	Analyzed and reported		Boat dock in figure 2.9.
Sea water	Analyzed and reported		Boat dock in figure 2.9.
Additional water samples from lakes around the airport.	Not analyzed, stored in the freezer.	Sampled in the same manner as the other water samples.	Not specified

3.4 Transport and Storage

Large plastic boxes previously washed with methanol on a piece of cloth were used to store the samples taken. To ensure good traceability the samples were labelled with a unique identifier before they were stored in the box, right after sampling.

The samples were transported to the Institute of Marine Research (IMR) in Bergen immediately following sampling and stored in a temperature controlled freezer at around – 20 ºC up until the day of sample pre-treatment and extraction.

3.5 Sample preparation and pre-treatment

Soil:

Firstly, the soil samples were taken out of the freezer to thaw for a few hours before they were transferred out of their bottles onto aluminum traces. They were set aside in a fume hood to air dry for seven days before they were put back into their corresponding bottles. Then, the soil was taken to a ball-mill machine used to mill the soil into a fine powder. Larger rocks and organic material (roots, plants etc.) were removed manually if noticed before the milling step.

The soil was then sieved trough a 1 mm sieve back into their original bottles (see figure 3.2), the rest was either milled once again or discarded. The milling equipment was washed with methanol on a cloth until visually clean between each sample.

Figure 3.2. *Milled and sieved soil sample in its original sampling container.*

Biota:

The sample preparation was done using guidelines of the IMR (27). Briefly, the fish was taken out of the freezer to thaw overnight. Afterwards the internal organs were removed, and the liver put aside into a small PP container. The rest of the organs were discarded. The rest of the fish was filleted and skinned and everything besides boneless fish muscle was discarded. The liver was homogenized with a stand mixer. The fish muscles were homogenized with a kitchen blender to a fine paste. Knives, scalpels, cutting boards, and blenders were washed

using tap water and soap in-between each sample. The samples were then stored in PP containers up to 100 mL in volume, in a plastic bag in a freezer until the extraction. Due to their small size, no liver was successfully extracted from the pollock and goldsinny wrasse.

Foam and water:

Foam and water samples were taken out of the freezer thaw before the extraction. No additional pretreatment was done.

3.6 Extraction

Soil:

The extraction method was based on previous study (28). In this study, zwitterionic PFAS were better extracted by the usage of acidified and alkali methanol, potentially increasing the amount of unknown PFAS which might be oxidized later in the TOPA and transformed into measurable product. The original extraction method was modified by increasing the time of sonication and changing the composition of the acidified and alkali methanol.

Approximately 1 gram of dry and homogenized soil sample was weighed into a 15 mL PP centrifuge tube. At this stage 9 ng of ISTD was added. The sample was first extracted with 0.5 % ammonium hydroxide methanol solution by adding 5 mL to the soil sample followed by vortexing for 10 minutes at 2500 rpm. Then the tube was sonicated at room temperature for 30 minutes before centrifugation at 4000 rpm for 10 minutes. The supernatant was then decanted to a new 15 mL PP centrifuge tube and extraction was repeated and followed by two more extractions with 0.5 % acetic acid methanol solution. The extracts were evaporated under a gentle stream of nitrogen gas on a heating plate set to 40 °C as the extractions continued and more extract was added. In total around 20 mL of methanol was used for the extraction of each sample. The samples were evaporated to around 5 mL before they were transferred to 5 mL syringes equipped with 0.45 µm nylon filters and were filtered into new centrifuge tubes. The evaporation was then continued until the extract were completely dry before the extracts were redissolved in 1 mL of 1:9 (v/v) water:methanol solution. The extractions for each spot and depth were done in triplicates. Afterwards, 200 µL of each sample was taken into LC vials for analysis, the rest of the sample was utilized in the TOPA (chapter 3.7).

For qualitative screening early in the method development process, discussed later in chapter 4.1, a slightly different method was utilized. Approximately 0.1 grams of soil was weighted

and extracted with 10 mL of methanol by vortexing at 2500 rpm for 15 minutes, sonication at room temperature for 30 minutes and centrifugation at 4000 rpm for 10 minutes. The supernant was filtered through 0.45 μm nylon syringe filters before evaporation to ~ 1 mL under a gentle stream of nitrogen gas without any heating. The sample was spiked with 2 ng of internal standards of PFOA and PFOS right after the weighting. However, ultimately the procedure was modified as described above due to difficulty of weighting the precise amount of sample and increasing possibly the detection of trace PFAS.

Biota:

Approximately 0.5 grams of homogenized fish liver and 1.0 grams of homogenized fish muscle (and proficiency test swine liver) was weighted into a 15 mL PP centrifuge tube. Then 9 ng of internal standard of PFOS was added to each tube. Then 4 mL of methanol was added to the tube followed by 15 minutes in a multirotator before the samples were put in a ultrasonic bath at room temperature for 60 minutes. Afterwards the samples underwent centrifugation for 10 minutes at 4000 rpm and decantation into a 5 mL syringes. The extracts were then filtered through a 0.45 μm nylon syringe filters into 50 mL PP centrifuge tubes, see figure 3.3 below. MilliQ water was added until the total volume was around 36 mL in each tube. The samples were then divided equally into two 20 mL ASPEC reagent tubes and underwent SPE extraction as presented in chapter 3.8.

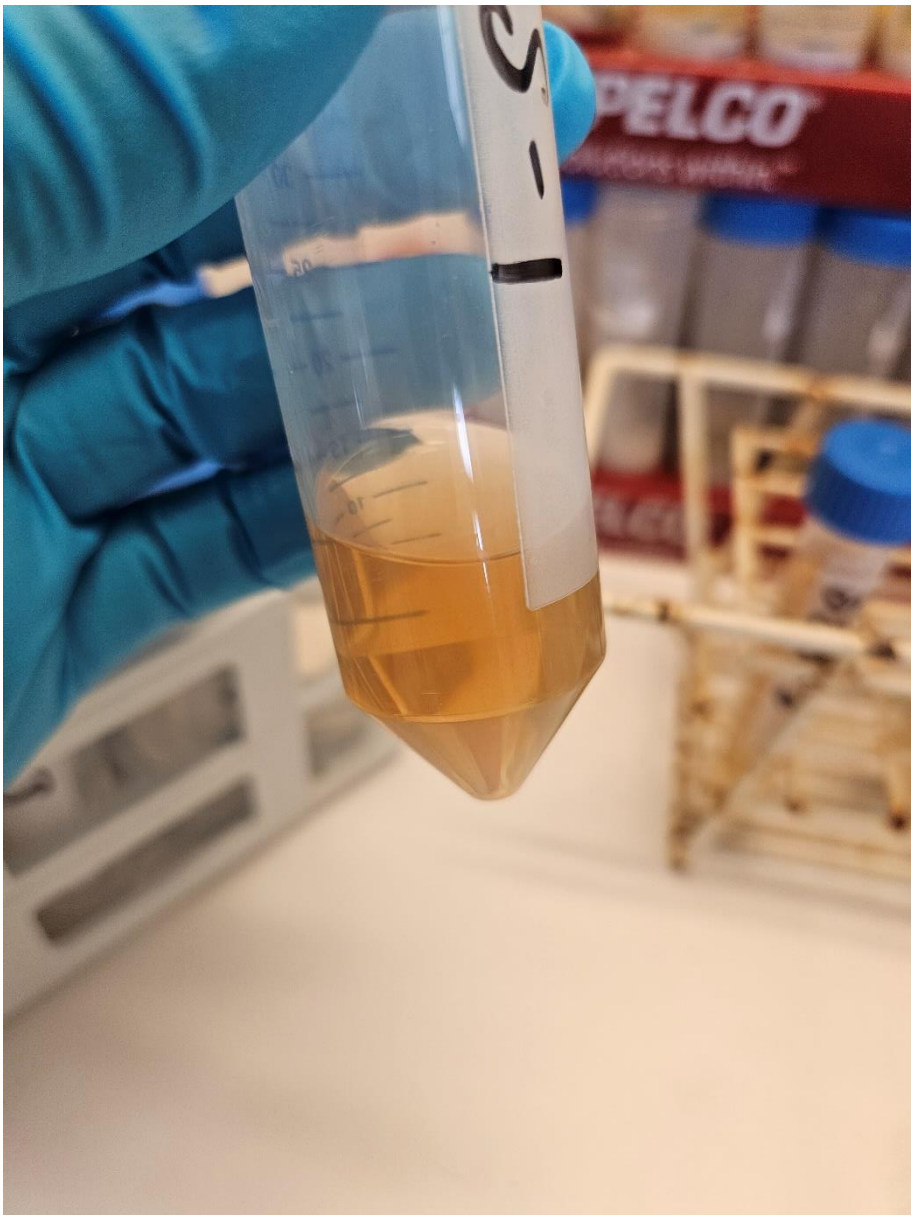


Figure 3.3. *Extract of a biota sample.*

Water and foam:

Foam and water samples underwent extraction presented in chapter 3.8.

3.7 TOPA

The previously mentioned extracts of the soil samples (chapter 3.6) were oxidized by adding 10 mL of 200 mM NaOH and 75 mM potassium persulfate solution before the extracts were vortexed for 30 seconds followed by heating in water bath at 85 °C for 6 hours. Samples were then taken out of the bath and allowed to cool down for around 30 minutes before one drop of hydrochloric acid was added to each tube. The samples were then vortexed for 30 seconds and

stored away in a fridge at 4 °C until the SPE WAX extraction or cleanup the next day (chapter 3.8). Samples from the surface, middle and bottom levels can be seen from left to right respectively in the figure 3.4 below.

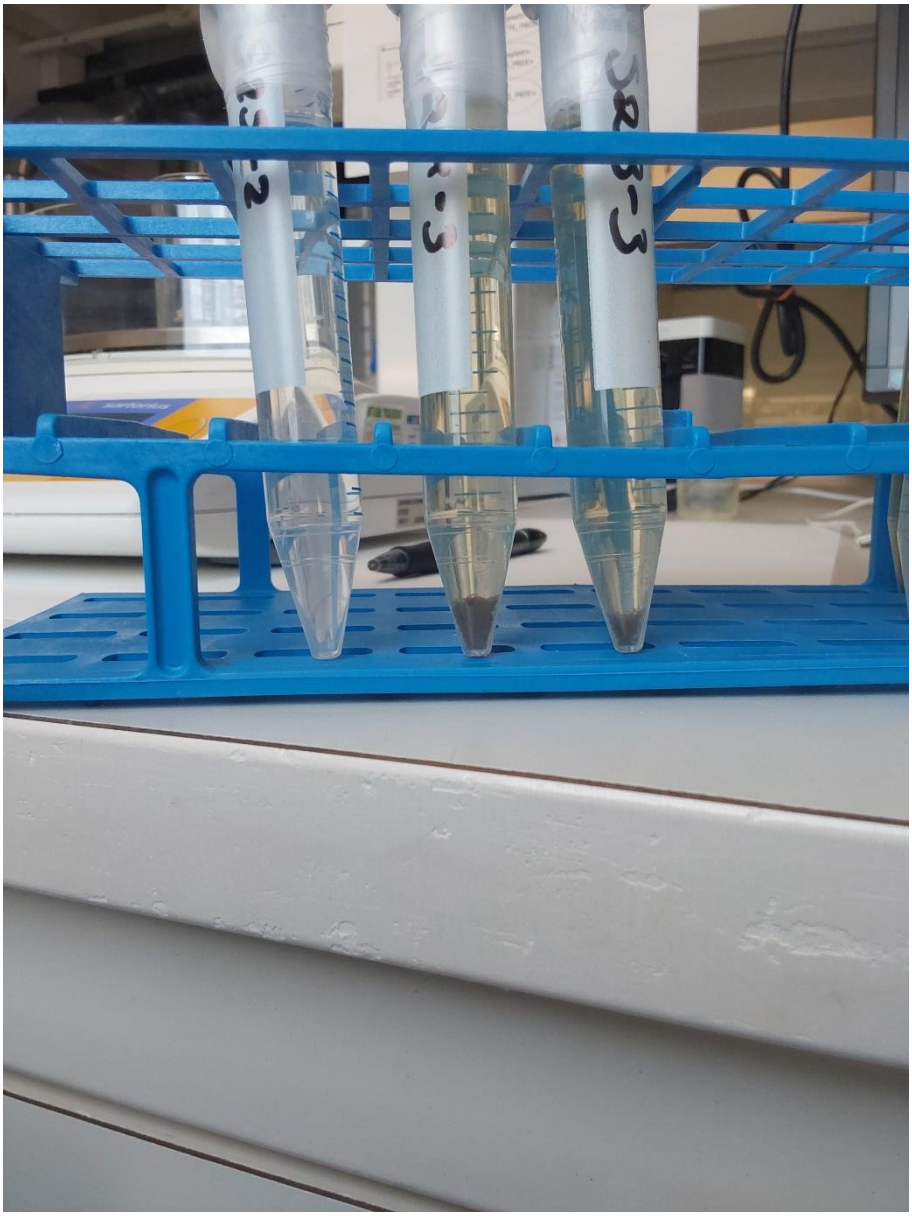


Figure 3.4. *Samples after the TOPA, surface sample to the left, mid−level sample in the middle and bottom sample to the right. The surface sample looks relatively clean compared to the others, the middle sample looks most dirty with most participate formed.*

An unknown precipitate was formed in some one of the TOPA samples. It can be seen in figure 3.4 above. Most of the precipitate was formed in mid and bottom level soil samples. Some was formed in the surface samples which cannot be seen in the figure above (due to picture quality), and no visible particles were detected in any of the blanks. There was also a difference in colour between the samples, compared to the blank samples, the surface sample

had minor colour to it, almost a little misty like. Mid and bottom level samples had brown/green colour just like in the figure above.

3.8 SPE WAX extraction

Two approaches of SPE WAX extraction were performed. Biota samples utilized the automated solid phase extraction cartridge (ASPEC) system, for automated SPE WAX extraction. The foam, water and soil samples underwent manual SPE WAX extraction. In all cases, column conditioning and wash steps were done in the same way. The extractions differ only in sample types and instrumentation. In the case of manual extraction, the flow rate was kept to around < 2 drops per second for all steps. Water samples were extracted in duplicates, around 300 - 400 mL of water per duplicate. Three WAX columns were used per duplicate to speed up the extraction. In some cases, the columns became clogged up with particles (sand etc.) and had to be unclogged by pouring some of the water out of the column. There was only enough foam sample (now water) for one duplicate, around 120 mL, also three columns were used for extraction.

For water and foam samples, 9 ng of internal standards of PFOS was added right before extraction. Picture of the manual SPE setup can be seen in figure 3.5 below.

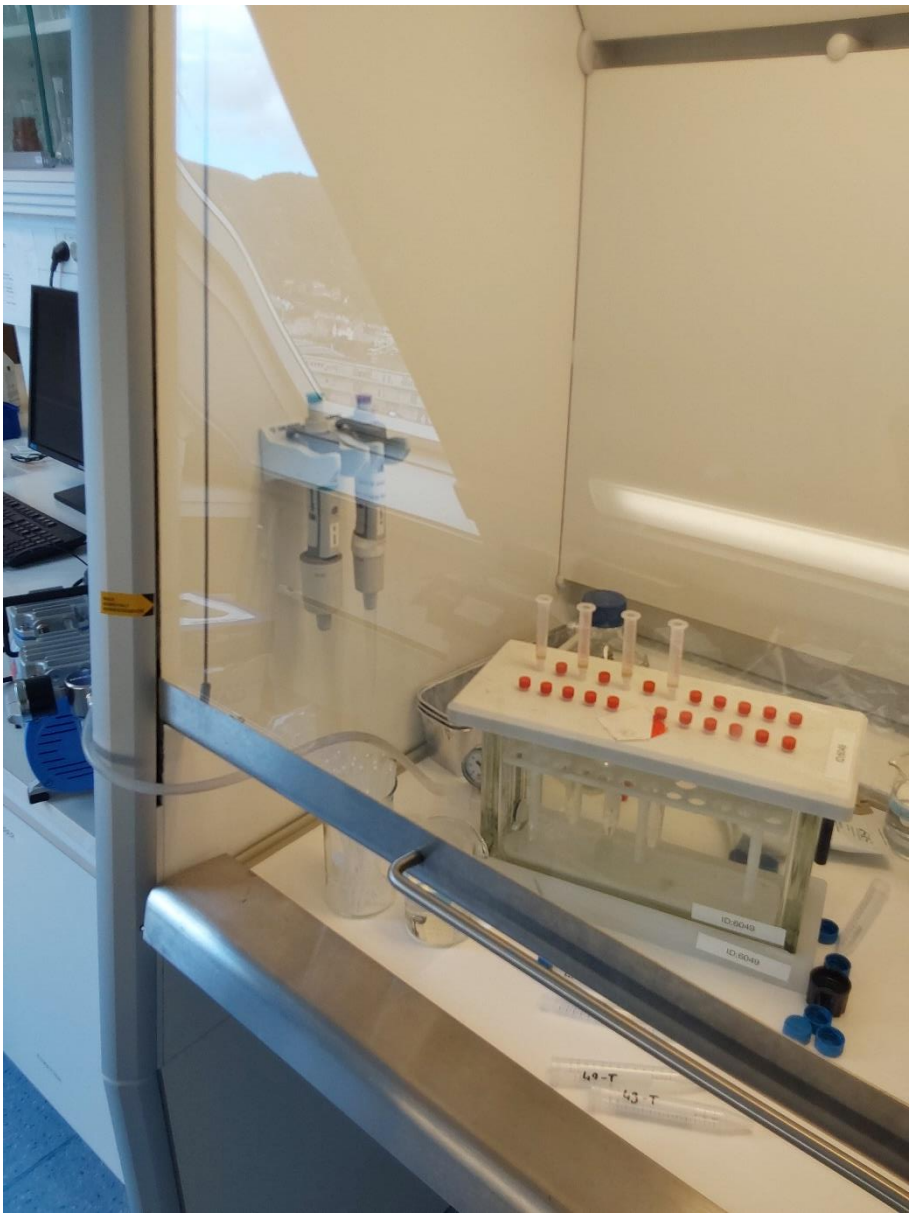


Figure 3.5. *The manual SPE extraction setup, the pump can be seen outside of the fume hood to the left connected to the container with a house. The SPE columns can be seen on top of the container, inside of the container are centrifuge tubes used for gathering up the waste.*

The SPE columns were conditioned by 5 mL 1 % (v/v) ammonium hydroxide in methanol, followed by 5 mL of methanol and 5 mL of MilliQ water. The sample was then carefully loaded onto the column. In the case of foam and water samples, a PP reservoir column was utilized to increase the volume capacity. The column was then washed with 5 mL 2 % (v/v) formic acid in water before the elution was done by 1 mL 1 % (v/v) ammonium hydroxide in methanol. The samples were then transferred into LC vials and were ready for instrument analysis.

3.9 Analytical method development

Originally, the method development was based on a previous study from 2012 which utilized the Ascentis® Express F5, 2.7 µm HPLC Column for separation of PFOS and PFOA isomers (26). The separation of PFOA and PFOS isomers achieved in the reference method can be seen in figure 3.7 below. The results of the method are discussed in chapter 4.1.

