# Perfluorinated Alkylated Substances (PFAS) in Arctic Char (*Salvelinus alpinus*): A case study from Svalbard

# Perfluorerte Alkylerte Substanser (PFAS) i Arktisk Røye (*Salvelinus alpinus*): En studie fra Svalbard

Maren Garsjø

Norwegian University of Life Sciences
Department of chemistry, biotechnology and food science
Master Thesis 60 credits 2013

# Preface

## Perfluorinated alkylated substances (PFAS) in Arctic char (*Salvelinus alpinus*): A case study from Svalbard, Norway

This thesis was written at the Department of Chemistry, Biotechnology and Food Science at the University of Life Sciences in Ås, Norway. The work was done in collaboration with the University Centre in Svalbard (UNIS).

Maren Garsjø

Ås, 27.11.2013

# ACKNOWLEDGEMENTS

Since I started studying at the University of Life Sciences (UMB), in Ås, I have always wanted to go to Svalbard. During the course of my master's degree I have had the opportunity to travel to Spitsbergen several times. During these visits I have attended classes and conducted field- and laboratory work, the results of which are contained in this thesis. The past two years have gone by so fast. Especially the last year, working on my thesis, has been exiting and very challenging. It has been two instructive years!

First of all I want to thank my supervisor, Professor Roland Kallenborn, for giving me the opportunity to conduct research on Svalbard and also for guidance while writing my thesis. Additionally, I would like to thank Dr. Dorte Herzke at NILU in Tromsø for assisting me with sample analysis, as well as guidance and encouraging words. The Governor of Svalbard granted my fieldwork. Thank you. The project was conducted in close collaboration with the University Centre in Svalbard (UNIS), Department of Arctic Technology in Longyearbyen. Funding for this project was provided from the Nordic Council of Ministers (NMR) and Arctic Monitoring and Assessment Programme (AMAP) through the research project "Combined effects of pollutants and climate change" as well the Research Council of Norway (RCN) funded project "Polyfluorinated compounds: Are point sources contaminating the environment-consequences for human exposure in China and Norway ". Thank you for helping this project financially. I also want to give thanks to David Huertas, who kindly provided fish samples for my project, and my fellow student Graham Gilbert for feedback on my thesis. Thank you, Trygve Almøy, for helping me with the statistical program.

I kindly acknowledge the help of Tatyana Drotikova and Pernilla Carlsson in the laboratory at UNIS, and also Sara Mollie Cohen for accompanying me to Kapp Linné for field wok. I guess I would have been lost on Svalbard if it were not for you ☺. Thank you so much! We had a lot of fun! Family and friends, thank you for giving me support, encouraging words and hugs through this period. I would probably not have made it without you believing in me and help me remember to have fun while working on this project. I have gained a lot of new experiences and I will bring them with me when I now start a new chapter in my life.

27th of November 2013
Maren Garsjø

# ABSTRACT

Perfluoroalkylated substances (PFAS) have been used globally, and transported to and detected in the Arctic. Since the Arctic is not expected to be the source for these substances, these contaminants have the potential to be transported via the atmosphere and/or the ocean from areas in lower latitudes. However local sources may also contribute. PFAS can affect organisms, and have toxic and carcinogenic potential. Hence, PFAS are of concern for the Arctic wildlife and humans living in Arctic areas.

The aim of the study is to increase the scientific knowledge about PFAS levels in Arctic char (*Salvelinus alpinus*) from Linnévatnet in western Svalbard, with regards to the potential exposure route for human consumers. In most of the high Arctic region, Arctic char is the only resident fish species in freshwaters, and it was therefore chosen as biomonitoring species in this project. Arctic char were caught in March 2013 and September 2010. Analysis of liver and fillet samples were conducted at the Norwegian Institute of Air Research (NILU) in Tromsø. Principal component analysis (PCA) was used to examine the relationship between liver and fillet samples and PFAS.