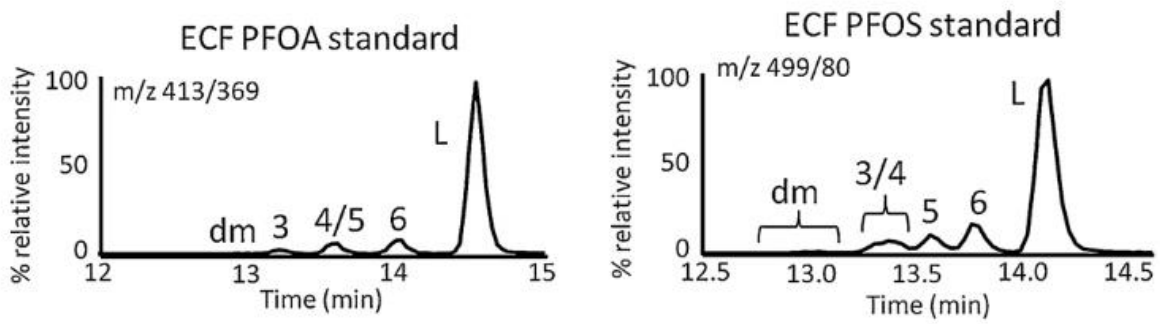


Figure 3.7. *Separation of PFOA and PFOS isomer from the reference study used as a starting point in method development in the thesis (26).*

The same column was acquired, and a modified method was set up, utilizing the same mobile phases as in the original method, the same MS parameters as mentioned later in chapter 3.10 and a different gradient program which can be found in table 3.5 below. The modified method used a slightly longer gradient program (12 minutes longer, 35 minutes total) on order to be able to separate longer chain PFCA and PFSA for other purposes in the future and to try and improve the separation of the isomers. Comparatively the reference method managed to separate several monomethyl PFOA and PFOS isomers in und er 23 minutes.

Table 3.5. *Most recent and successful gradient program used for separation of PFOA and PFOS isomers in the thesis method.*

Time	Flow (mL/min)	% Methanol	Curve	Oven temperature	Autosampler temperature
0 to 5 minutes	0.250	15	5	60 ºC	4 ºC
5 to 10 minutes	0.250	15 to 60	5	60 ºC	4 ºC
10 to 15 minutes	0.250	60 to 80	5	60 ºC	4 ºC
15 to 25 minutes	0.250	100	5	60 ºC	4 ºC
25 to 35 minutes	0.250	15	5	60 ºC	4 ºC
Total time:					
35 minutes			Injection volume: 10 µL		

Sadly, after some issues discussed later (chapter 4.1), another method had to be developed based on the Acquity UPLC® BEH C18 column which was available at the laboratory at the time. The new method on the C18 column was developed using the MS parameters from the previous method (chapter 3.10). The method (gradient and mobile phases) was based on an already existing in house method at the IMR and previous experiences with the F5 column.

It was assumed that lower flow rate and longer gradient program will have a better chance of separation the target analytes. Theoretically, by increasing the time the analytes spend in the column (k term, chapter 2.8), the resolution of the separation should increase. The temperature could not be increased any further due to the recommended limit of 60 ºC for both columns. The flow rate was decreased to 0.200 mL/min and the gradient was prolonged in hope to better the separation of the isomers. The LC parameters of the new method are shown in table 3.6 below.

Table 3.6. LC parameters of the newly developed method used with the C18 column.

Time	Flow (mL/min)	Methanol based mobile phase	Curve	Oven temperature	Autosampler temperature
0 to 5 minutes	0.200	10%	5	60 ºC	4 ºC
5 to 30 minutes	0.200	10 % to 100 %	5	60 ºC	4 ºC
30 to 40 minutes	0.200	100%	5	60 ºC	4 ºC
40 to 41 minutes	0.200	100% to 10 %	5	60 ºC	4 ºC
41 to 50 minutes	0.200	10%	5	60 ºC	4 ºC
Total time:					
50 minutes			Injection volume: 10 µL		

3.10 MS method development

The analytes of interest were detected by the Orbitrap Exploris 120 series mass spectrometer. The MS method was developed from the default settings already existing in the instrument. The method was developed by following the in-software tooltips on different parameters and analysing standard solutions of PFOS and PFOA isomer at concentrations of 1 to 50 ng/mL. The parameter was changed one a at time until visually good sensitivity was achieved. The final settings can be found in appendix E.

3.11 Quality control and method validation parameters

Selectivity

Selectivity of the method describes its ability to accurately measure an analyte in the presence of interference (contamination, noise, etc.) (39).

In this thesis, different blank samples were used to check for methods ability to selectively measure the analyte. Instrument blanks consisting of pure methanol injections were evaluated visually for any cross contamination or carry over effect at the retention time of target analytes. They were injected two times at the start of the analysis to prime the column and always at the end of a set of replicates (about every third injection). If carry over was detected, the samples were investigated for any signs of systematic increase or decrease in concentration and signal.

Field blanks consisting of either MilliQ water for water and biota samples, clean sand for soil samples or air, were collected using the same containers that were used for sampling. The field blanks were transported to the sampling sites and opened for the whole duration of sampling before they were transported back and stored together with the real samples. Field blanks were analyzed for any potential contamination to other samples.

Procedural blanks consisting of clean sand and no matrix (just solvents) were extracted the same way as the real samples and analyzed for potential contamination originating from the extraction and solvents.

Linearity and measuring range:

Calibration standards containing target analytes at concentrations ranging from 0.02 to 50 ng/mL were prepared and spiked with 9 ng of ISTD. These were used to create calibration curves using the internal standard method and linear regression. The criteria for linearity were set to $R^2 \geq 0.99$. Individual concentrations and chromatograms were excluded based on their responses and visual form of the peaks respectively to satisfy the criteria (34). Amounts beyond the highest concentration in the calibration curve were reported as estimates (Est).

Limit of detection (LOD) and Limit of Quantification (LOQ)

LOD is the estimated lowest concentration which can be differentiated from noise by the method. LOQ is the lowest concentration which can be detected and quantified by the method (39).

In this work, LOD is calculated by multiplying the standard error of the y intercept by a value of 3.3 and dividing it by the slope of the regression curve. LOQ was calculated in the same manner but multiplied by a factor of 10 instead of 3.3. The regression was done from calibration standards of low concentrations (up to 1 ng/mL) in the range of LOQ and LOD. It's based on the ICH method (46).

Precision:

Precision is a measure of repeatability and agreement between individual tests (34, 39). In this thesis it is measured by calculating the standard deviation (SD, formula 3.1) and mean values ($\bar{x}$, formula 3.2) between the replicates of each sample from which relative standard deviation percentage (RSD %, formula 3.3) is calculated.

Formula 3.1. Sample standard deviation used for samples with three replicates, n = 3.

$$\mathrm{SD} = \frac{\Sigma(x_i - \bar{\mathrm{x}})}{n-1}$$

Formula 3.2. Arithmetic mean (average) calculated by the sum series of observations divided by the number of observations (n) and where x (observations) can be e.g., concentration (c).

$$\bar{\mathrm{x}} = \frac{\Sigma x_i}{n}$$

Formula 3.3. Relative standard deviation percentage.

$$RSD\ \% = \frac{\mathrm{SD}}{\bar{\mathrm{x}}} * 100\ \%$$

The criteria of precision set in this thesis were based on the 1 part per billion (ppb) RSD % value proposed by the Horwitz equation multiplied by 2/3 (39). It was rounded up to 100 ± 30 % RSD.

Accuracy (trueness)

Accuracy is the measure of the degree of agreement between measured value and known true value (39). It's calculated as the recovery of known amount of analyte (e.g. spiked) from a sample, see formula 3.4 below.

Formula 3.4: The equation used to calculate recovery percentage.

$$Recovery\ (\%) = \frac{Measured\ amount}{Theorecial\ amount} * 100\ \%$$

Two samples were utilized to assess the accuracy of the method. Quality control sample consisting of standard 9 ng standard solutions (native and ISTD) to assess the accuracy of the instrument method. Proficiency test sample of pork liver acquired from the European Union Reference Laboratory (EURL), extracted the same as biota samples (40).

The criteria for accuracy set for this method was set in accordance to the recommendations from the Codex Alimentarius Commission Procedural Manual (45). The recovery criteria for concentrations in ng/g range is 40 - 120 % recovery of target analyte.

3.12 Data handling and quantitation

The analyte concentrations were calculated using the internal standard method. For all analytes, the internal standard of PFOS was used. Calibration curves were plotted by the ratios of analyte signal divided by internal standard signal on the y-axis and the theoretical (spiked) concentration of standard solutions. The calibration was done automatically in TraceFinder software but was presented by Excel in this thesis. The data used was exported directly from TraceFinder.

Analytes coeluting (not chromatographically resolved) were quantified together as one peak/compound. The data was compared by the average of measured concentrations between the replicates of each sample type. Standard deviations were are reported together with the average amounts. Concentrations of soil, fish and liver were recalculated to ng per dry weight (d.w.) or wet weight (w.w.) by dividing the measured concentrations by the weighted weight before calculating the reported average concentrations (for weight data see Appendix F).

Concentrations below LOD were treated as noise and were not reported. Concentrations below LOQ and above LOD were reported and tagged accordingly. LOD and LOQ criteria were checked and applied to the final, average concentration.

The relative change % between average concentrations in samples before and after TOPA were calculated by formula 3.5 below. The differences were presented in chapter 4.3 and showcased in table 4.2.

Formula 3.5: Relative change % used to assess the difference in average concentrations between samples before and after TOPA.

$$\%\ Change = \frac{C_{TOPA} - C_{before\ TOPA}}{C_{before\ TOPA}} * 100\ \%$$

4. Results

4.1 Chromatographic separation in the first method

The chromatographic separation of target PFOS and PFOA isomers using the F5 column can be seen in figures 4.1 and 4.2 below.

PFOA and PFOS separation was partial with several of the isomers coeluting. In total 4 to 5 out of 8 PFOA isomers eluted as individual or mixed peaks. As for PFOS, all of the isomers besides P45/P35DMHxS coeluted but some separation was achieved.

However, in next experiments the column became unstable, and the peaks overlapped. Efforts were made to revive the column (washing and conditioning) in hope that it would bring back the stability and separation. The column was judged unusable and too time consuming to work on for this project. Therefore, a C-18 column (Acquity UPLC® BEH C18 column, 130Å, 1.7 µm, 2.1 mm x 50 mm) was utilized to continue the study.

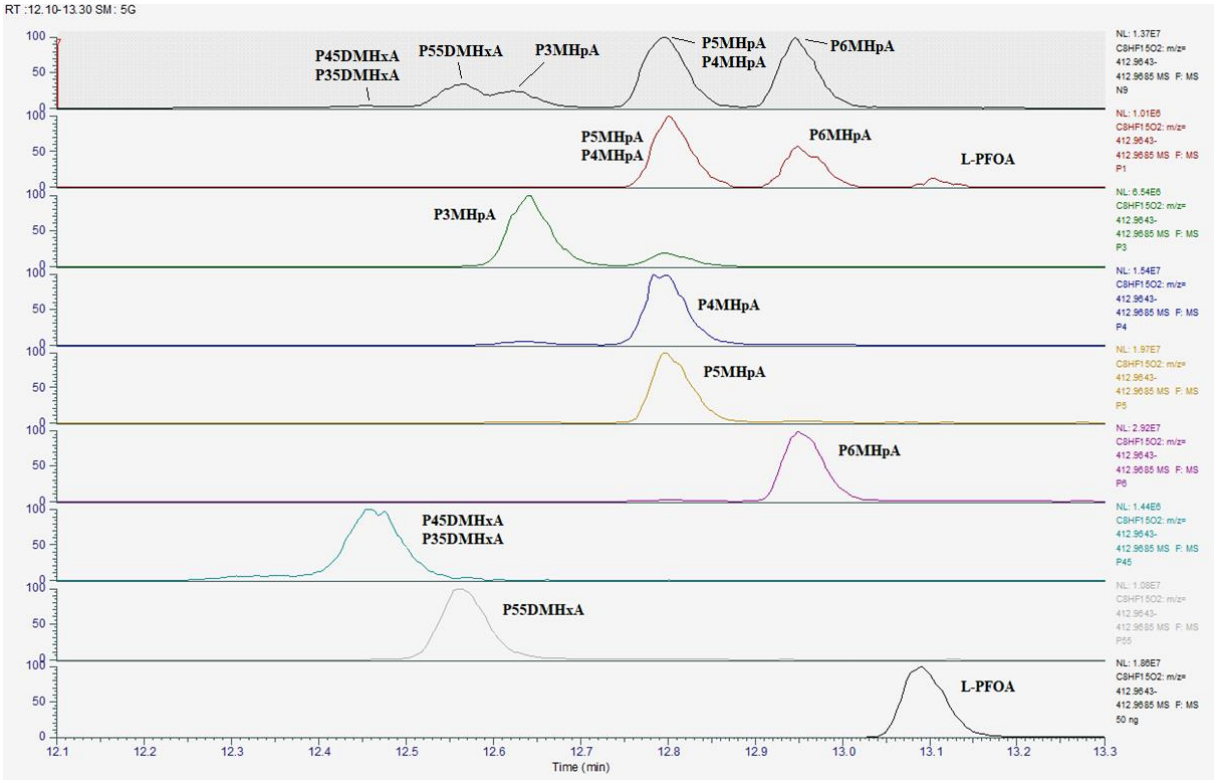


Figure 4.1. *Chromatographic separation of all available nonlinear PFOA isomer mixture at the top and their corresponding individual solutions going downwards, linear PFOA chromatogram can be seen in the bottom chromatogram. All injected at 50 ng/mL.*

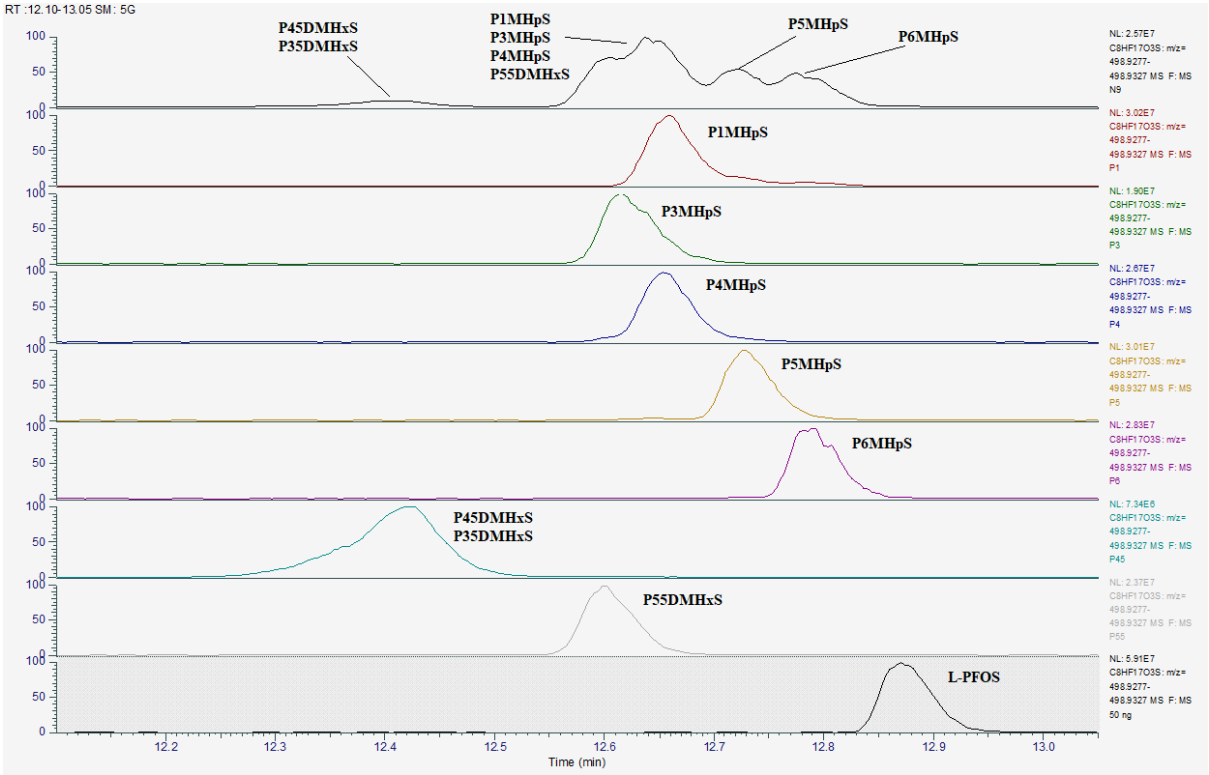


Figure 4.2. *Chromatographic separation of all available nonlinear PFOS isomers mixture at the top and their corresponding individual solutions going downwards, linear PFOS chromatogram can be seen in the bottom chromatogram. All injected at 50 ng/mL.*

Before the column was deemed unusable it was used to analyse a soil sample from the most southern spot in figure 3.1 (mixed soil sample, S1 black circle) for qualitative screening. The chromatographic results can be seen in figures 4.3 and 4.4 below. The chromatogram in figure 4.3 show peaks in the retention time for P55DMHxA, P3MHpA, P5/P4MHpA, P6MHpA and linear PFOA detected in the soil sample. No peak for P45/P35DMHxA was detected. The chromatograms in figure 4.4 shows peaks for P1/P3/P4MHpS, P5MHpS, P6MHpS and linear PFOS detected in the soil sample. These peaks are not completely resolved and several of the isomers coelute. No peak for P45/P35DMHxS was detected.

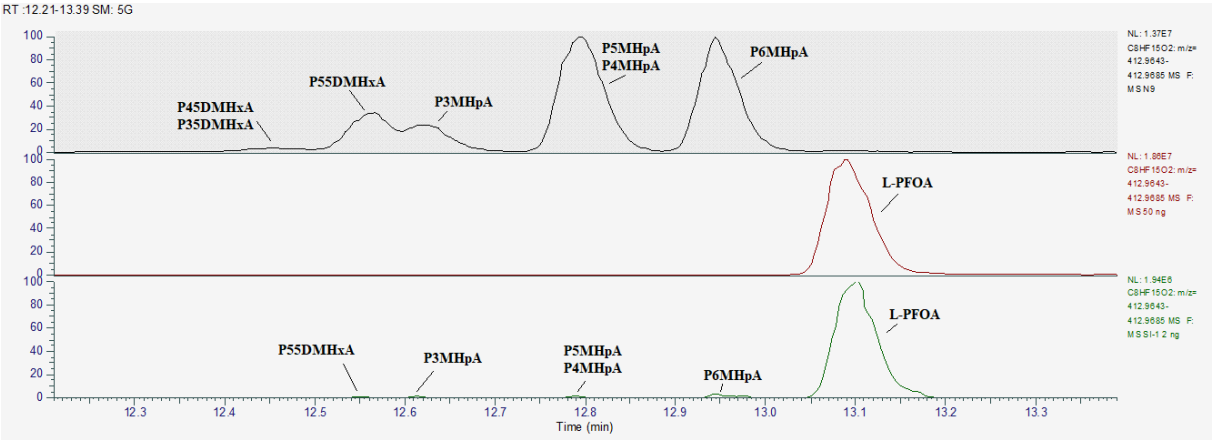


Figure 4.3. *Chromatographic separation of the isomer mixture at the top, chromatogram of the linear PFOA in the middle and the chromatographic separation of the real soil sample at the bottom.*

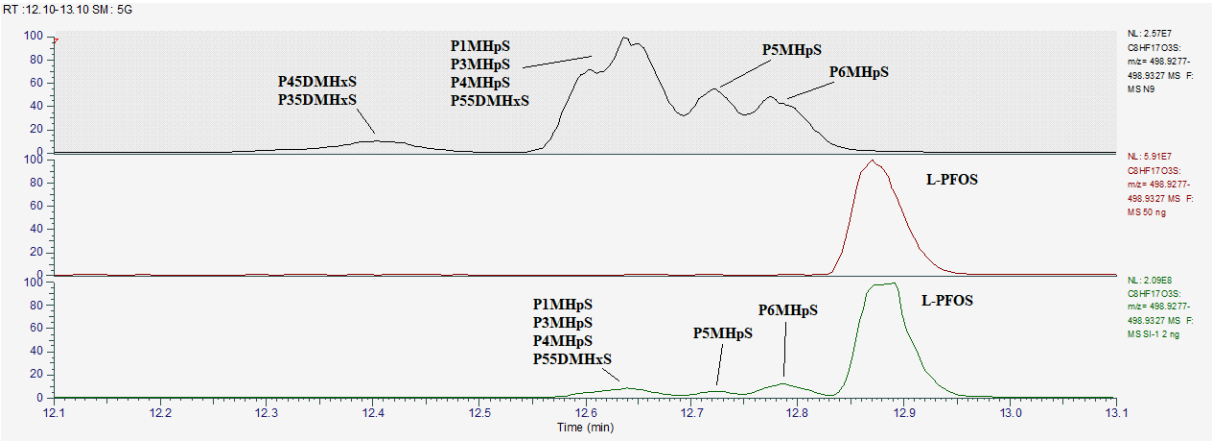


Figure 4.4. *Chromatographic separation of the isomer mixture at the top, chromatogram of the linear PFOS in the middle and the chromatographic separation of the real soil sample at the bottom.*

4.2 Chromatographic separation in current method

The chromatographic separation in the current method can be seen in figures 4.5 and 4.6 for PFOS and PFOA respectively.

It utilizes the previously mentioned (chapter 4.1) Acquity UPLC® BEH C18 column. Linear, P5 and P6 PFOS coeluted together, therefore they have been measured as one peak. P55DMHxS coeluted with all remaining monomethyl PFOS isomers and was also measured with the others as one peak. In the case of PFOA, the linear didn't coelute with any of the other isomers and the monomethyl and dimethyl isomers coeluted separately from each other.

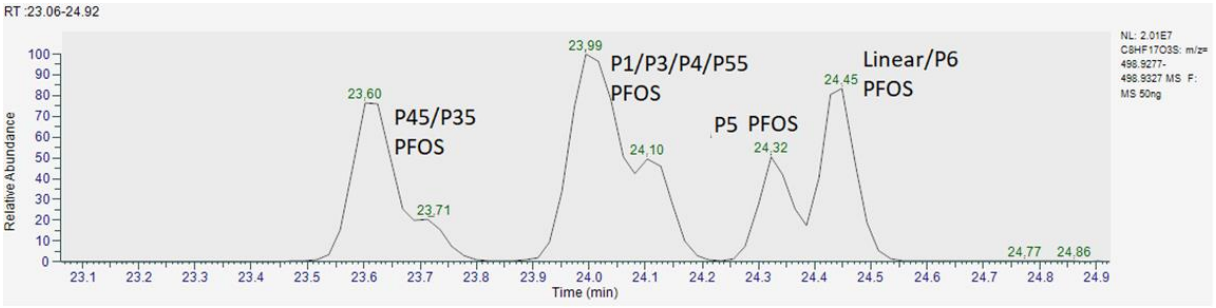


Figure 4.5. *PFOS separation achieved on the C18 column injected at 50 ng/mL.*

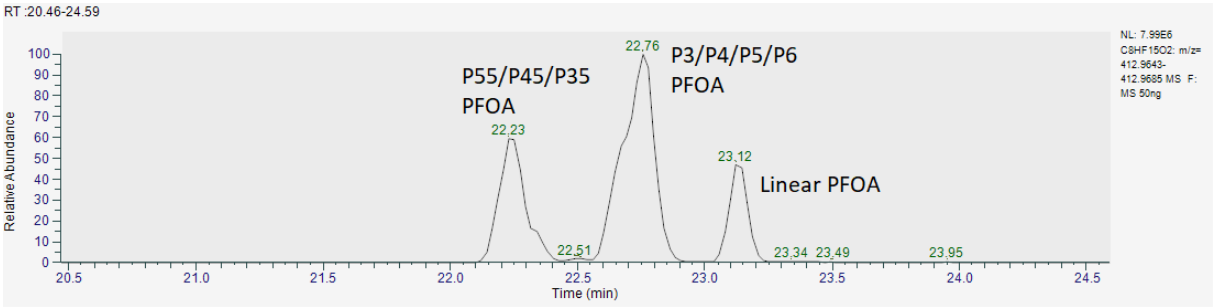


Figure 4.6. *PFOA separation achieved on the C18 column injected at 50 ng/mL.*

4.3 Findings in soil samples

Table 4.1 shows an overview of the findings in soil samples both before and after TOPA. No dimethyl PFOA was found in soil samples. Dimethyl PFOS was found in around half of the samples. Monomethyl and linear isomers of both PFOS and PFOA were the most common, found consistently in all soil samples.

The highest PFOA concentration before and after TOPA was found in mid-level soil, with the highest amount of both linear and monomethyl PFOA at 108 ng/g and 3.77 ng/g respectively (before TOPA) and 448 ng/g and 20.19 ng/g (after TOPA). Note that the reported amount of linear PFOA is an estimate as the calculated amount was beyond the calibration range. The lowest amounts of PFOA were found in the surface soil.

For PFOS the highest concentrations before TOPA were found in the mid-level soil at an estimated amount of 17940 ng/g (linear/P6/P5) and 1624 ng/g (P1/P3/P4/P55). The highest amount of P45/P35 PFOS before TOPA was found in bottom soil (738 ng/g). The lowest amount of PFOS of all type was found in surface soil. In TOPA samples, average PFOS concentrations varied depending on analyte type and sample depth.

Table 4.1. Overview over the average concentrations and their standard deviations of both PFOS and PFOA in soil samples, before and after TOPA. Concentrations are given in ng/g d.w. Concentration beyond calibration are reported as estimates (Est).

	Surface soil		**Mid-level soil**		**Bottom soil**	
	n = 3		n = 3		n = 3	
	Average conc.	SD	Average conc.	SD	Average conc.	SD
Linear PFOA	0.568	0.065	108 (Est)	5.70	73.6 (Est)	7.65
Linear PFOA after TOPA	4.11	0.196	448 (Est)	51.8	313 (Est)	61.4
Monomethyl PFOA	0.006	0.003	3.77	0.239	2.44	0,519
Monomethyl PFOA after TOPA	0.292	0.134	19.7	2.10	16.5	2.98
Dimethyl PFOA	ND	NA	ND	NA	ND	NA
Dimethyl PFOA after TOPA	ND	NA	ND	NA	ND	NA
Linear/P6/P5 PFOS	14.9	1.91	17940 (Est)	733	4198 (Est)	249
Linear/P6/P5 PFOS after TOPA	15.4	2,43	19736 (Est)	1371	4404 (Est)	209
P1/P3/P4/P55 PFOS	0.489	0.050	1624 (Est)	27.8	1080 (Est)	131
P1/P3/P4/P55 PFOS after TOPA	0.589	0.056	1152 (Est)	48.6	738 (Est)	59.1
P45/P35 PFOS	0.016	0.003	NF	NA	39.6	11.4
P45/P35 PFOS after TOPA	NF	NA	27.9	2.06	25.3	4.49

Table 4.2 shows an overview of the difference in the measured (and estimated) amounts (% change) of both PFOS and PFOA in samples from before and after TOPA.

PFOA concentrations increased the most after TOPA, ranging from 314 % to 4766 % increase. The highest increase was found for the monomethyl PFOA in surface soil, at 4766 % increase. The second highest was linear PFOA also in surface soil, at 623 % increase. The lowest increase for PFOA was for the linear isomer at 314 % in mid-level soil.

Linear/P6/P5 PFOS increased slightly by 3 % to 10 % at all depths. P1/P3/P4/P55 PFOS increased by 20 % in the surface soil (highest increase for PFOS) but decreased by 29 % and 31 % in mid-level and bottom level soil respectively. P45/P35 PFOS decreased completely after TOPA in surface soil, appeared after TOPA (no % change could be calculated) in mid-level soil and decreased by 36 % in bottom soil.

Table 4.2. *Overview of % change in concentration between before and after TOPA soil samples at different depths.*

Sample	Surface soil	Mid-level soil	Bottom soil
Linear PFOA	623 % increase	314 % increase	325 % increase
Monomethyl PFOA	4766 % increase	422 % increase	576 % increase
Dimethyl PFOA	NA	NA	NA
Linear/P6/P5 PFOS	3 % increase	10 % increase	4 % increase
P1/P3/P4/P55 PFOS	20 % increase	29 % decrease	31 % decrease
P45/P35 PFOS	100 % decrease*	NA**	36 % decrease

*No analytes were detected in the TOPA sample.

**Analytes appeared in the sample after TOPA. No increase was calculated.

4.4 Findings in water and foam samples

Table 4.3 shows an overview of average concentrations and their standard deviations of target analytes in water sand foam samples.

No PFOA was found in the ground water, river water and foam samples. Linear and monomethyl PFOA was found in the sea water sample.

Linear/P6/P5 and P1/P3/P4/P55 PFOS isomers were found in the sea water samples. Some PFOS was found in river water and foam sample and was estimated by external calibration due to missing internal standard. Only the linear/P6/P5 PFOS was found in these samples.

Table 4.3. *An overview of average concentrations and their standard deviations of target analytes in water and foam samples. Concentrations are given in ng/mL.*

	Ground water		**River water**		**Sea water**		**Foam sample**	
	n = 2		n = 2		n = 2		n = 1	
	Average conc.	SD	Average conc.	SD	Average conc.	SD	Average conc.	SD
Linear PFOA	ND	NA	ND	NA	0.284	0.005	ND	NA
Monomethyl PFOA	ND	NA	ND	NA	0.007	0.003	ND	NA
Dimethyl PFOA	ND	NA	ND	NA	ND	NA	ND	NA

Linear/P6/P5 PFOS	ND	NA	0.46*	NA**	0.51	0.05	1.62*	NA**
P1/P3/P4/P55 PFOS	ND	NA	ND	NA	0.065	0.023	ND	NA
P45/P35 PFOS	ND	NA	ND	NA	ND	NA	ND	NA

*Calculated by external calibration method due to missing internal standard.
**No standard deviation and mean were calculated due to only one replicate containing target analyte.

4.5 Findings in biota samples

Table 4.4 shows an overview of findings in biota sample. The findings are presented as calculated average concentration of target analytes.

Linear PFOA was the only PFOA detected in all of the biota samples. It was detected in three of the five liver samples, ranging in concentration between 0.59 ng/g to 0.95 ng/g.

Linear/P6/P5 PFOS was detected in all of the biota samples. In mackerel muscle the concentrations ranged between 0.25 ng/g and 0.39 ng/g. In mackerel liver the concentrations ranged from 1.37 ng/g to 2.43 ng/g. Other PFOS isomers (P1/P3/P4/P55 only) were found in two of the liver samples and only in one replicate of each sample (no mean concentration could be calculated). The concentrations ranged from 0.024 ng/g to 0.042 ng/g. Linear/P6/P5 PFOS was also detected in muscles of Pollock and Goldshiny wrassle at concentrations of 0.367 ng/g and 0.112 ng/g respectively. No other PFOS was detected in these fish.

Table 4.4. *An overview of findings in biota samples. Concentrations are given in ng/g w.w.*

Sample	Average conc. Linear PFOA (n=2)	Average conc. Monomethyl PFOA (n=2)	Average conc. Dimethyl PFOA (n=2)
Mackerel 1, muscle.	ND	ND	ND
Mackerel 2, muscle.	ND	ND	ND
Mackerel 3, muscle.	ND	ND	ND
Mackerel 4, muscle.	ND	ND	ND
Mackerel 5, muscle.	ND	ND	ND
Mackerel 1, liver.	0.59*	ND	ND
Mackerel 2, liver.	ND	ND	ND
Mackerel 3, liver.	ND	ND	ND
Mackerel 4, liver.	0,95	ND	ND
Mackerel 5, liver.	0,79	ND	ND
Pollock muscle	ND	ND	ND
Goldsinny wrasse	ND	ND	ND

Sample	Average conc. Linear/P6/P5 PFOS (n=2)	Average conc. P1/P3/P4/P55 PFOS (n=2)	Average conc. P45/P35 PFOS (n=2)
Mackerel 1, muscle.	0.39	ND	ND
Mackerel 2, muscle.	0.25	ND	ND
Mackerel 3, muscle.	0.25	ND	ND
Mackerel 4, muscle.	0.31	ND	ND
Mackerel 5, muscle.	0.37	ND	ND
Mackerel 1, liver.	4.02	0.042*	ND
Mackerel 2, liver.	2.90	ND	ND
Mackerel 3, liver.	3.28	ND	ND
Mackerel 4, liver.	4.76	0.024*	ND
Mackerel 5, liver.	4.00	ND	ND
Pollock muscle	0.367	ND	ND
Goldsinny wrasse	0.112	ND	ND

*Only one replicate contained the target analyte and mean could not be calculated. The calculated amount from this replicate was reported directly.

4.6 Quality control and method validation results

Sensitivity results (blank samples)

Table 4.5 shows an overview of contaminants found in several of the blank samples. Additionally cross contamination was detected in most of the instrument blanks. Mostly peaks for linear/P6/P5 PFOS were found, linear PFOA was rarely present. Other isomers were barely detectable with peaks consisting of only several data points.

It must be noted that one of the TOPA blanks was lost during the heating process (crack in the centrifuge tube). Blanks where the ISTD was not present or present in minor amounts compared to real samples were estimated by external calibration method by comparing the response of the blanks to the response of the standards in the calibration curve.

Field blanks used for soil sampling show minor linear/P6/P5 PFOS contamination, estimated to be below 0.05 ng/mL. No PFOA contamination was found.

Field blanks used for fishing and water sampling show some degree of linear/P6 PFOS contamination, containing around 0.164 ng/mL. Some linear PFOA contamination was also found, estimated to be < 0.1 ng/mL.

Linear/P6/P5 PFOS was found in the procedural blank containing clean sand from before TOPA, estimated to be < 0.05 ng/mL. After TOPA, linear/P6/P5 PFOS was estimated to be < 20 ng/mL. Additionally, P1/P3/P4/P55 PFOS was detected and estimated to be < 0.5 ng/mL. No PFOA was detected in any of procedural blanks.

Table 4.5. *Overview of the calculated and estimated contamination levels found in field and procedural blanks.*

	Analyte found	Average calculated amount in ng/mL	Average response	Estimated amount by external calibration
Field blank, (soil sampling) n = 3.	Linear/P6/P5 PFOS	0.164	123079	< 0.05 ng/mL
Field blank, (soil sampling) n = 3.	PFOA	NA	NA	NA
Field blank fishing and water sampling, n =2.	Linear/P6/P5 PFOS	NA	218425	< 0.05 ng/mL
Field blank fishing and water sampling, n =2.	Linear PFOA	NA	29583*	< 0.1 ng/mL
Blank with matrix, pre TOPA, n = 2.	Linear/P6/P5 PFOS	NA	53808	< 0.05 ng/mL
Blank with matrix, pre TOPA, n = 2.	PFOA	NA	NA	NA
Blank TOPA, n = 1**	Linear/P6/P5 PFOS	NA	25494279	< 20 ng/mL
Blank TOPA, n = 1**	P1/P3/P4/P55 PFOS	NA	1156431	< 0.5 ng/mL
Blank TOPA, n = 1**	PFOA	NA	NA	NA

*No average was calculated due response in only one replicate.
**Due to loss of one of the blanks during the heating process, only one blank remained.

Linearity and measurement range results

Table 4.6 shows an overview of linear range and linearity of each analyte group. The linear range was shorter for PFOA who's signal was weaker at the lower end of the calibration curve

compared to PFOS. Linear/P6/P5 PFOS showed weaker at the lower end compared to other P1/P3/P4/P55 and P45/P35 isomers.

Table 4.6. *Calibration data for PFOA and PFOS isomers acquired in this thesis.*

Analyte group	Correlation coefficient (R^2)	Linear range (ng/mL)
Linear PFOA	0.999433	0.1 - 50
Monomethyl PFOA	0.997220	0.1 - 50
Dimethyl PFOA	0.999728	0.1 - 50
Linear/P6/P5 PFOS	0.999406	0.05 - 50
P1/P3/P4/P55 PFOS	0.998784	0.02 - 50
P45/P35 PFOS	0.999051	0.02 - 50

LOD and LOQ results

Table 4.7 shows an overview of the LOD and LOQ results.

Table 4.7. *Results of the LOD and LOQ for all target analytes.*

Analyte	LOD (ng/mL)	LOQ (ng/mL)
Linear PFOA	0.003	0.008
Monomethyl PFOA	0.002	0.005
Dimethyl PFOA	0.002	0.005
Linear/P6/P5 PFOS	0.004	0.013
P1/P3/P4/P55 PFOS	0.003	0.009
P45/P35 PFOS	0.001	0.004

Precision results

Table 4.8 shows an overview of the relative standard deviations of each soil samples. Table 4.9 and 4.10 show the relative standard deviations of water/foam and biota samples respectively.

Values that fell outside of the set criteria (100 ± 30%) were marked by a hashtag "#". Briefly monomethyl PFOA in surface soil both before and after TOPA ranged from 52.6 % to 45.7 % respectively. One water sample, P1/P3/P4/P55 PFOS in sea water at 36.2 % RSD.

Table 4.8. *RSD % values for each soil sample, before and after TOPA.*

	Surface soil RSD	Mid-level soil RSD	Bottom soil RSD
Linear PFOA	11.5 %	5.26 %	10.4 %
Linear PFOA TOPA	4.79 %	11.5 %	19.6 %
Monomethyl PFOA	# 52.6 %	6.32 %	21.2 %

Monomethyl PFOA TOPA	# 45.7 %	10.7 %	18.0 %
Dimethyl PFOA	NA	NA	NA
Dimethyl PFOA TOPA	NA	NA	NA
Linear/P6/P5 PFOS	12.9 %	4.08 %	5.94 %
Linear/P6/P5 PFOS TOPA	15.7 %	6.94 %	4.75 %
P1/P3/P4/P55 PFOS	10.3 %	1.71 %	12.1 %
P1/P3/P4/P55 PFOS TOPA	9.49 %	4.21 %	8.01 %
P45/P35 PFOS	18.8 %	NA	28.9 %
P45/P35 PFOS TOPA	NA	7.37 %	17.7 %

Table 4.9. *RSD % values of biota samples.*

Sample	**RSD % Linear PFOA**	**RSD % Monomethyl PFOA**	**RSD % Dimethyl PFOA**
Mackerel 1, muscle.	ND	ND	ND
Mackerel 2, muscle.	ND	ND	ND
Mackerel 3, muscle.	ND	ND	ND
Mackerel 4, muscle.	ND	ND	ND
Mackerel 5, muscle.	ND	ND	ND
Mackerel 1, liver.	NA*	ND	ND
Mackerel 2, liver.	ND	ND	ND
Mackerel 3, liver.	ND	ND	ND
Mackerel 4, liver.	ND	ND	ND
Mackerel 4, liver.	16.2 %	ND	ND
Mackerel 5, liver.	13.0 %	ND	ND
Pollock muscle	ND	ND	ND
Goldsinny wrasse	ND	ND	ND
Sample	**RSD % Linear/P6/P5 PFOS**	**RSD % P1/P3/P4/P55 PFOS**	**RSD % P45/P35 PFOS**
Mackerel 1, muscle.	9.4 %	ND	ND
Mackerel 2, muscle.	3.7 %	ND	ND
Mackerel 3, muscle.	1.4 %	ND	ND
Mackerel 4, muscle.	1.1 %	ND	ND
Mackerel 5, muscle.	5.0 %	ND	ND
Mackerel 1, liver.	0.62 %	NA*	ND
Mackerel 2, liver.	3.5 %	ND	ND
Mackerel 3, liver.	15.5 %	ND	ND
Mackerel 4, liver.	5.5 %	NA*	ND
Mackerel 5, liver.	3.4 %	ND	ND
Pollock muscle	2.0 %	ND	ND

Goldsinny wrasse	0.29 %	ND	ND

*Only one replicate was detected so no RSD % was calculated.

Table 4.10. *RSD % values for water samples.*

	Ground water RSD %	**River water RSD %**	**Sea water RSD %**	**Foam sample RSD %**
Linear PFOA	ND	ND	1.74 %	ND
Monomethyl PFOA	ND	ND	28.6 %	ND
Dimethyl PFOA	ND	ND	ND	ND
Linear/P6/P5 PFOS	ND	NA	10.9 %	NA
P1/P3/P4/P55 PFOS	ND	ND	# 36.2 %	ND
P45/P35 PFOS	ND	ND	ND	ND

Accuracy (trueness) results

Table 4.11 shows the results of the quality control sample used to estimate accuracy (% recovery) of the method.