The results showed that perfluorobutanoic acid (PFBA) and perfluorohexanoic acid (PFHxA) were the most abundant PFAS compounds detected in all samples. In 2010, PFBA in fillet samples showed the highest concentration (8.3 ng/g wet weight; ww). The fluortelomer 6:2FTS showed highest concentration (5.3 ng/g ww) in liver sample from 2013. The short-chained PFAS compounds (4-6 carbons) contributed to 78 % of ΣPFAS in the 2010 measurements, while the long-chained PFAS (7-13 carbons) together accounted for 56.8% of the ΣPFAS in 2013. In general, the concentrations were relatively low. There was a decrease in levels of PFAS in fillet samples from 2010 to 2013, while the highest levels were detected mainly in liver samples from 2013. This is consistent with previous studies indicating that PFAS binds to proteins in the blood and bioaccumulate in the liver. Because of the location of Linnévatnet and the low concentrations of PFAS, long-rang atmospheric transport is the main pathway for these contaminants instead of local sources. Based on previous studies, perfluorooctanoic sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were expected to have higher concentrations and to be the dominating contaminants, but this was not the case in the present study. This indicates that temporal trends and spatial distribution of PFAS

should be continually monitored. The present study is, to our knowledge, the first to report levels of PFAS in freshwater fish in Svalbard.

# SAMMENDRAG

Perfluoroalkylerte substanser (PFAS) har blitt spredt globalt, og blant annet detektert i Arktis. Siden Arktis ikke forventes å være kilden for slike stoffer, er det grunn til å tro at disse miljøgiftene har blitt transportert via atmosfæren eller havet, fra områder i mer urbane områder. Lokale kilder kan ikke utelukkes. PFAS kan påvirke levende organismer, og har toksisk og karsinogent potensial. Dette gir grunn til bekymring for miljøet, dyrelivet og menneskene.

Målet med studien var å undersøke nivåene av PFAS i Arktisk røye (*Salvelinus alpinus*), fra innsjøen Linnévatnet på Svalbard. Det var i tillegg ønskelig å få kunnskap om nivåene av PFAS i Arktisk røye, da arten er en potensiell eksponeringsrute for mennesker som spiser fisk. I de nordligste områdene i Arktis er Arktisk røye den eneste stasjonære fiskearten i ferskvann. Derfor ble denne arten undersøkt i dette prosjektet. Arktisk røye ble fisket under feltarbeid i mars 2013 og september 2010. Lever- og filétprøver ble brukt til undersøkelsen av PFAS. Analysene ble utført ved Norsk Institutt for Luftforskning (NILU) i Tromsø. Prinsipal komponent analyse (PCA) ble brukt til å undersøke forholdet mellom lever- og filétprøver og PFAS forbindelsene.

Resultatene viste at perfluorert butansyre (PFBA) og perfluorert heksansyre (PFHxA) var de forbindelsene som var detektert i alle prøvene. I 2010 viste PFBA høyeste konsentrasjon (8.3 ng/g våtvekt) i filét prøve. Fluortelomer 6:2 FTS viste høyest konsentrasjon (5.3 ng/g våtvekt) i lever prøve fra 2013. De kortkjedete PFAS forbindelsene (4-6 karboner) bidro til 78 % av ΣPFAS i 2010 målingene, mens de langkjedete PFAS forbindelsene (7-13 karboner) til sammen stod for 56,8% av ΣPFAS i 2013. Generelt var konsentrasjonene relativt lave. Likevel indikerte resultatene en nedgang i nivåer av PFAS i filét prøver fra 2010 til 2013, men de høyeste nivåene ble hovedsakelig påvist i lever prøver fra 2013. Dette er i overensstemmelse med tidligere studier som indikerer at PFAS bindes til proteiner i blodet og bioakkumulerer i leveren. På grunn av den geografiske lokaliteten til Linnévatnet, samt de lave konsentrasjonene av PFAS, er det grunn til å tro at lang-transport via atmosfæren er den viktigste ruten for disse miljøgiftene, i stedet for lokale kilder. Basert på tidligere studier, var det forventet å få høyere konsentrasjoner av perfluorooktan sulfonat (PFOS ) og perfluoroktan syre (PFOA), samt å være de mest dominerende forbindelsene, men det var ikke tilfelle i denne studien. Dette viser at det grunn for videre forskning og til å undersøke

tidsmessige trender og distribusjon av disse miljøgiftene. Studien som foreligger er, så vidt vi vet, den første til å rapportere nivåer av PFAS i ferskvannsfisk på Svalbard.