The recovery of the QC sample for PFOS was within the given limit (40 - 120 %). The recovery of the QC sample for PFOA was also within the limit set by the method.

Table 4.11. *Quality control samples of linear PFOS and PFOA and their recoveries.*

Analyte	**Theoretical amount (ng)**	**Measured amount (ng)**	**Recovery %**
PFOS	9.00	5.83	64.7
PFOA	9.00	10.03	111.4

Table 4.12 shows the results of the proficiency test.

The recovery of PFOS from the proficiency test sample was within the criteria of recovery. The recovery of PFOA way outside the criteria of recovery.

Table 4.12. *Overview of the results from the proficiency test.*

Analyte	**Theoretical amount (ng/g w.w.)**	**Total average measured amount (ng/g w.w.)**	**Recovery**
Total PFOS	29.8	32.71	109.7 %
Total PFOA	0.847	2.417	283.6 %

5. Discussion

5.1 Chromatographic separation

C18 column

Separation of all target analytes was not completely achieved in the current method on the C18 column. The current method separates all of the target PFOA analytes into three main groups; the linear, monomethyl and dimethyl isomers. PFOS isomers has proven to be more problematic to separate then PFOA. P6MHpS and P5MHpS coeluted with its linear isomer as well as the P55DMHxS coeluted with the remaining monomethyl isomers. A complete separation was not expected, as the usual separation on C18 columns reported in other studies also struggles with separation of some of the isomers (14). The separation achieved on this column was enough to get some overview of the variety of contamination in the soil samples. It has also revealed some valuable information about the differences in transport of different PFAS trough the soil (discussed below).

The separation of linear/P6 PFOS from P5MHpS could have been improved with lower injection volume as it would possibly result in slimmer peaks. Possibly a 5 µL injection as opposed to the current 10 µL. It might have also been useful in separation of other isomers. Improving MS parameters might also yield better resolutions as the method wasn't fully optimized with only PFOA and PFOS in mind. The initial plan was to add other compounds e.g. short chain PFSAs and PFCAs and their potential precursors which would be especially interesting for TOPA.

F5 column

Separation on the F5 column was comparatively much better and efficient then on the C18 column. It was very similar to the reference study, and seemingly increasing the run time didn't affect the separation much, meaning other PFAS could also have been analysed.

The linear, P6MHpA, P4MHpA and P5MHpA were all separated the same way as in the reference study. The P55DMHxA and P3MHpA coeluted, furthermore they were separated from P45DMHxA and P35DMHxA. The reference study utilized P24DMHxA as their only dimethyl PFOA isomer, not used in this thesis. Therefore, it is hard to compare the current results with the results in the reference study. Arguably, the current separation managed to separate more dimethyl compounds which is better.

PFOS separation was arguably more difficult to optimize. The pattern of separation was the same with the P1MHpS and P55DMHxS coeluting together with P3MHpS/P4MHpS.

However, the dimethyl PFOS in the reference study was P34DMHxS which was not utilized in this thesis, which eluted in the same pattern as the P45DMHxS/P35DMHxS utilized in this thesis. Overall, both separation in this thesis and the reference study are very similar.

The cause of the degradation of the column is unknown, perhaps the clean-up method used for the samples was inefficient resulting in clogging up the column and destroying it in the process. It is unlikely that pressure of the pump would harm the column as the maximum pressure was set 10 % below the maximum allowed by the manufacturer. Storage was also no different than for the other columns, it was always washed and filled with pure organic solvent (methanol in this case), plugged, and stored in its original container inside of a cupboard or on the laboratory desk. The washings consisted of pure water and methanol, starting at 10 to 15 % methanol, and increasing to 100 % in a span of 2-3 hours. The recommended organic solvent for storage of the column was 100 % acetonitrile, which was impossible to achieve due to the pump on the LC system having issues with pumping pure acetonitrile, methanol was chosen instead. This might have contributed to the degradation of the column, but was unlikely the sole reason for it, as methanol is very commonly used for storage of such columns, especially when they are routinely used.

Chromatography summarized

In the case of both columns, several isomers coeluted meaning they would share similar chemical and physical characteristics due to similar structure. There is an obvious pattern where the shorter chain isomers are less retained and elute quicker. This is most likely due to them being more soluble as suggested in the previous study made at Flesland airport (17). More soluble compounds will be less retained by the stationary phase, resulting in lower retention times. There are also groupings of approximately dimethyl, monomethyl and linear isomers eluting together, probably due to similar structure and chemical properties associated with it. Its shows that shorter compounds are less retained by the stationary phase, it might mean that they are more polar and more attracted to the mobile phase due to their structure.

Also, one similarity between the F5 and C18 column was that PFOA isomers were easier to separate then PFOS, suggesting that the carboxyl group was a better match for the reversed phase chemistry in the stationary phase and the mobile phase. As mentioned in chapter 2.4, PFOA is a weaker Brønsted acid compared to PFOS. This might have results in PFOA being less polar as its better at retaining its hydrogen atom. This results in PFOA being more retained by the nonpolar stationary phase, positively affecting the separation (resolution) due

to the increased retention factor in the resolution equation (chapter 2.8, formula 2.1). PFOA is also significantly lighter then PFOS, which could also have been a factor in the separation.

5.2 Findings in soil samples

Figures 5.1 and 5.2 show a visual representation of the findings for PFOA and PFOS respectively in soil samples before TOPA.

As shown in the results section (and figures 5.1 and 5.2) surface soil was overall the least contaminated. It must be mentioned again that the surface of the training site was covered by fresh soil and gravel due to historical use of AFFF. This makes the comparison between the surface and deeper layers much more difficult. With that in mind, PFAS was found in some quantities at the surface of the training site. The training site was not in use after the "cover-up". It might suggest that the transport of PFAS happens both up and down the soil, or it's coming down (e.g. with rain) from the elevated forest floor right beside the training site. No reports were found to support the claim that PFAS might travel upwards in the soil.

Overall, the most amount of PFOS and PFOA was found in the mid-level soil samples. The concentrations decrease in the bottom level soil samples. The only exception to this trend was the P45/P35 PFOS which was only found in the surface and bottom soil. The data show that the deposition of linear and monomethyl isomers trough the soil is very similar for both PFOS and PFOA.

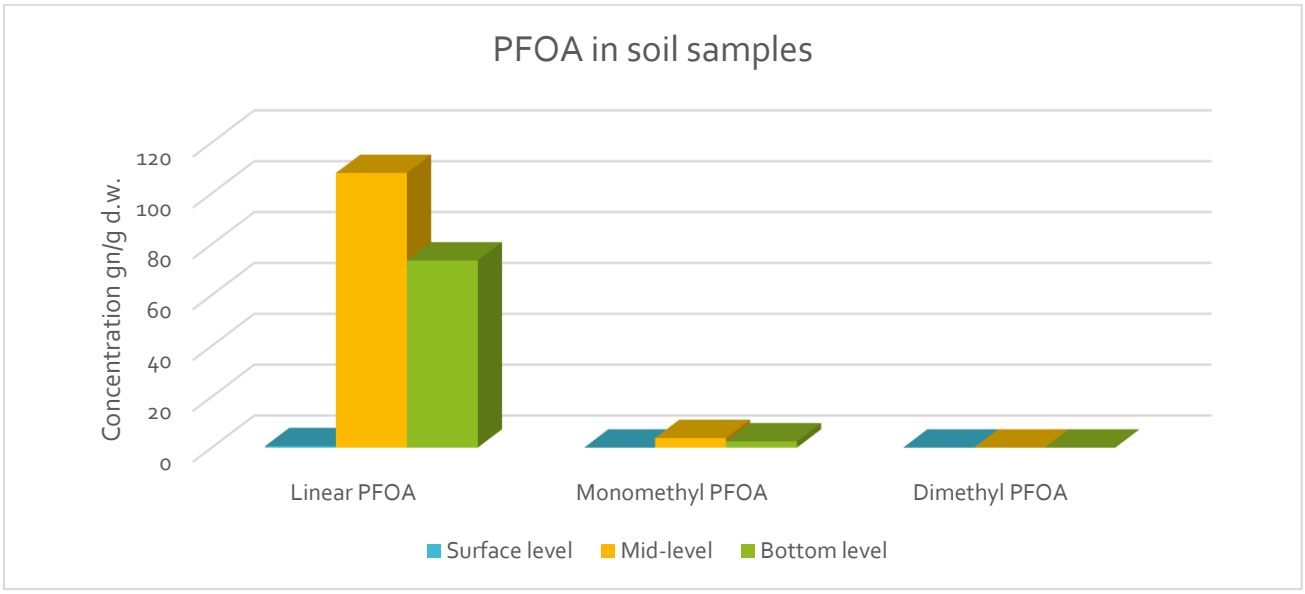


***Figure 5.1**. Visual overview of results for PFOA in soil samples before TOPA.*

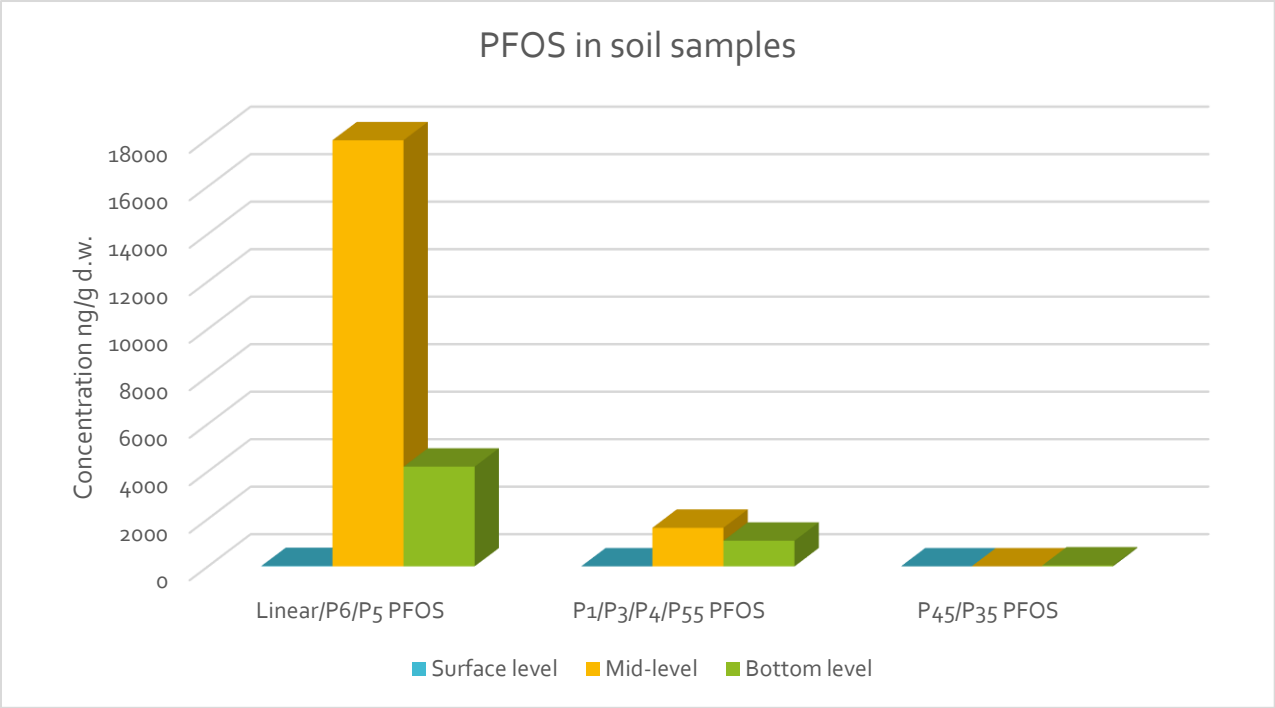


Figure 5.2. *Visual overview of results for PFOS in soil samples before TOPA.*

The amount of total branched isomers relative to linear PFOA and lienar/P6/P5 PFOS can be seen in table 5.1 below. Additionally, the amounts of branched isomers in ECF and previous study (at centre of different hotspot, same airport) are shown for comparison.

The data shows that the linear isomers are the most dominant at the surface. As the depth increases so does the branched isomers. It was shown in the previous study that linear PFOA and PFOS are being found enriched in soil and that branched isomers are more water soluble (17). The gravel and sand at the surface of the hotspot retains less water than the soil found in the deeper levels. This would explain why more branched isomers are being found in the deeper layers. Another way of explaining this in LC terms might be that branched isomers are less retained by the stationary phase (soil) and more attracted to the mobile phase (water, rain, etc.) and over time the branched isomers go deeper into the soil compared to the linear isomers. PFOA are more water soluble then PFOS as discussed in chapter 2.4 which would also explain why less branched PFOA is being found compared to branched PFOS.

It might also be that PFAS found in the deeper layers is older, created by ECF in the 2000s, and that the more linear composition of PFAS found in the surface was made by telomerization.

Overall the origin of PFAS used at this site is most likely a mix of ECF and telomerization as more branched isomers were found in the previous study (at the surface), matching the composition of ECF (17).

Table 5.1 *Overview of the distribution between linear and branched isomers in soil samples, previous study and theoretical ECF production.*

	Surface soil	Mid-level soil	Bottom soil	ECF	Previous study (17)
Linear PFOA	99 %	97 %	97 %	~ 78 %	80 %
Branched PFOA	1 %	3 %	3 %	~ 22 %	20 %
Linear PFOS*	97 %	92 %	79 %	~ 70 %	63 %
Branched PFOS	3 %	8 %	21 %	~ 30 %	37 %

*In the case of soil samples, its linear/P6/P5 PFOS.

No dimethyl PFOA was found in these soil samples, while a small peak could be seen in the chromatogram from the screening experiments on the F5 column (chapter 4.1, figure 4.3) nothing was found in this soil.

TOPA

A visual representation of the data from TOPA experiments (table 4.1 in chapter 4.3) can be seen in figures 5.3 and 5.4. Figure 5.3 shows the PFOA results and figure 5.4 shows PFOS results. Figure 5.5 shows an overview of the % change data from table 4.2 in chapter 4.3.

TOPA has proven to be an effective tool at transforming unknown PFAS into PFCAs. All of the PFAS analytes expect the dimethyl PFAO increased in concentration. The highest relative increase in concentration was at 4766 % for monomethyl PFOA in surface soil (see figure 5.5). In other studies, TOPA usually increases the total PFAS content by around 20 % to 800 % which was about the same for all the other analytes in this thesis (43). The high % increase for this one isomer must be due to the initial low concentration detected. It must be noted that the data proves that TOPA transforms unknown precursors into both linear and branched products. The data also suggests that TOPA is more effective at transforming unknown precursors into branched products of PFOA as their % change increase is higher than that of linear PFOA (figure 5.5).

PFOS was less affected by the assay. Linear/P6/P5 PFOS increased slightly (10 % maximum). P45/P35 PFOS completely disappeared after TOPA, probably due to the already

low concentration (0.016 ng) present in the sample. It might have been both due to TOPA or the clean-up method (WAX SPE). In the mid-level sample, P45/P35 increased in from zero concentration up to 27.9 ng/g. It has also caused a decrease in concentration of the same isomer in the bottom soil. This might have an explanation. As mentioned earlier, the mid-level soil seems to be the most contaminated, meaning that it's safe to assume that it might contain more precursors (then the other soil) which can then transform into e.g. P45/P35 PFOS under TOPA. The decrease in concentration for P45/P35 and P1/P3/P4/P55 PFOS might be to the increased dose of oxidation agents and less precursors present to be oxidized. Houtz and Sedlak recommended a lower amount which didn't degrade any PFOS, but no testing was done on higher amounts of oxidant (20).

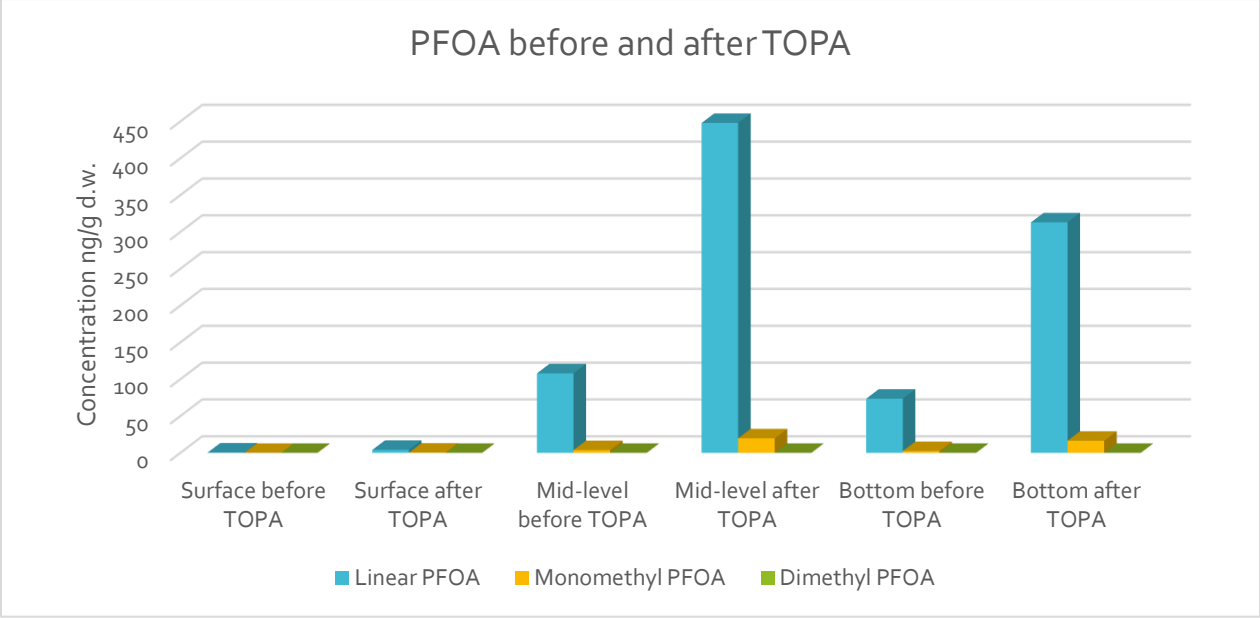


Figure 5.3. *Visal representation of the data from table 4.1 in chapter 4.3. PFOA in soil samples before and after TOPA.*

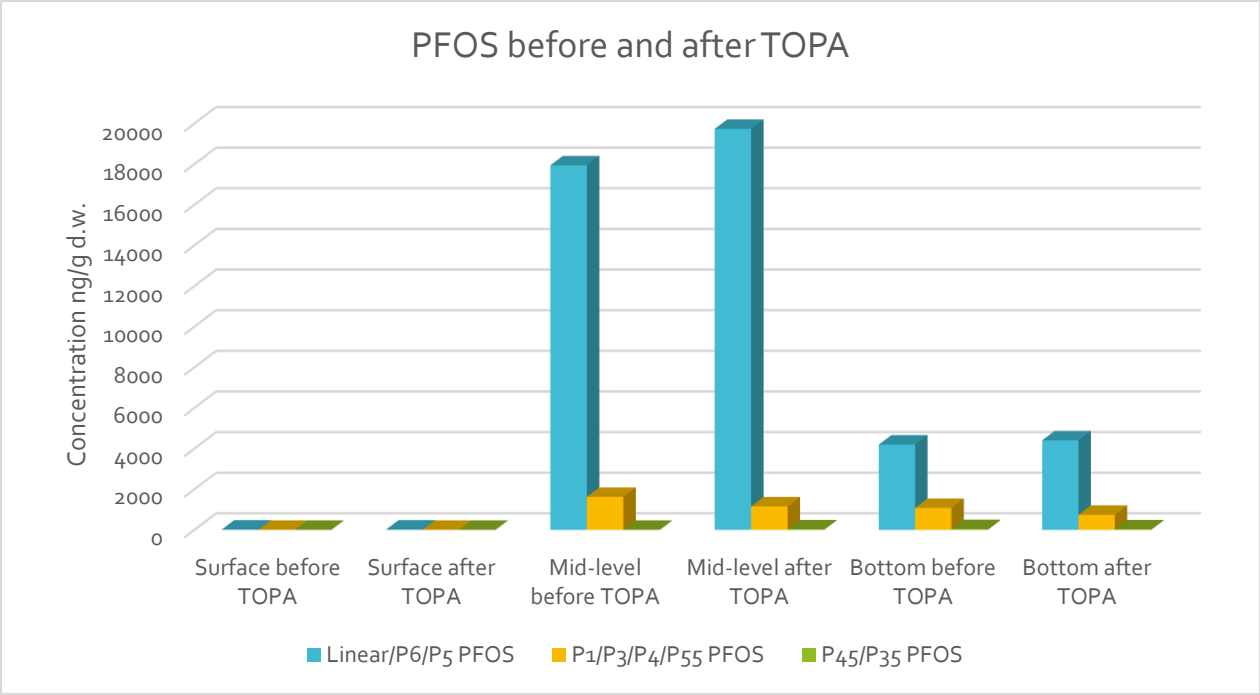


Figure 5.4. *Visal representation of the data from table 4.1 in chapter 4.3. PFOS in soil samples before and after TOPA.*

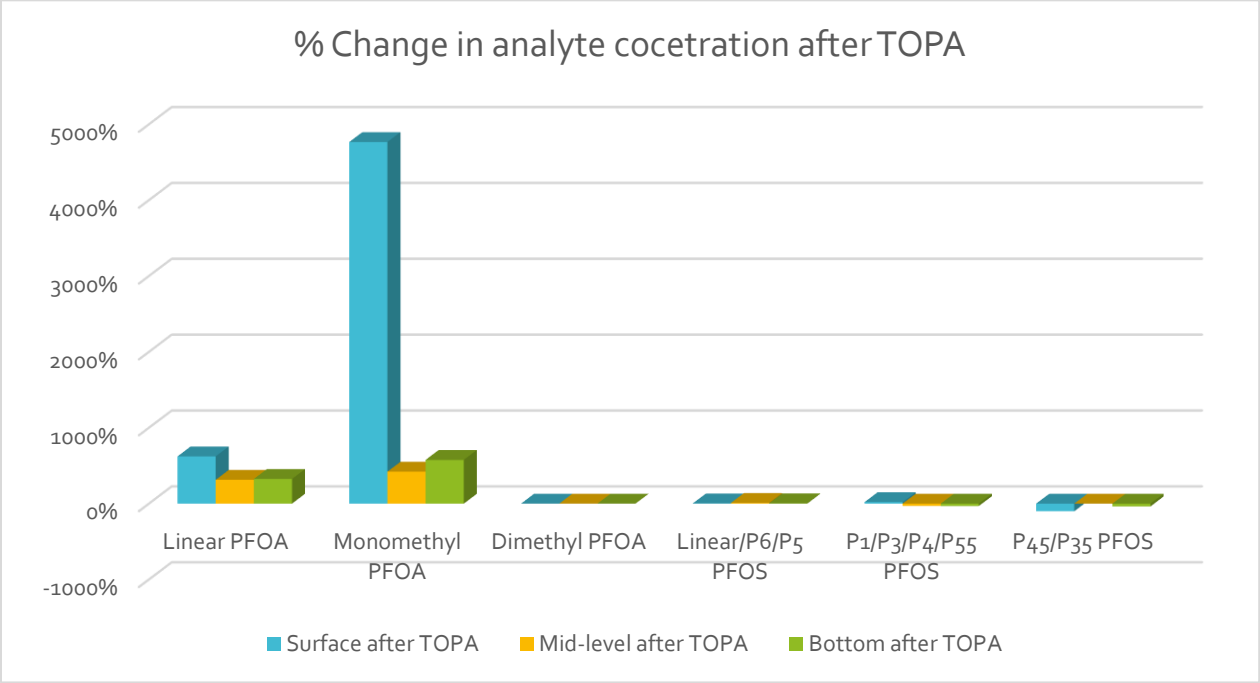


Figure 5.5. *Visual representation of the data (soil) from table 4.2 in chapter 4.3. It shows % change in analyte concentration between samples before and after TOPA.*

5.3 Findings in water samples

A visual representation of the findings from foam and water samples can be seen in figure 5.6 below.

PFOA and PFOS was found in sea water just outside the airport which consisted of a total of 0.58 ng/mL PFOS (11 % branched isomers) and 0.29 ng/mL PFOA (2 % branched isomers). Branched isomers of both PFAS were present indicating some level of ECF production of PFAS. The amount of branched isomers match with the amounts found in the soil samples at the airport; PFOA branched 1 – 3 % and PFOS branched 3 – 21 %. It is also possible that potential degradation of some PFOS and PFOA precursors into branched isomers was the cause of the presence of the isomers.

Linear/P6/P5 PFOS was detected in foam and river water samples. No ISTD was detected in these samples (and ground water sample), and the amount of PFOS was calculated manually by external calibration. The lack of ISTD is very concerning as it indicates a possible problem with the extraction method. The manually calculated amounts were 0.46 ng/mL in river water and 1.62 ng/mL in foam sample. It not surprising to find more PFAS in the foam sample as PFAS are known surfactants (34).

If the contamination originates from the firefighting site, it would be logical to expect some PFOA and PFOS in the ground water nearby. This was not the case as no target analytes were found. Due to this it’s difficult to argue that the contamination in the sea water comes from the firefighting site. Some PFOS was found in the river water coming into the sea, but no branched isomers were detected. It is also possible that the contamination originates from different sources, perhaps the dockyard nearby or other industrial buildings around the airport.

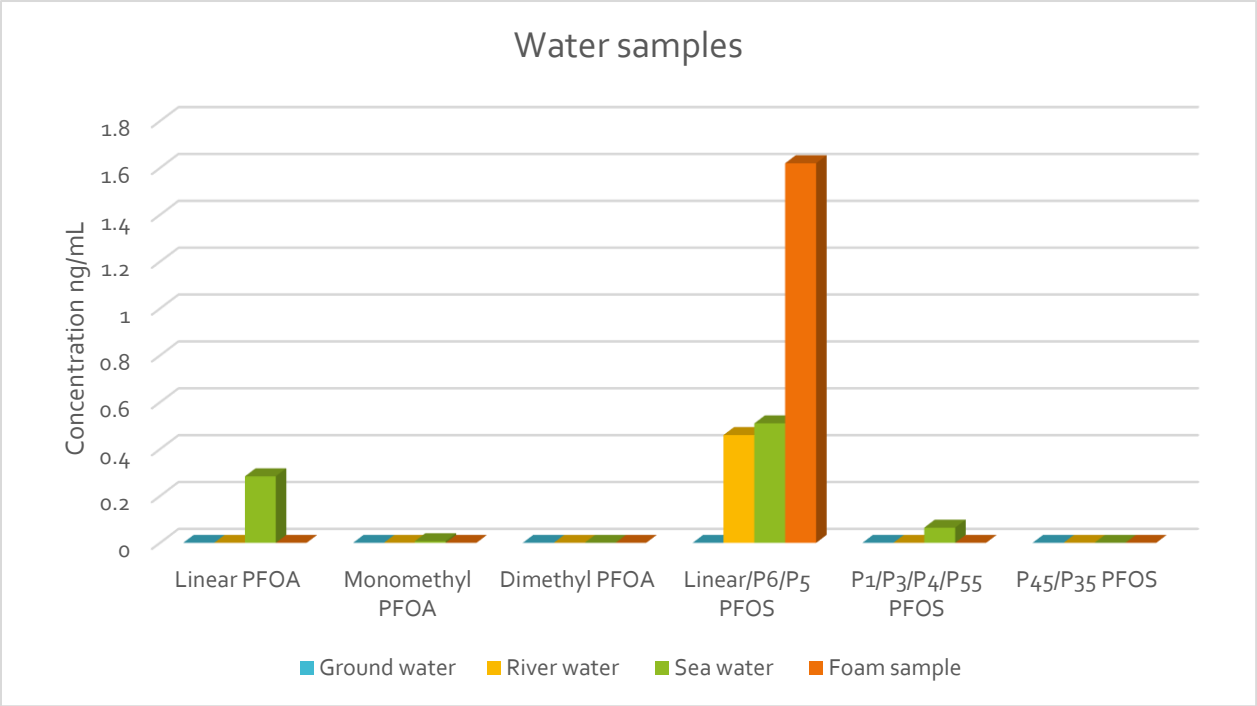


Figure 5.6. *Visual representation of the findings from the foam and water samples.*

5.4 Findings in biota samples

A visual representation of the results of the biota samples can be seen in figure 5.7 below.

PFOS was found in all the biota samples with the majority of it being the linear/P6/P5 isomers and some trace amounts of P1/P3/P4/P55 PFOS in two of the livers. Linear PFOA was also found in small amounts in three of the fish livers. The livers contained ten to twelve times more PFOS then their muscle counterparts. It is known that the liver is the target organ for bioaccumulation of PFAS, which is supported by our findings (8). The overall amount of PFAS in a small amount of each sample (1 gram muscle and 0.5 gram liver) is high, as eating 100 grams of contaminated fish muscle would result in an intake of 25 ng to 39 ng of PFOS alone (assuming potential contamination from other PFAS as well).

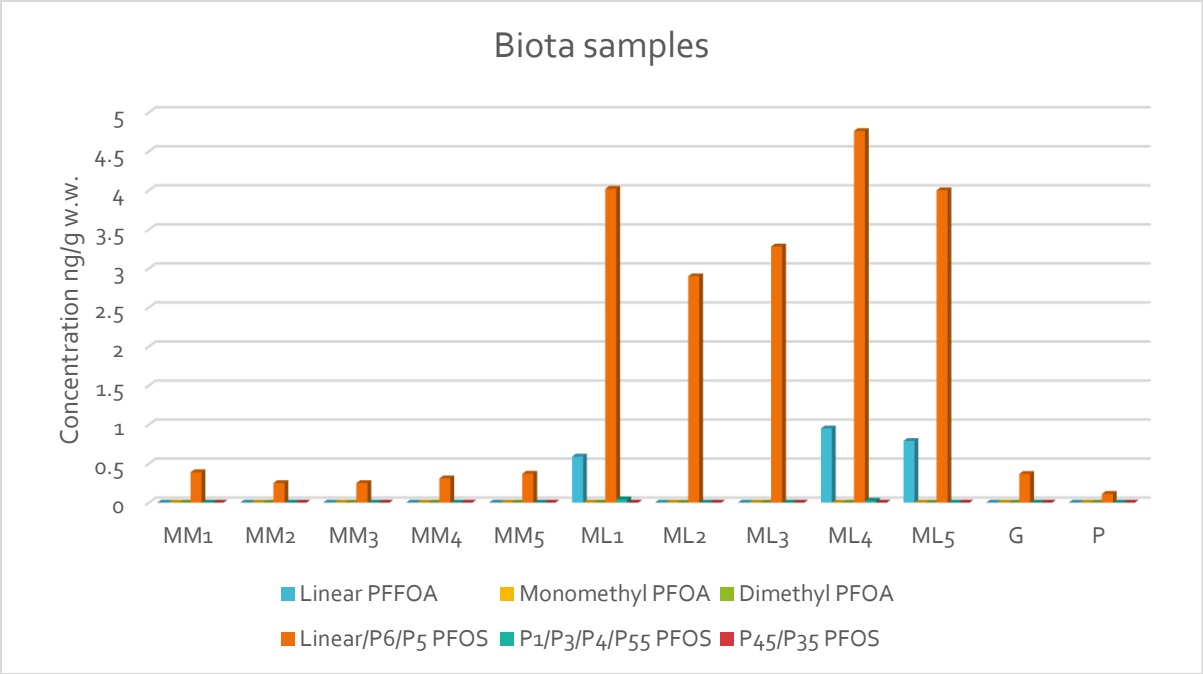


Figure 5.7. *A visual representation of the biota results. MM stands for mackerel muscle, ML stands for mackerel liver, G stands for goldshinny wrasse (muscle) and P stands for pollock (muscle).*

A person with a body weight of 100 kg would need to consume 440 ng PFAS to be at the tolerable weekly intake (4,4 ng kg^{-1}) which translates to around 1128 grams to 1760 grams of this contaminated fish muscle (36). If we continue with the assumption that some PFNA or PFHxS might also be present (which contribute to the TWI) the amount of fish a person would be able to consume would be lower.

The origin of the contamination in these fish might come from the sea water which was probably contaminated by the water coming from the airport or by a different source (discussed in chapter 5.3).

5.5 Homogenization and extraction methods

As discussed in chapter 5.3, there are reasons to assume that the extraction done on the water samples was inefficient. The lack of ISTD was one thing but also the analyte being present is just one of the replicates. Comparatively to the biota extraction which was very similar, no such issues were present, suggesting that the automated WAX SPE is a more reliable choice. The manual SPE extraction was time consuming and prone to many errors. It is possible that the pressure of the pump was too high for the small SPE columns, or that the speed at which the sample was pumped through the column was too high. Perhaps the sand and debris in the water samples contributed to the loss of ISTD in addition to a very large dilution (9 ng in

around 300 - 400 mL of water). A sonication and filtration of the water prior to SPE extraction might have prevented this. On the other hand, the TOPA samples were also extracted manually without any of these issues, which brings up the question if the TOPA results are reliable enough to be trusted, or maybe it's just the case for water samples.

The soil could have been homogenized further, a smaller grid on the sieve could have been utilized to possible better the extraction as smaller sand particles might have yielded more PFAS during sonication.

5.6 Quality control and validation parameters

Selectivity (blank samples)

Instrument blanks:

Some PFAS carryover was detected in instrument blanks. All the samples were investigated to look for any signs of decrease or increase in concentration between the replicates. No obvious signs were noticed. In addition, the carry over didn't affect precision as most of the samples were within the set criteria. Some carry over was expected with such high amounts of analytes in the sample. Overall, the method performed well.

Field blanks:

Some contamination was also found in the field blanks (soil sampling), the levels of contamination were small, negligible even compared to the amount of PFOS and PFOA found in the real samples. The source of this contamination might have come from the sampling site, the air or gotten inside when the containers were closed. It might have also come from the rain, as it was rainy on the day of the sampling. Other possibilities are the extraction method or solvents but its unlikely due to other samples being clean from these analytes. The only reagent used in the field blanks that was not used anywhere else was the sand, which might have been contaminated and is further discussed below.

Procedural blanks:

PFOS contamination found in the procedural blanks after TOPA was estimated to be < 20 ng/mL for the linear/P6/P5 PFOS and < 0.5 ng/mL for the P1/P3/P4/P55 PFOS. Before TOPA the contamination was estimated to be < 0.05 ng/mL for the linear/P6/P5 PFOS and none for the P1/P3/P4/P55 PFOS.

This level of contamination is not insignificant. If this contamination comes from solvents used for TOPA, other samples like e.g. surface soil would probably show a larger difference

in concentration (only 3 % difference, ~ 1 ng/mL) between samples from before and after TOPA. With this in mind it can be assumed that the contamination doesn't come from the solvents as it would show up at about the same level in other samples.

These blanks were not spiked, meaning no cross contamination from standards. Some possible sources of contamination are listed and discussed below;

1. One possible source of contamination is the clean soil, used only for the procedural blanks and field blanks which were also contaminated at similar level.
2. Another possible source of contamination could be a small mistake e.g. dirt on glove, in the fume hood or exchanging the cork of the blank sample with one of the real samples. With such high amounts of analyte in the samples, even the smallest mistakes can lead to high amounts of contamination.

As for other samples before and after TOPA, no outliers, decreasing/increasing trends or unexplained high standard deviations were detected which would signify an issue with the analysis and the method. It is assumed that there was a mistake made with this blank sample which caused the sudden spike in PFOS concentration after TOPA, or the clean sand used as matrix was contaminated.

Linearity and measurement range

The linearity of all target analytes was well within the given limits ($R^2 \geq 0.99$). The calibration range was realistically too small for the amounts of PFAS wound in soil samples. The range could have been increased or the samples diluted. Diluting the samples come with a risk that analytes with low/trace concentration would be undetected. Increasing the range of calibration is a more costly and time-consuming process, it would also require additional quality control samples at higher levels of calibration.

LOD and LOQ

The values for LOD and LOQ for all target analytes were estimated to be very low. This is mostly since little to no noise could be detected using the Orbitrap system. Due to this, LOD and LOQ was estimated from the calibration curves and regression statistic.

LOD and LOQ were not tested, and the values should be validated by analysing samples at those concentration levels. No concentrations below LOQ and LOD were detected.

Precision

For precision, only a handful of samples were not within the given limit. Two of the soil samples with the highest RSD % were also the lowest in concentration. Higher RSD % values are often expected at lower concentration levels. The last one was for one of the isomers in the sea water, this sample was also lower in concentration, meaning that the lower precision is not surprising. The criteria were set to match 1 ppb corresponding to a concentration of 1 ng/mL. Concentrations below this are not expected to always match these criteria. Overall, the method performed well when it comes down to precision.

Accuracy (trueness)

Accuracy was measured in two ways, first the quality control samples which measures the accuracy of the instruments and calibration. Both PFOA and PFOS was recovered within the given limits from the quality control sample (40 – 120 % recovery).

The second was the proficiency test provided by the EURL. The sample of pork liver was extracted in the same way as the fish muscle, providing an accuracy test for the instrument and extraction method. Only PFOS was recovered in accordance with the set criteria. PFOA was recovery was outside of the set criteria, at 283.6 %. To check if the use of PFOS as internal standard could be the main contributor to the issue PFOA concertation was estimated by external calibration (using average signal and weight of the replicates) and resulted in concertation of 4.79 ng/g w.w. corresponding to 565 % recovery. It suggests that PFOS ISTD was not the main issue.

6.0 Conclusions

The current study attempted to develop a working method for analysis of a wide variety of sample types and analytes. The separation on the C18 column provided some valuable information about the variety of contamination around and inside of the Flesland airport. The method showed good linearity and precision for target analytes. The accuracy of the method was also within the given limits with the expectation of PFOA in the proficiency test, which should be improved further. The study has also provided insights into the efficiency of separation on the F5 column and future possibilities associated with it.

The contamination found around the firefighting site was beyond the calibration used in this method, resulting in several samples being reported as estimates. The data showed that branched isomers of PFOA and PFOS increase in amount with depth, suggesting historical use of ECF AFFF. The more linear composition found at the surface of the hotspot suggest recent use of telomerization AFFF. Another possibility is that branched isomers are not as retained by the gravel and soil and are getting washed down faster compared to their linear counterparts due to their increased solubility. The results were different from the findings in the previous study done at the same airport where the branched composition of PFOA and PFOS matched the theoretical ECF composition (17).

TOPA has shown that there are a lot more PFAS waiting to be revealed. It is unknown if the oxidation is complete, it safe to assume that with such high concentrations many of the PFAS precursors were left unoxidized. Contamination in sea water and fish samples is very alarming as it proves that PFAS can be found far away from such hotspots (1.35 km). The amount of PFAS found in the fish is high enough to be potentially harmful for people and wildlife consuming these fish few times a week.

Issues with the extraction of river, ground and foam water led to difficulties with pinpointing the contamination in the sea to the firefighting site. It is very probable that the PFOS found in river water originates from the airport but also it might be other sources of PFAS which we don’t know about (e.g. the boat dock, other industries nearby).

At the end the study proved the following hypotheses;

I. PFOA and PFOS isomer profiles depend on the type of PFAS source and the history of PFAS production. As several PFOA and PFOS isomers were detected which are known to be mass produced by the ECF synthesis method until the

2000s. By comparison to the theoretical amounts of branched isomers produced by the ECF and the previous study done on this airport, the data obtained in this study suggests historical use of ECF AFFF and more recent use of telomerization AFFF. Additionally, possible leaching of branched isomers throughout the soil at a higher rate compared to the linear isomers. Further analysis of other soil and water samples could confirm this statement.