# TABLE OF CONTENTS

# INDEX OF FIGURES, TABLES AND APPENDIX

## FIGURES



## TABLES



## APPENDIX

# ABBREVIATIONS

| | |
|---|---|
| APCI | Atmospheric pressure chemical ionization |
| APFO | Perfluorooctanoate |
| APPI | Atmospheric pressure photoionization |
| bl | Blank |
| bw. | Body weight |
| C | Carbon |
| ECF | Electrochemical fluorination |
| ECI | Electrospray ionization |
| FOSA | Perfluoro octansulfonamide |
| FTOHs | Fluorotelomer alcohols |
| FTS | Fluorotelomer sulfonates |
| g | Gram |
| GC/MS | Gas chromatography/mass spectrometry |
| GJIC | Gap junction intercellular communication |
| HPLC | High-performance liquid chromatography |
| IKBM | Department of Chemistry, Biotechnology and Food Science |
| ISTD | Internal standard |
| LOQ | Limit of quantification |
| MeOH | Methanol |
| MS | Mass spectrometry |
| MS/MS | Tandem mass spectrometry |
| *n* | number of carbon atoms |
| NA | Not available |
| N-EtPFOSE | 2(N -ethyl perfluorooctane sulfonamido)ethanol |
| ng | Nanogram |
| $NH_4OAc_{aq}$ | Aqueous ammonium acetate |
| NILU | Norwegian Institute of Air Research |
| NPI | Norwegian Polarinstitute |
| PCB | Polychlorinated biphenyls |
| PFAS | Perfluorinated alkylated substances |
| PFBA | Perfluorobutanoate |
| PFBS | Perfluorobutane sulfonate |

| | |
|---|---|
| PFCs | Perfluoro carbons (as defined via the Kyoto protocol) |
| PFCAs | Perfluorinated carboxylated acids |
| PFDcA | Perfluorodecanoate |
| PFDcS | Perfluorodecane sulfonate |
| PFDoA | Perfluorododecanoate |
| PFHpA | Perfluoroheptanoate |
| PFHpS | Perfluoroheptane sulfonate |
| PFHxA | Perfluorohexanoate |
| PFHxS | Perfluorohexane sulfonate |
| PFNA | Perfluorononanoate |
| PFOA | Perfluorooctanoic acid / Perfluorooctanoate |
| PFOS | Perfluorooctan sulfonate |
| PFOSA | Perfluorooctane sulfonamide |
| PFPA | Perfluoropentanoate |
| PFPS | Bis-(3-pentafluorophenylpropyl)-sulfide |
| PFSAs | Perfluoroalkylsulfonic acid / perfluoroalkylsulfonates |
| PFTeA | Perfluorotetradecanoate |
| PFTrA | Perfluorotridecanoate |
| PFUnA | Perfluoroundecanoate |
| POP | Persistent organic pollution |
| PP | Polypropylene |
| Q | Quadrupole |
| q | RF-only Quadrupole as collision cell |
| QqQ | Tripple quadrupole |
| qstd | Quantification standard |
| R | General symbol of an alkyl group |
| RF | Radio frequency |
| rpm | Revolutions per minute |
| RSTD | Recovery standard |
| Spl | Sample |
| SVOC | Semi-volatile organic compounds |
| t | Metric tons |
| TDI | Tolerable daily intake |

| | |
|---|---|
| TSP LC/MS | Thermospray liquid chromatography coupled to mass spectrometry; (TSP = Thermo Fisher Trademark) |
| UHPLC-MSMS | Ultrahigh pressure liquid chromatography triple-quadrupole mass-spectrometry |
| UMB | University of Life Sciences |
| UNIS | The University Center in Svalbard |
| USB | Ultrasonic bath |
| V | Voltage |
| W | Winter (used for fish age) |
| ww | wet weight |
| WWTP | Waste water treatment plant |
| 3,7 brPFDcA | 3,7-dimethyl-branched perfluorodecanoic acid |

# 1 Introduction

## 1.1 Perfluorinated Alkylated Substances

Perfluorinated Alkylated substances (PFAS) are a group of anthropogenic compounds characterized by unique physico-chemical properties. Such properties include: low surface energy, high surface-active properties as well as thermal and chemical inertness (Key et al. 1997). Because of these unique and desired properties, PFAS have been manufactured over 50 years and used in different industrial and consumer products, such as emulsifiers, surfactants, fire-fighting foam, electrical products and for clothing that needs waterproofing and insulation (Holmstrom et al. 2005; Houde et al. 2006; Kelly et al. 2009; Sinclair et al. 2006). Additionally, due to their lipophobic and hydrophobic properties, PFAS are employed as stain and grease repellents. The liberal application of PFAS in industrial processes and manufacturing of consumer goods has resulted in their introduction into natural environments. Today, PFAS are ubiquitous, to greater or lesser degrees, in the biosphere and hydrosphere. Of particular interest is the occurrence of PFAS in biota and emissions from point sources such as households and industry (Clara et al. 2008; Key et al. 1997; Schuetze et al. 2010).