II. The degradation of unknown PFAS precursors was isomer specific. Both linear and branched isomers of PFOA increased in concertation after TOPA. The data obtained from the TOPA experiments show that branched isomers of PFOA oxidize at a higher rate relative to the initial amount detected (compared to the linear). Meaning that overtime the amount of branched isomers could increase relative to the linear isomer, making it difficult to differentiate between ECF and telomerization origin of production. This also indicates a possible higher amount of branched precursors in the soil.

III. Targeted analysis of PFAS underestimate the level of contamination. TOPA has proven that large quantities of unknown precursors were present and in the samples.

7.0 Future work

7.1 Sampling

More data could have been gathered about the biota samples (weigh, gender, etc.). Additionally, more fish samples of different species, which might have been difficult to acquire for a novice fisherman.

Soil samples could have been gathered at smaller intervals as the amount of soil need for extraction was only around 1 gram, and even then, the amount of PFAS was beyond the calibration range. Smaller intervals of sampling could have better the overview of deposition of different PFAS in the soil. Additionally soil samples from deeper levels would give more insight into the leaching of branched vs linear isomers into the soil.

7.2 Sample workup

The drying of soil in a fume hood was a longsome and tedious process which also took a lot of space. It might have also led to some cross contamination as dust could travel from one sample to another.

Soil samples homogenization could have been improved. A sieve with smaller pores could have been utilized to make the sample more homogenous as the amount of sample wasn't a problem. Additional testing on how homogenization soil samples affect extraction of target analytes. This could be very useful information for future method development and validation.

Water extraction by manual WAX SPE could have been improved. Firstly, filtration and sonication of the samples prior to WAX SPE to ensure less clogging of the columns and possibly more analytes extracted. Filtration could have possibly been done by paper filters assuming they are PFAS free. More internal standard added as the dilution factor in such large volume of sample is very high (must be later corrected in calculations of concentrations). Manual SPE is also a very tedious process, taking several hours. Larger SPE columns could decrease the time of extraction. Testing the performance of the extraction by spiked experiments, especially the mentioned extractions by manual WAX SPE as they didn't perform well.

7.3 Analysis

Further optimalization of LC and MS parameters. The time of analysis on the C18 column is very long. Additional work to reduce the run time as well as improving the separation should

be done. This might have been achieved by reducing the injection volume. Other possibilities are using a temperature gradient and using other mobile phases. The same can be done with the F5 column in addition to establishing the correct way to take care of it as it has shown to be sensitive (easy to destabilize). MS parameters can be improved as they were set up with other compounds in mind (short/long PFSA and PFCA and precursors).

Addition of shorter chain PFCAs and PFSAs and their precursors would reveal a much more accurate description of the effects of TOPA on real samples.

There are several samples that have been gathered and not utilized in this study, these are safely stored at the IMR and might prove a better overview of the and spread and levels of contamination around the airport and into the sea.

7.4 Quality

Accuracy of the extraction of PFOA could have been improved as it was not within the given criteria. LOD and LOQ should be validation further as they were not tested in this work. Other parameters were well within the given limits.

7.5 Other

It should be noted that in the chromatograms showing separation of target isomers on a real sample (chapter 4.1, figures 4.3 and 4.4), a small peak for dimethyl PFOA is visible. Additionally, a large peak (compared to the others) for dimethyl PFOS isomers can be seen in the other chromatogram. This suggest that dimethyl PFOA and PFOS are present in some quantities in the soil at the airport but were not detected with the current method or in this particular sample. Additional work on all the other gathered samples would reveal a bigger picture for the contamination at the airport.

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Appendix

A – Calibration data

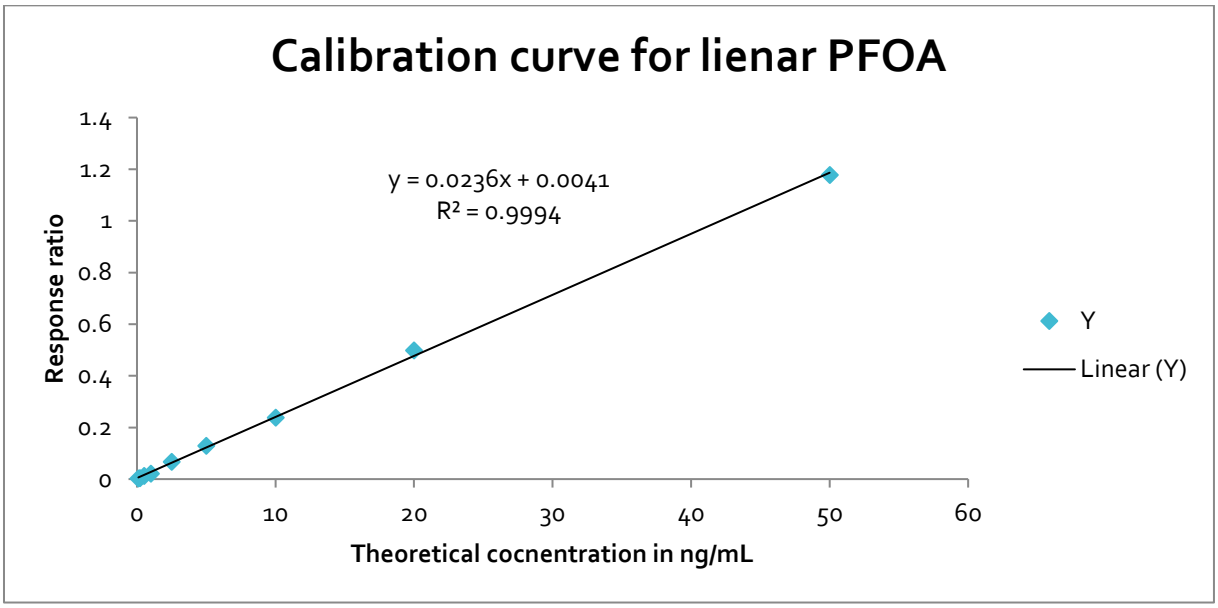

Figure A.1. Calibration curve for linear PFOA.

Table A.1. Regression data for linear PFOA.

Regression Statistics	
Multiple R	0.999716759
R Square	0.999433598
Adjusted R Square	0.999352684
Standard Error	0.009857637
Observations	9

Table A.2. Calibration data for linear PFOA.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
0.1 ng	23.13	30761	0.084	15345559	0.002
0.2 ng	23.13	56479	0.179	13262871	0.004
0.5 ng	23.13	209864	0.485	18200731	0.012
1 ng	23.14	290363	0.879	13903112	0.021
2.5 ng	23.14	914600	2.825	13622321	0.067
5 ng	23.14	1859374	5.439	14381950	0.129
10 ng	23.14	2948858	10.004	12400856	0.238
20 ng	23.14	6424289	20.938	12908701	0.498
50 ng	23.12	19430047	49.566	16492235	1.178

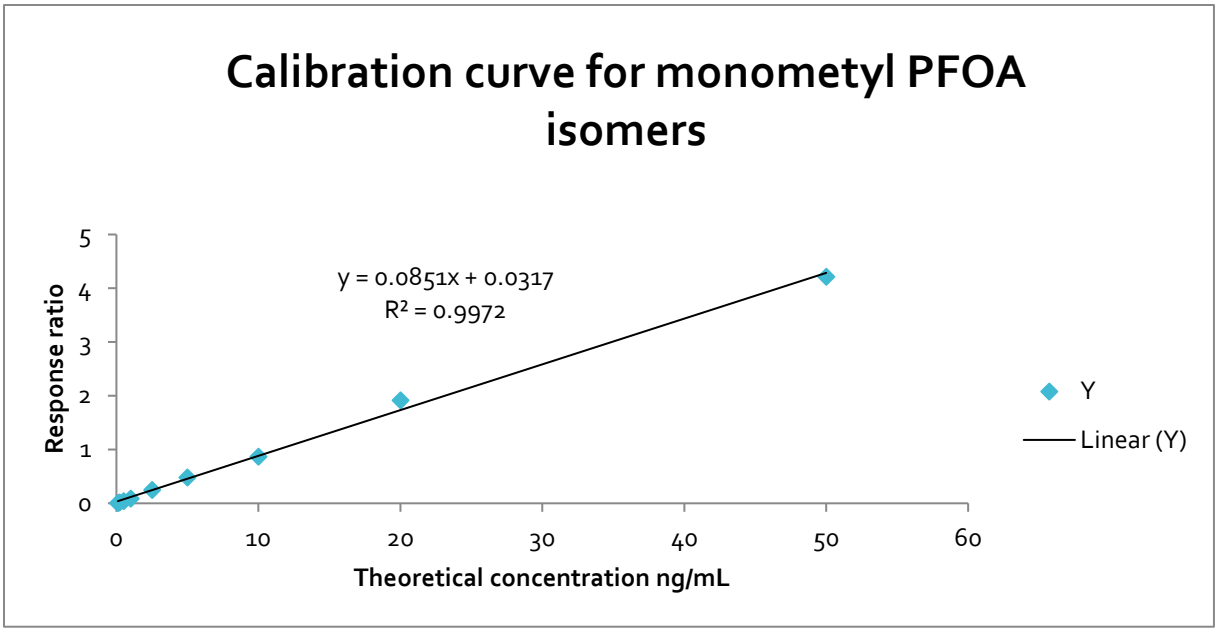

Figure A.2. Calibration curve for monomethyl PFOA isomers.

Table A.3. Regression data for monomethyl PFOA isomers.

Regression Statistics	
Multiple R	0.99860923
R Square	0.997220394
Adjusted R Square	0.996823307
Standard Error	0.078655502
Observations	9

Table A.4. Calibration data for monomethyl PFOA isomers.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
0.1 ng	22.78	113330	0.086	15345559	0.007
0.2 ng	22.76	200698	0.176	13262871	0.015
0.5 ng	22.76	790791	0.505	18200731	0.043
1 ng	22.77	1211668	1.013	13903112	0.087
2.5 ng	22.77	3381921	2.886	13622321	0.248
5 ng	22.75	6928391	5.601	14381950	0.482
10 ng	22.77	10794753	10.121	12400856	0.87
20 ng	22.76	24729885	22.273	12908701	1.916
50 ng	22.76	69489224	48.987	16492235	4.213

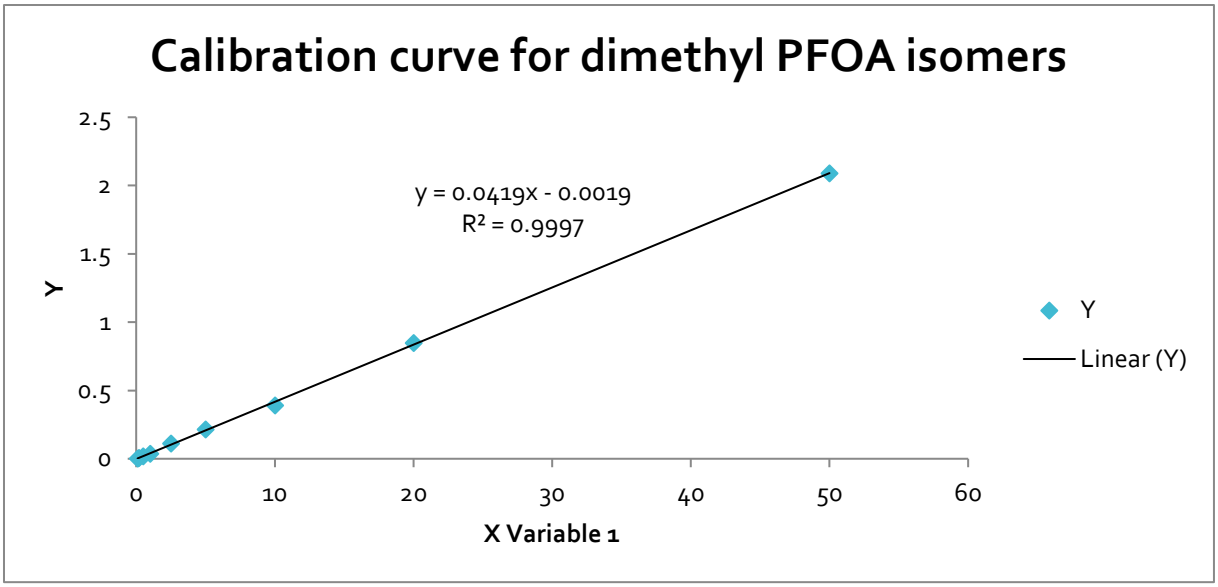

Figure A.3. Calibration curve for dimethyl PFOA.

Table A.5. Regression data for dimethyl PFOA.

Regression Statistics	
Multiple R	0.999864
R Square	0.999728
Adjusted R Square	0.9996892
Standard Error	0.0120894
Observations	9

Table A.6. Calibration data for dimethyl PFOA isomers.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
0.1 ng	22.25	50262	0.078	15345559	0.003
0.2 ng	22.26	88426	0.159	13262871	0.007
0.5 ng	22.24	331328	0.435	18200731	0.018
1 ng	22.24	526125	0.905	13903112	0.038
2.5 ng	22.26	1511137	2.654	13622321	0.111
5 ng	22.23	3104569	5.164	14381950	0.216
10 ng	22.24	4836144	9.33	12400856	0.39
20 ng	22.25	10952330	20.297	12908701	0.848
50 ng	22.23	34465229	49.994	16492235	2.09

B – PFOS calibration data

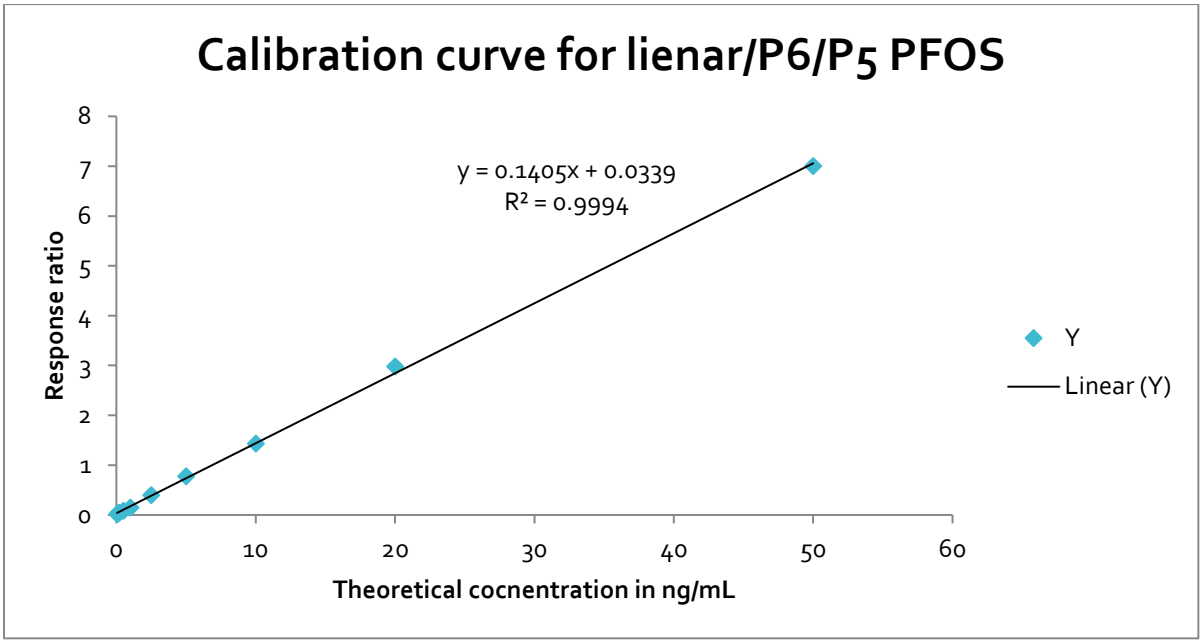

Figure B.1. Calibration curve of linear/P6/P5 PFOS.

Table B.1. Calibration data for linear/P6/P5 PFOS.

Filename	Actual RT	Area	Calculated Amt.	ISTD Response	Response Ratio
0.05 ng	24.46	248106	0.126	13889419	0.018
0.1 ng	24.45	404198	0.186	15345559	0.026
0.2 ng	24.45	556231	0.296	13262871	0.042
0.5 ng	24.45	1436727	0.558	18200731	0.079
1 ng	24.44	2028393	1.031	13903112	0.146
2.5 ng	24.44	5344330	2.773	13622321	0.392
5 ng	24.44	11142593	5.476	14381950	0.775
10 ng	24.44	17755083	10.12	12400856	1.432
20 ng	24.44	38464425	21.062	12908701	2.98
50 ng	24.45	115466886	49.488	16492235	7.001

Table B.2. Regression data for linear/P6/P5 PFOS.

Regression Statistics	
Multiple R	0.999703107
R Square	0.999406303
Adjusted R Square	0.999332091
Standard Error	0.057216976
Observations	10

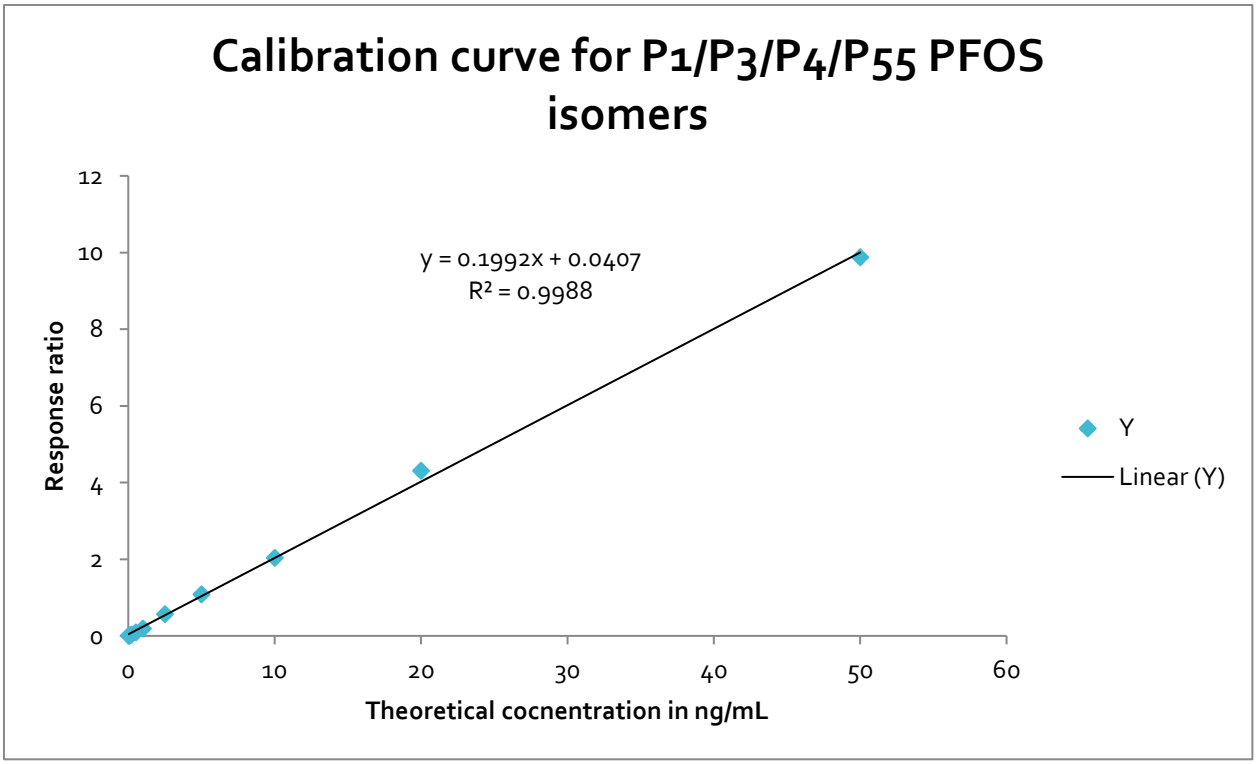

Figure B.2. Calibration curve of P1/P3/P4/P55 PFOS isomers.