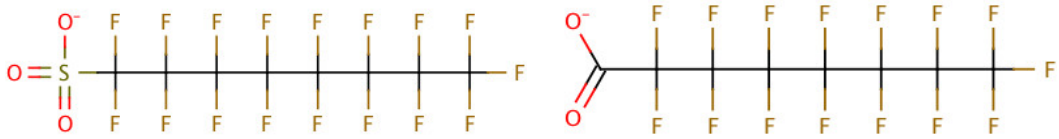
Levels of perfluoroalkyl substances (PFAS) have been globally detected in different environmental matrices, but the distribution, transport and fate of PFAS to the Arctic, especially for the European Arctic, are still not fully understood. This makes scientists question what may be the origin and transport pathways for these compounds.

### *1.1.1 Physical-chemical properties and structure*

Per- and polyfluoroalkyl compounds (PFAS) consist of a perfluoroalkyl chain with a hydrophilic end group. The chain can vary in length, from n = 4 to n = 18 (Sturm & Ahrens 2010). In these compounds, the majority of the hydrogen atoms are replaced with fluoride atoms. The general structural formula is $F(CF_2)_nR$ , where R represents an alkyl group and *n* is the number of ($CF_2$) in the chain (Clara et al. 2008; Kissa 2001; Lau et al. 2007; Parsons et al. 2008; Stock et al. 2010). Perfluoroalkylated compounds are characterized by strong carbon-fluorine bonds, present due to high electronegativity of the C-F bond. These compounds are therefore highly resistant to biodegradation,

photolysis, hydrolysis and metabolism (Buck et al. 2011; Giesy et al. 2001; Hansen et al. 2001).

At present, there are several different classes of perfluoroalkyl compounds (PFCs) (Stock et al. 2010). The most known groups of PFCs are the perfluoroalkylsulfonic acid or perfluoroalkylsulfonates (PFSAs) and perfluorocarboxylic acids or perfluorocaboxylates (PFCAs); which include compounds such as perfluoroctaneslufonic acid (PFOS) and perfluorooctane carboxylic acid (PFOA), respectively. Both of these compounds have very low pKa values and dissociates in the environment (Conder et al. 2008; Kissa 2001; Schenker et al. 2008; Stock et al. 2010). A generalized diagram of PFOS and PFOA is provided in Figure 1.



PFOS PFOA

**Figure 1:** Chemical Structure of PFOS and PFOA
Drawn by Maren Garsjø (ChemIDplus Downloaded 25.09.2013).

In organic chemistry, the carbon-fluorine bond is the strongest observed. The strength of this bond is attributed to a strong polarization. Fluorine attracts electrons, has a high ionization potential, low polarizability and is the most electronegative element known (Kissa 2001; Smart 2001). The reason perfluorinated alkylated substances are typified by high thermal and chemical stability is the strength of the C-F bond (~460 kJ/mol). In other words, the C-F bond is extremely resistant to chemical and heat attacks (3MCompany 2000b; Butt et al. 2010; Key et al. 1997; Lau et al. 2007). PFCAs and PFSAs dissociate completely in water, and the solubility of the perfluoroalkyl chain usually decreases with increasing length. PFOS is less water-soluble than PFOA (Stock et al. 2010).

### *1.1.2 Manufacturing and production*

In the environment, some PFAS can occur naturally, but this happens rarely. On the other hand, PFOS and PFOA are exclusively anthropogenic compounds (Kissa 1994; Paul et al. 2009). In the commercial manufacturing of PFAS, there are two processes necessary to mention; telomerization and electrochemical fluorination (ECF). To better understand the environmental occurrence and behavior of PFAS, it is useful to describe these two techniques briefly.

*Electrochemical fluorination (ECF)*

Electrochemical fluorination (ECF) involves the replacement of hydrogen atoms with fluorine atoms in a hydrocarbon chain (3MCompany 1999). This is possible to carry out in the presence of anhydrous hydrogen fluoride (HF) and undergo electrolysis (Buck et al. 2011). During ECF, hydrogen atoms are replaced by fluorine, and the process is provided in Figure 2. However, in some compounds, such as carboxylic halides and sulfonyl halides, the functional groups containing hydrogen atoms are retained. As the hydrogen atoms are evolved at the cathode, the voltage from the electric current used in electrolysis is results in fluorination to occurrence at the anode. Rearrangements and breakage of the C-chains occurs because of the free-radical nature of the process. This results in a mixture of linear and branched isomers and homologues of perfluorinated compounds (Benskin et al. 2011; Buck et al. 2011; Lau et al. 2007; Paul et al. 2009). The production has mainly been 6-, 8- and to some extent 10-carbon perluoroalkane sulfonyl derivatives and products from these compounds (3MCompany 2000a).