Table B.3. Calibration data for P1/P3/P4/P55 PFOS isomers.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
0.1 ng	24.02	283483	0.092	15345559	0.018
0.02 ng	24.04	75220	0.032	11636359	0.006
0.2 ng	24.02	497809	0.187	13262871	0.038
0.05 ng	24	120917	0.043	13889419	0.009
0.5 ng	24.02	1762499	0.483	18200731	0.097
1 ng	24.01	2747981	0.986	13903112	0.198
2.5 ng	24.01	7723514	2.829	13622321	0.567
5 ng	24.01	15685548	5.442	14381950	1.091
10 ng	24.01	25238830	10.156	12400856	2.035
20 ng	24.01	55665974	21.518	12908701	4.312
50 ng	23.99	162947440	49.302	16492235	9.88

Table B.4. Regression data for P1/P3/P4/P55 PFOS isomers.

Regression Statistics	
Multiple R	0.999392
R Square	0.998784
Adjusted R Square	0.998649
Standard Error	0.111279
Observations	11

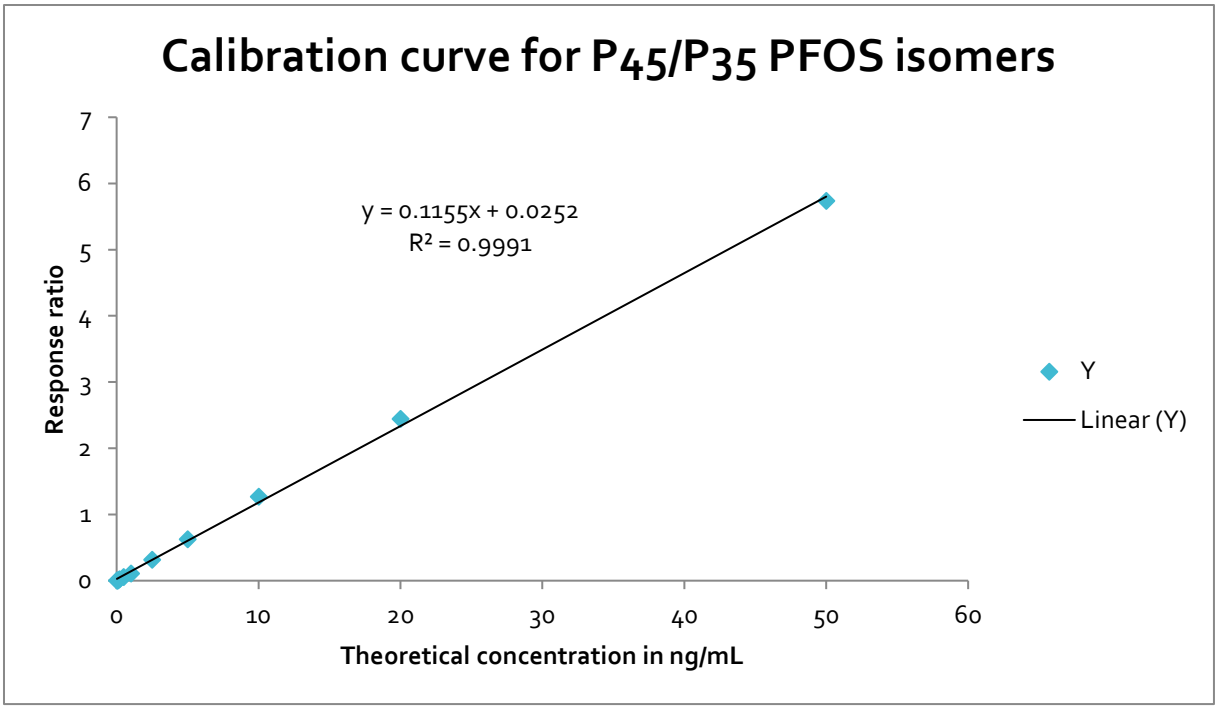

Figure B.3. Calibration curve for P45/P35 PFOS isomers.

Table B.5. Calibration data for P45/P35 PFOS isomers.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
0.02 ng	23.63	20591	0.015	11636359	0.002
0.1 ng	23.63	156149	0.088	15345559	0.01
0.05 ng	23.61	53092	0.033	13889419	0.004
0.2 ng	23.63	271579	0.176	13262871	0.02
0.5 ng	23.63	992521	0.469	18200731	0.055
1 ng	23.61	1537789	0.951	13903112	0.111
2.5 ng	23.61	4352743	2.748	13622321	0.32
5 ng	23.62	9030654	5.401	14381950	0.628
10 ng	23.62	15732333	10.912	12400856	1.269
20 ng	23.62	31571344	21.037	12908701	2.446
50 ng	23.6	94625261	49.352	16492235	5.738

Table B.6. Regression data for P45/P35 PFOS isomers.

Regression Statistics	
Multiple R	0.999526
R Square	0.999051
Adjusted R Square	0.998946
Standard Error	0.056985
Observations	11

C – Raw data PFOA

Table C.1. Raw data for the linear PFOA isomer in soil samples. The SX samples come from the surface soil, MX samples from the mid-level, BX samples from the bottom level (X is the number of the replicate). Samples with T at the end are TOPA samples. Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g d.w.
S1	23.12	578539	0.529	45999482	0.013	0.518
S2	23.11	632422	0.547	48647691	0.013	0.545
S3	23.11	559765	0.639	36847553	0.015	0.642
S1 T	23.3	1271168	4.22	12672954	0.1	4.13
S2 T	23.3	1278656	4.3	12509586	0.102	4.28
S3 T	23.3	1332763	3.873	14476701	0.092	3.89
M1	23.26	26642866	110.54	10140352	2.627	103.21
M2	23.25	22684917	104.371	9144271	2.481	107.15
M3	23.3	31654051	119.485	11145699	2.84	114.44
M1 T	23.28	53271253	416.909	5375824	9.909	389.27
M2 T	23.29	48874227	461.138	4459046	10.961	473.44
M3 T	23.28	54005973	504.419	4504464	11.989	483.62
B1	23.11	32435712	79.327	17202705	1.886	76.57
B2	23.12	21972799	65.395	14136278	1.554	64.87
B3	23.11	32116165	80.942	16693246	1.924	79.27
B1 T	23.29	45117606	338.838	5602059	8.054	327.06
B2 T	23.26	33237718	247.923	5640375	5.893	245.95
B3 T	23.28	36507873	374.166	4105016	8.893	366.47

Table C.2. Raw data for the monomethyl PFOA isomers in soil samples. The SX samples come from the surface soil, MX samples from the mid-level, BX samples from the bottom level (X is the number of the replicate). Samples with T at the end are TOPA samples. Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g d.w.
S1	23.45	36282	0.009	45999482	0.001	0.009
S2	22.75	10668	0.003	48647691	0	0.003
S3	23.49	15731	0.005	36847553	0	0.005
S1 T	23.62	189127	0.174	12672954	0.015	0.170
S2 T	23.63	468777	0.436	12509586	0.037	0.434
S3 T	23.62	333440	0.268	14476701	0.023	0.269
M1	22.75	3298237	3.782	10140352	0.325	3.53
M2	22.76	2902183	3.69	9144271	0.317	3.78
M3	22.75	4008500	4.181	11145699	0.36	4.00
M1 T	22.91	8558930	18.51	5375824	1.592	17.28
M2 T	22.91	7613356	19.851	4459046	1.707	20.38
M3 T	22.91	8608445	22.219	4504464	1.911	21.30
B1	22.73	4126550	2.789	17202705	0.24	2.69

B2	22.74	2259079	1.858	14136278	0.16	1.84
B3	22.73	4083733	2.844	16693246	0.245	2.78
B1 T	22.9	9074224	18.832	5602059	1.62	18.17
B2 T	22.89	6386530	13.164	5640375	1.132	13.05
B3 T	22.91	6585219	18.651	4105016	1.604	18.26

Table C.3. Raw data for the dimethyl PFOA isomers in soil samples. The SX samples come from the surface soil, MX samples from the mid-level, BX samples from the bottom level (X is the number of the replicate). Samples with T at the end are TOPA samples. Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g d.w.
S1	N/F	N/F	N/F	45999482	N/F	N/F
S2	N/F	N/F	N/F	48647691	N/F	N/F
S3	N/F	N/F	N/F	36847553	N/F	N/F
S1 T	N/F	N/F	N/F	12672954	N/F	N/F
S2 T	N/F	N/F	N/F	12509586	N/F	N/F
S3 T	N/F	N/F	N/F	14476701	N/F	N/F
M1	N/F	N/F	N/F	10140352	N/F	N/F
M2	N/F	N/F	N/F	9144271	N/F	N/F
M3	N/F	N/F	N/F	11145699	N/F	N/F
M1 T	N/F	N/F	N/F	5375824	N/F	N/F
M2 T	N/F	N/F	N/F	4459046	N/F	N/F
M3 T	N/F	N/F	N/F	4504464	N/F	N/F
B1	N/F	N/F	N/F	17202705	N/F	N/F
B2	N/F	N/F	N/F	14136278	N/F	N/F
B3	N/F	N/F	N/F	16693246	N/F	N/F
B1 T	N/F	N/F	N/F	5602059	N/F	N/F
B2 T	N/F	N/F	N/F	5640375	N/F	N/F
B3 T	N/F	N/F	N/F	4105016	N/F	N/F

Table C.4. Raw data of linear PFOA in fish samples. The naming can be explained by the following; M(mackerel)L(liver sample) 1 (fish n) and A (replicate, n = 2, A and B). Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g w.w.
MMus 1 A	N/F	N/F	N/F	30567161	N/F	N/F
MMus 1 B	N/F	N/F	N/F	26496078	N/F	N/F
MMus 2 A	N/F	N/F	N/F	31101477	N/F	N/F
MMus 2 B	N/F	N/F	N/F	28244275	N/F	N/F
MMus 3 A	N/F	N/F	N/F	27295328	N/F	N/F
MMus 3 B	N/F	N/F	N/F	26956222	N/F	N/F
MMus 4 A	N/F	N/F	N/F	29425166	N/F	N/F
MMus 4 B	N/F	N/F	N/F	31470667	N/F	N/F
MMus 5 A	N/F	N/F	N/F	28257770	N/F	N/F
MMus 5 B	N/F	N/F	N/F	27372843	N/F	N/F

MLiv 1 A	22.98	207981	0.304	28820566	0.007	0.304
MLiv 1 B	N/F	N/F	N/F	23946633	N/F	N/F
MLiv 2 A	N/F	N/F	N/F	24840785	N/F	N/F
MLiv 2 B	N/F	N/F	N/F	18288333	N/F	N/F
MLiv 3 A	N/F	N/F	N/F	32865641	N/F	N/F
MLiv 3 B	N/F	N/F	N/F	27983919	N/F	N/F
MLiv 4 A	23.0	407349	0.603	28419686	0.014	0.603
MLiv 4 B	23.04	292756	0.384	32084598	0.009	0.384
MLiv 5 A	22.96	242738	0.382	26707723	0.009	0.382
MLiv 5 B	22.98	250664	0.409	25796835	0.01	0.409
GSW A	N/F	N/F	N/F	25397213	N/F	N/F
GSW B	N/F	N/F	N/F	28721740	N/F	N/F
Pollock A	N/F	N/F	N/F	28065559	N/F	N/F
Pollock B	N/F	N/F	N/F	27278455	N/F	N/F

Table C.5. Raw data of monomethyl PFOA in fish samples. The naming can be explained by the following; M(mackerel)L(liver sample) 1 (fish n) and A (replicate, n = 2, A and B). Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g w.w.
MMus 1 A	N/F	N/F	N/F	30567161	N/F	N/F
MMus 1 B	N/F	N/F	N/F	26496078	N/F	N/F
MMus 2 A	N/F	N/F	N/F	31101477	N/F	N/F
MMus 2 B	N/F	N/F	N/F	28244275	N/F	N/F
MMus 3 A	N/F	N/F	N/F	27295328	N/F	N/F
MMus 3 B	N/F	N/F	N/F	26956222	N/F	N/F
MMus 4 A	N/F	N/F	N/F	29425166	N/F	N/F
MMus 4 B	N/F	N/F	N/F	31470667	N/F	N/F
MMus 5 A	N/F	N/F	N/F	28257770	N/F	N/F
MMus 5 B	N/F	N/F	N/F	27372843	N/F	N/F
MLiv 1 A	N/F	N/F	N/F	28820566	N/F	N/F
MLiv 1 B	N/F	N/F	N/F	23946633	N/F	N/F
MLiv 2 A	N/F	N/F	N/F	24840785	N/F	N/F
MLiv 2 B	N/F	N/F	N/F	18288333	N/F	N/F
MLiv 3 A	N/F	N/F	N/F	32865641	N/F	N/F
MLiv 3 B	N/F	N/F	N/F	27983919	N/F	N/F
MLiv 4 A	N/F	N/F	N/F	28419686	N/F	N/F
MLiv 4 B	N/F	N/F	N/F	32084598	N/F	N/F
MLiv 5 A	N/F	N/F	N/F	26707723	N/F	N/F
MLiv 5 B	N/F	N/F	N/F	25796835	N/F	N/F
GSW A	N/F	N/F	N/F	25397213	N/F	N/F
GSW B	N/F	N/F	N/F	28721740	N/F	N/F
Pollock A	N/F	N/F	N/F	28065559	N/F	N/F
Pollock B	N/F	N/F	N/F	27278455	N/F	N/F

Table C.6. Raw data of dimethyl PFOA in fish samples. The naming can be explained by the following; M(mackerel)L(liver sample) 1 (fish n) and A (replicate, n = 2, A and B). Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g w.w.
MMus 1 A	N/F	N/F	N/F	30567161	N/F	N/F
MMus 1 B	N/F	N/F	N/F	26496078	N/F	N/F
MMus 2 A	N/F	N/F	N/F	31101477	N/F	N/F
MMus 2 B	N/F	N/F	N/F	28244275	N/F	N/F
MMus 3 A	N/F	N/F	N/F	27295328	N/F	N/F
MMus 3 B	N/F	N/F	N/F	26956222	N/F	N/F
MMus 4 A	N/F	N/F	N/F	29425166	N/F	N/F
MMus 4 B	N/F	N/F	N/F	31470667	N/F	N/F
MMus 5 A	N/F	N/F	N/F	28257770	N/F	N/F
MMus 5 B	N/F	N/F	N/F	27372843	N/F	N/F
MLiv 1 A	N/F	N/F	N/F	28820566	N/F	N/F
MLiv 1 B	N/F	N/F	N/F	23946633	N/F	N/F
MLiv 2 A	N/F	N/F	N/F	24840785	N/F	N/F
MLiv 2 B	N/F	N/F	N/F	18288333	N/F	N/F
MLiv 3 A	N/F	N/F	N/F	32865641	N/F	N/F
MLiv 3 B	N/F	N/F	N/F	27983919	N/F	N/F
MLiv 4 A	N/F	N/F	N/F	28419686	N/F	N/F
MLiv 4 B	N/F	N/F	N/F	32084598	N/F	N/F
MLiv 5 A	N/F	N/F	N/F	26707723	N/F	N/F
MLiv 5 B	N/F	N/F	N/F	25796835	N/F	N/F
GSW A	N/F	N/F	N/F	25397213	N/F	N/F
GSW B	N/F	N/F	N/F	28721740	N/F	N/F
Pollock A	N/F	N/F	N/F	28065559	N/F	N/F
Pollock B	N/F	N/F	N/F	27278455	N/F	N/F

Table C.7. Linear PFOA, water samples results. Sea A/B are sea water samples. FOAM is the foam sample. Elv A/B are samples of river water going into the sea by the dockyard. Site A/B are water samples of ground water near the hotspot. Concentrations given in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
Sea A	23.29	537885	0.287	78775743	0.007
Sea B	23.26	377132	0.28	56685497	0.007
FOAM	N/F	N/F	N/F	N/F	N/F
Elv A	N/F	N/F	N/F	N/F	N/F
Elv B	N/F	N/F	N/F	N/F	N/F
Site A	N/F	N/F	N/F	N/F	N/F
Site B	N/F	N/F	N/F	N/F	N/F

Table C.8. Monomethyl PFOA, water samples results. Sea A/B are sea water samples. FOAM is the foam sample. Elv A/B are samples of river water going into the sea by the dockyard. Site A/B are water samples of ground water near the hotspot. Concentrations given in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
Sea A	22.92	60897	0.009	78775743	0.001
Sea B	22.89	21959	0.005	56685497	0
FOAM	N/F	N/F	N/F	N/F	N/F
Elv A	N/F	N/F	N/F	N/F	N/F
Elv B	N/F	N/F	N/F	N/F	N/F
Site A	N/F	N/F	N/F	N/F	N/F
Site B	N/F	N/F	N/F	N/F	N/F

Table C.9. Dimethyl PFOA, water samples results. Sea A/B are sea water samples. FOAM is the foam sample. Elv A/B are samples of river water going into the sea by the dockyard. Site A/B are water samples of ground water near the hotspot. Concentrations given in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
Sea A	N/F	N/F	N/F	78775743	N/F
Sea B	N/F	N/F	N/F	56685497	N/F
FOAM	N/F	N/F	N/F	N/F	N/F
Elv A	N/F	N/F	N/F	N/F	N/F
Elv B	N/F	N/F	N/F	N/F	N/F
Site A	N/F	N/F	N/F	N/F	N/F
Site B	N/F	N/F	N/F	N/F	N/F

Table C.10. Blanks samples analysed for linear PFOA. B T 2 was not analysed (lost during extraction). Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
B T	N/F	N/F	N/F	N/F	N/F
B T 2	x	x	x	x	x
BUM 1	N/F	N/F	N/F	N/F	N/F
BUM 2	N/F	N/F	N/F	N/F	N/F
FB1	N/F	N/F	N/F	5703317	N/F
FB2	N/F	N/F	N/F	5211637	N/F
FB3	N/F	N/F	N/F	4948357	N/F
FBA	23.29	29583	N/A	N/F	N/F
FBB	N/F	N/F	N/F	127876	N/F

Table C.11. Blanks samples analysed for monomethyl PFOA. B T 2 was not analysed (lost during extraction). Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
B T	N/F	N/F	N/F	N/F	N/F
B T 2	x	x	x	x	x

BUM 1	N/F	N/F	N/F	N/F	N/F
BUM 2	N/F	N/F	N/F	N/F	N/F
FB1	N/F	N/F	N/F	5703317	N/F
FB2	N/F	N/F	N/F	5211637	N/F
FB3	N/F	N/F	N/F	4948357	N/F
FBA	N/F	N/F	N/F	N/F	N/F
FBB	N/F	N/F	N/F	127876	N/F

Table C.12. Blanks samples analysed for dimethyl PFOA. B T 2 was not analysed 8lost during extraction). Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
B T	N/F	N/F	N/F	N/F	N/F
B T 2	x	x	x	x	x
BUM 1	N/F	N/F	N/F	N/F	N/F
BUM 2	N/F	N/F	N/F	N/F	N/F
FB1	N/F	N/F	N/F	5703317	N/F
FB2	N/F	N/F	N/F	5211637	N/F
FB3	N/F	N/F	N/F	4948357	N/F
FBA	N/F	N/F	N/F	N/F	N/F
FBB	N/F	N/F	N/F	127876	N/F

Table C.13. Quality control sample, PFOA. Concentration (calculated amount) is shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
QC 9 ng/mL (Linear)	23.13	35815405	10.03	150224569	0.238

Tabell C.14. Proficiency tests results for PFOA. Concentrations are shown in mg/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g w.w.
PT 1 Linear PFOA	23.23	712962	1.387	21625708	0.033	2.657
PT 2 Linear PFOA	23.23	671956	1.17	24158368	0.028	2.422
PT 3 Linear PFOA	23.22	699762	1.086	27101310	0.026	2.172
PT 1 (P1/P3/P4/P55)	N/F	N/F	N/F	21625708	N/F	N/F
PT 2 (P1/P3/P4/P55)	N/F	N/F	N/F	24158368	N/F	N/F
PT 3 (P1/P3/P4/P55)	N/F	N/F	N/F	27101310	N/F	N/F
PT 1 (P45/P35)	N/F	N/F	N/F	21625708	N/F	N/F
PT 2 (P45/P35)	N/F	N/F	N/F	24158368	N/F	N/F

PT 3 (P45/P35)	N/F	N/F	N/F	27101310	N/F	N/F

D – Raw data PFOS

Table D.1. Raw data for the linear/P6/P5 PFOS in soil samples. The SX samples come from the surface soil, MX samples from the mid-level, BX samples from the bottom level (X is the number of the replicate). Samples with T at the end are TOPA samples. Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g d.w.
S1	24.43	87911794	13.509	45999482	1.911	13.23
S2	24.43	99248248	14.421	48647691	2.04	14.37
S3	24.42	87970088	16.875	36847553	2.387	16.97
S1 T	24.6	23826293	13.289	12672954	1.88	13.01
S2 T	24.59	27313072	15.433	12509586	2.183	15.38
S3 T	24.59	36396584	17.771	14476701	2.514	17.87
M1	24.33	26276395564	18316.182	10140352	2591.271	17101
M2	24.34	23000866613	17779.409	9144271	2515.331	18253
M3	24.35	30368574870	19259.246	11145699	2724.69	18465
M1 T	24.52	14824731396	19492.339	5375824	2757.667	18199
M2 T	24.53	12802473067	20294.303	4459046	2871.124	20835
M3 T	24.51	13409240477	21041.817	4504464	2976.878	20173
B1	24.38	11107510450	4563.964	17202705	645.684	4404
B2	24.4	7903986137	3952.149	14136278	559.128	3920
B3	24.37	10296722104	4359.939	16693246	616.82	4269
B1 T	24.56	3562543978	4495.052	5602059	635.935	4338
B2 T	24.56	3406853524	4269.408	5640375	604.012	4235
B3 T	24.55	2750966247	4736.882	4105016	670.147	4638

Table D.2. Raw data for the P1/P3/P4/P55 PFOS isomers in soil samples. The SX samples come from the surface soil, MX samples from the mid-level, BX samples from the bottom level (X is the number of the replicate). Samples with T at the end are TOPA samples. Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g d.w.
B1	24.13	4079245982	1183.247	17202705	237.128	1141
B2	24.14	2655662490	937.411	14136278	187.862	929
B3	24.14	3994901300	1194.147	16693246	239.312	1169
B1 T	24.25	878911775	782.871	5602059	156.891	754
B2 T	24.24	767224352	678.745	5640375	136.024	672
B3 T	24.25	661819165	804.483	4105016	161.222	787
M1	24.15	3469210875	1707.144	10140352	342.119	1593
M2	24.14	2911939645	1589.007	9144271	318.444	1631
M3	24.15	3841018618	1719.616	11145699	344.619	1648
M1 T	24.25	1266731234	1175.796	5375824	235.635	1097
M2 T	24.26	1035521956	1158.804	4459046	232.23	1188

M3 T	24.26	1103110797	1221.992	4504464	244.893	1170
S1	24.1	4064443	0.441	45999482	0.088	0.431
S2	24.09	4947848	0.508	48647691	0.102	0.506
S3	24.09	3886442	0.526	36847553	0.105	0.529
S1 T	24.28	1370715	0.54	12672954	0.108	0.528
S2 T	24.26	1606644	0.641	12509586	0.128	0.639
S3 T	24.27	1735761	0.598	14476701	0.12	0.601

Table D.3. Raw data for the P45/P35 PFOS isomers in soil samples. The SX samples come from the surface soil, MX samples from the mid-level, BX samples from the bottom level (X is the number of the replicate). Samples with T at the end are TOPA samples. Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g d.w.
S1	23.6	71288	0.013	45999482	0.002	0.012
S2	23.59	99698	0.018	48647691	0.002	0.017
S3	23.59	77486	0.018	36847553	0.002	0.018
S1 T	N/F	N/F	N/F	12672954	N/F	N/F
S2 T	N/F	N/F	N/F	12509586	N/F	N/F
S3 T	N/F	N/F	N/F	14476701	N/F	N/F
M1	N/F	N/F	N/F	10140352	N/F	N/F
M2	N/F	N/F	N/F	9144271	N/F	N/F
M3	N/F	N/F	N/F	11145699	N/F	N/F
M1 T	23.76	17256601	27.611	5375824	3.21	25.78
M2 T	23.77	14242392	27.474	4459046	3.194	28.20
M3 T	23.77	16320533	31.165	4504464	3.623	29.88
B1	23.68	91074708	45.538	17202705	5.294	43.95
B2	23.65	44148386	26.863	14136278	3.123	26.64
B3	23.67	95717941	49.32	16693246	5.734	48.30
B1 T	23.75	18799444	28.865	5602059	3.356	27.86
B2 T	23.75	13350889	20.36	5640375	2.367	20.19
B3 T	23.75	13696212	28.699	4105016	3.336	28.10

Table D.4. Raw data of linear/P6/P5 PFOS in biota samples. The naming can be explained by the following; M(mackerel)Mus(muscle or Liv for liver sample) 1 (fish n) and A (replicate, n = 2, A and B). GSW stands for goldsinny wrasse. Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g w.w.
MMus 1 A	24.52	1961876	0.454	30567161	0.064	0.416
MMus 1 B	24.53	1302114	0.347	26496078	0.049	0.364
MMus 2 A	24.52	1210097	0.275	31101477	0.039	0.255
MMus 2 B	24.53	967755	0.242	28244275	0.034	0.242
MMus 3 A	24.53	1087982	0.282	27295328	0.04	0.254
MMus 3 B	24.53	921282	0.242	26956222	0.034	0.249
MMus 4 A	24.52	1433592	0.344	29425166	0.049	0.307

MMus 4 B	24.53	1437362	0.323	31470667	0.046	0.312
MMus 5 A	24.5	1434226	0.359	28257770	0.051	0.379
MMus 5 B	24.52	1393818	0.36	27372843	0.051	0.353
MLiv 1 A	24.39	8432965	2.068	28820566	0.293	4.04
MLiv 1 B	24.4	7486845	2.21	23946633	0.313	4.00
MLiv 2 A	24.42	4616175	1.314	24840785	0.186	2.84
MLiv 2 B	24.46	3687714	1.425	18288333	0.202	2.98
MLiv 3 A	24.38	7032678	1.513	32865641	0.214	2.92
MLiv 3 B	24.35	7612152	1.923	27983919	0.272	3.64
MLiv 4 A	24.37	10455206	2.6	28419686	0.368	4.58
MLiv 4 B	24.39	10216026	2.251	32084598	0.318	4.95
MLiv 5 A	24.41	8168851	2.162	26707723	0.306	4.10
MLiv 5 B	24.43	6688851	1.833	25796835	0.259	3.91
Pollock A	24.55	1364219	0.344	28065559	0.049	0.362
Pollcok B	24.54	1405710	0.364	27278455	0.052	0.373
GSW A	24.55	390682	0.109	25397213	0.015	0.112
GSW B	24.55	444474	0.109	28721740	0.015	0.112

Table D.5. Raw data of P1/P3/P4/P55 PFOS in biota samples. The naming can be explained by the following; M(mackerel)Mus(muscle or Liv for liver sample) 1 (fish n) and A (replicate, n = 2, A and B). GSW stands for goldsinny wrasse. Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g w.w.
MMus 1 A	N/F	N/F	N/F	30567161	N/F	N/F
MMus 1 B	N/F	N/F	N/F	26496078	N/F	N/F
MMus 2 A	N/F	N/F	N/F	31101477	N/F	N/F
MMus 2 B	N/F	N/F	N/F	28244275	N/F	N/F
MMus 3 A	N/F	N/F	N/F	27295328	N/F	N/F
MMus 3 B	N/F	N/F	N/F	26956222	N/F	N/F
MMus 4 A	N/F	N/F	N/F	29425166	N/F	N/F
MMus 4 B	N/F	N/F	N/F	31470667	N/F	N/F
MMus 5 A	N/F	N/F	N/F	28257770	N/F	N/F
MMus 5 B	N/F	N/F	N/F	27372843	N/F	N/F
MLiv 1 A	N/F	N/F	N/F	28820566	N/F	N/F
MLiv 1 B	24.04	199494	0.042	23946633	0.008	0.076
MLiv 2 A	N/F	N/F	N/F	24840785	N/F	N/F
MLiv 2 B	N/F	N/F	N/F	18288333	N/F	N/F
MLiv 3 A	N/F	N/F	N/F	32865641	N/F	N/F
MLiv 3 B	N/F	N/F	N/F	27983919	N/F	N/F
MLiv 4 A	24.03	135684	0.024	28419686	0.005	0.0423
MLiv 4 B	N/F	N/F	N/F	32084598	N/F	N/F
MLiv 5 A	N/F	N/F	N/F	26707723	N/F	N/F
MLiv 5 B	N/F	N/F	N/F	25796835	N/F	N/F
GSW A	N/F	N/F	N/F	25397213	N/F	N/F
GSW B	N/F	N/F	N/F	28721740	N/F	N/F

Pollock A	N/F	N/F	N/F	28065559	N/F	N/F
Pollock B	N/F	N/F	N/F	27278455	N/F	N/F

Table D.6. Raw data of P45/P35 PFOS in biota samples. The naming can be explained by the following; M(mackerel)Mus(muscle or Liv for liver sample) 1 (fish n) and A (replicate, n = 2, A and B). GSW stands for goldsinny wrasse. Concentrations (calculated amount) are shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g w.w.
MMus 1 A	N/F	N/F	N/F	30567161	N/F	N/F
MMus 1 B	N/F	N/F	N/F	26496078	N/F	N/F
MMus 2 A	N/F	N/F	N/F	31101477	N/F	N/F
MMus 2 B	N/F	N/F	N/F	28244275	N/F	N/F
MMus 3 A	N/F	N/F	N/F	27295328	N/F	N/F
MMus 3 B	N/F	N/F	N/F	26956222	N/F	N/F
MMus 4 A	N/F	N/F	N/F	29425166	N/F	N/F
MMus 4 B	N/F	N/F	N/F	31470667	N/F	N/F
MMus 5 A	N/F	N/F	N/F	28257770	N/F	N/F
MMus 5 B	N/F	N/F	N/F	27372843	N/F	N/F
MLiv 1 A	N/F	N/F	N/F	28820566	N/F	N/F
MLiv 1 B	N/F	N/F	N/F	23946633	N/F	N/F
MLiv 2 A	N/F	N/F	N/F	24840785	N/F	N/F
MLiv 2 B	N/F	N/F	N/F	18288333	N/F	N/F
MLiv 3 A	N/F	N/F	N/F	32865641	N/F	N/F
MLiv 3 B	N/F	N/F	N/F	27983919	N/F	N/F
MLiv 4 A	N/F	N/F	N/F	28419686	N/F	N/F
MLiv 4 B	N/F	N/F	N/F	32084598	N/F	N/F
MLiv 5 A	N/F	N/F	N/F	26707723	N/F	N/F
MLiv 5 B	N/F	N/F	N/F	25796835	N/F	N/F
GSW A	N/F	N/F	N/F	25397213	N/F	N/F
GSW B	N/F	N/F	N/F	28721740	N/F	N/F
Pollock A	N/F	N/F	N/F	28065559	N/F	N/F
Pollock B	N/F	N/F	N/F	27278455	N/F	N/F

Table D.7. Linear/P6/P5 PFOS, water samples results. Sea A/B are sea water samples. FOAM is the foam sample. Elv A/B are samples of river water going into the sea by the dockyard. Site A/B are water samples of ground water near the hotspot. Concentrations given in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
Sea A	24.57	5530284	0.551111111	78775743	0.07
Sea B	24.56	3411233	0.472222222	56685497	0.06
FOAM	24,49	2829350	N/F	N/F	N/F
Elv A	24,58	198599	N/F	N/F	N/F
Elv B	N/F	N/F	N/F	N/F	N/F
Site A	N/F	N/F	N/F	N/F	N/F
Site B	N/F	N/F	N/F	N/F	N/F

Table D.8. P1/P3/P4/P55 PFOS, water samples results. Sea A/B are sea water samples. FOAM is the foam sample. Elv A/B are samples of river water going into the sea by the dockyard. Site A/B are water samples of ground water near the hotspot. Concentrations given in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
Sea A	24,25	1273292	0,081	78775743	0,016
Sea B	24,12	547881	0,048	56685497	0,01
FOAM	N/F	N/F	N/F	N/F	N/F
Elv A	N/F	N/F	N/F	N/F	N/F
Elv B	N/F	N/F	N/F	N/F	N/F
Site A	N/F	N/F	N/F	N/F	N/F
Site B	N/F	N/F	N/F	N/F	N/F

Table D.9. P45/P35 PFOS, water samples results. Sea A/B are sea water samples. FOAM is the foam sample. Elv A/B are samples of river water going into the sea by the dockyard. Site A/B are water samples of ground water near the hotspot. Concentrations given in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
Sea A	N/F	N/F	N/F	78775743	N/F
Sea B	N/F	N/F	N/F	56685497	N/F
FOAM	N/F	N/F	N/F	N/F	N/F
Elv A	N/F	N/F	N/F	N/F	N/F
Elv B	N/F	N/F	N/F	N/F	N/F
Site A	N/F	N/F	N/F	N/F	N/F
Site B	N/F	N/F	N/F	N/F	N/F

Table D.10. Linear/P6/P5 PFOS blank samples.

Filename	Actual retention time	Area	Calculated amount	ISTD Response	Response ratio
B T	24.56	25494279	N/F	N/F	N/F
B T 2	x	X	x	x	x
BUM 1	24.59	49273	N/F	N/F	N/F
BUM 2	24.57	58343	N/F	N/F	N/F
FB1	24.43	133351	0.165	5703317	0.023
FB2	24.45	129718	0.176	5211637	0.025
FB3	24.42	106168	0.152	4948357	0.021
FBA	24.59	164677	N/A	N/F	N/F
FBB	24.57	272172	15.044	127876	2.128

Table D.11. P1/P3/P4/P55 PFOS blank samples.

Filename	Actual retention time	Area	Calculated amount	ISTD Response	Response ratio
B T	24.24	1156431	N/F	N/F	N/F
B T 2	x	X	x	x	x
BUM 1	N/F	N/F	N/F	N/F	N/F
BUM 2	N/F	N/F	N/F	N/F	N/F
FB1	N/F	N/F	N/F	5703317	N/F
FB2	N/F	N/F	N/F	5211637	N/F
FB3	N/F	N/F	N/F	4948357	N/F
FBA	N/F	N/F	N/F	N/F	N/F
FBB	N/F	N/F	N/F	127876	N/F

Table D.12. P45/P35 PFOS blank samples.

Filename	Actual retention time	Area	Calculated amount	ISTD Response	Response ratio
B T	N/F	N/F	N/F	N/F	N/F
B T 2	x	X	x	x	x
BUM 1	N/F	N/F	N/F	N/F	N/F
BUM 2	N/F	N/F	N/F	N/F	N/F
FB1	N/F	N/F	N/F	5703317	N/F
FB2	N/F	N/F	N/F	5211637	N/F
FB3	N/F	N/F	N/F	4948357	N/F
FBA	N/F	N/F	N/F	N/F	N/F
FBB	N/F	N/F	N/F	127876	N/F

Table D.13. Quality control sample, PFOS. Concentration (calculated amount) is shown in ng/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio
QC 9 ng/mL (Linear/P6/P5)	24.43	123931935	5.831	150224569	0.825

Tabell D.14. Proficiency tests results for PFOS. Concentrations are shown in mg/mL.

Filename	Actual RT	Area	Calculated Amt	ISTD Response	Response Ratio	ng/g w.w.
PT 1 (Linear/P6/P5)	24.36	57463986	18.782	21625708	2.657	35.98
PT 2 (Linear/P6/P5)	24.39	49941536	14.612	24158368	2.067	30.25
PT 3 (Linear/P6/P5)	24.36	58154369	15.168	27101310	2.146	30.34

PT 1 (P1/P3/P4/P55)	24.16	2581618	0.596	21625708	0.119	0.596
PT 2 (P1/P3/P4/P55)	24.15	2365683	0.489	24158368	0.098	0.489
PT 3 (P1/P3/P4/P55)	24.14	2804733	0.516	27101310	0.103	0.516
PT 1 (P45/P35)	N/F	N/F	N/F	21625708	N/F	N/F
PT 2 (P45/P35)	N/F	N/F	N/F	24158368	N/F	N/F
PT 3 (P45/P35)	N/F	N/F	N/F	27101310	N/F	N/F

E - MS parameters

Table E.1. Mass spectrometer parameters used in this thesis.

Scan Parameters	**Value/status**
Settings	**House method**
Method duration	50 min
Expected LC peak Width (s)	10
Mild Trapping	Off
Internal Mass Calibration	EASY-IC
Mode	Scan-to-Scan
Full Scan + dyn. Exc. + targ. Mass.	
Orbitrap resolution	60000
Scan Range (m/z)	100 13000
RF Lens (%)	70
AGC Target	Standard
Maximum Injection Time Mode	Auto
Microscans	5
Data Type	Profile
Polarity	Negative
Source Fragmentation	FALSE
Use EASY-IC	On
Number of Scans	1
Dynamic Exclusion Mode	Custom
Exclude after n times	1
Exclusion Duration (s)	2
Mass Tolerance	ppm
Low	10
High	10
Exclude isotopes	TRUE
Mass List Type	m/z
Time Mode	Retention Time Window
Include Intensity Treshhold	Off
Mass Tolerance	ppm
Low	10

High	3
Set Collision Energy per Compound	Off
Perform dependant scan on most intense ion if no targets are found	On
MS2 Scan properties	
Isolation Windown (m/z)	1.5
Isolation Offset	Off
Collision Energy Type	Normalized
HCD Collision Energies (%)	20, 60, 100
Orbitrap resolution	30000
Scan Range Mode	Define m/z range
First Mass (m/z)	79 - 750
AGC Target	Standard
Maximum Injection Time Mode	Auto
Microscans	1
Data Type	Profile
Use EASY-IC	On
Ion Source parameters	
Ion Source Type	H-ESI
Spray Voltage	Static
Positive Ion	3500
Negative Ion	3000
Gas Mode	Static
Seath Gas (Arb)	40
Aux Gas (Arb)	10
Sweep Gas (Arb)	1
Ion Transfer Tube Temp (Celsius)	320
Vaporaizer Temp (Celsius)	300
APPI Lamp	Not in Use
Use Ion Source Settings from Tune	Off
FAIMS Mode	Not Installed

F - Weights of samples

Table F.1. Overview of the weighed samples used in this project. S stands soil surface (soil), M for mid-level, B for bottom level. PT standard for proficiency test. MM stands for mackerel muscle, GS stands for goldshinny wrassle muscle, L stands for pollock muscle. ML stands for mackerel liver.

Sample	Dry weight (grams)	Wet weight (grams)
S1	1.021	X
S2	1.003	X
S3	0.994	X
M1	1.071	X
M2	0.974	X
M3	1.043	X
B1	1.036	X

B2	1.008	X
B3	1.021	X
MM11	X	1.091
MM12	X	0.952
MM21	X	1.077
MM22	X	0.999
MM31	X	1.112
MM32	X	0.973
MM41	X	1.119
MM42	X	1.035
MM51	X	0.948
MM52	X	1.021
ML11	X	0.512
ML12	X	0.552
ML21	X	0.463
ML22	X	0.478
ML31	X	0.518
ML32	X	0.528
ML41	X	0.568
ML42	X	0.455
ML51	X	0.527
ML52	X	0.469
GS1	X	0.971
GS2	X	0.975
L1	X	0.950
L2	X	0.977
PT1	X	0.522
PT2	X	0.483
PT3	X	0.500