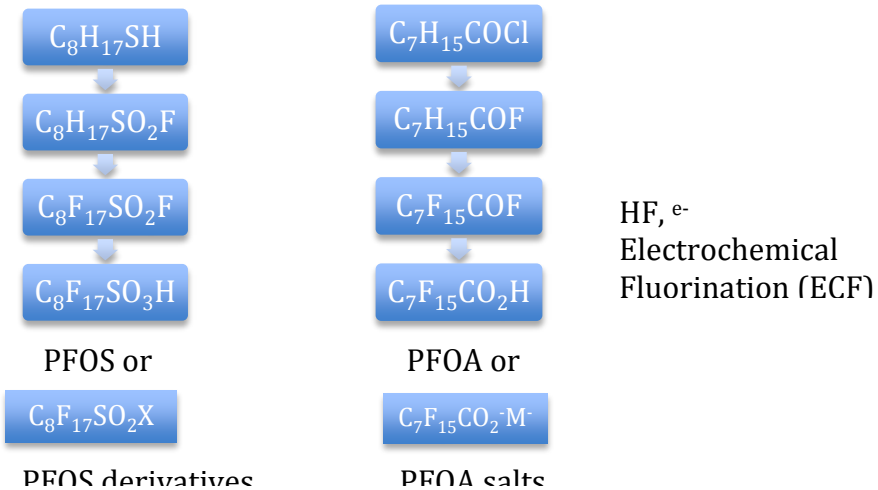



**Figure 2:** Electrochemical fluorination synthesis
ECF produces PFOS, PFOA and their derivatives. Alkanesulfonyl fluoride and alkanecarbonyl fluoride can be used as starting materials in an ECF process, and be converted to their perfluorinated counterparts. Perfluorosulfonyl fluoride and perfluorocarbonyl fluorides can, through hydrolysis, be converted to PFSAs and PFCAs respectively (Kissa 2001; Stock et al. 2010). Modified from Buck et al. (2011).

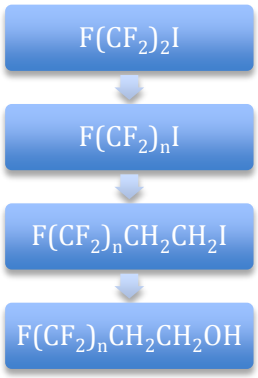
*Telomerization*

Telomerization is defined as a radical process (Lehmler 2005). The process is provided in Figure 3. This is a polmerization reaction that yields a telomer or a fluorotelomer, which is a fluorocarbon-based oligomer consisting of a few monomers. This reaction occurs between a telogen, such as perfluoroethyl iodide, and a taxogen - unsaturated molecules such as tetrafluoroethylene (Buck et al. 2011; Kissa 2001). Perfluoroethyl iodide and tetrafluoroethylene are usually the start materials in telomerization.

$$YZ + nA \rightarrow Y\text{-}(A)_n\text{–}Z$$

Telogen Taxogen Telomer

Pentafluoroethyl iodide is generally accepted to be the most significant telogen in telomerization. In commercial telomerization reactions, tetrafluoroethylene oligomers are most frequently utilized as a taxogen. The product of this reaction is perfluoroalkyl iodide polymer (Kissa 2001; Stock et al. 2010). In other words, the reaction will yield perfluoroalkyl iodides with longer perfluorinated chains (Buck et al. 2011).




1) Pentafluoroethyl iodide,PFEI
+(n-2)/2 $CF_2=CF_2$, Tetrafluoroethylene, TFE

2) Perfluoroalkyl iodide, PFAI "Telomer A"

+ $CF_2=CF_2$, Ethylene

3) n:2 Fluorotelomer iodide, FTI "Telomer B"
4) n:2 Fluorotelomer alcohol, FTOH

**Figure 3:** The telomerization synthesis
Telomerization produces FTOH. First the perfluoroalkyl iodides have to react with ethylene to form perfluoroalkylethyl iodides as mentioned above. Then through hydrolysis, the compound can readily be converted to FTOHs and PFCAs. In the two first steps, the telomerization occurs. Modified from Buck et al. (2011).

### *1.1.3 Surfactants*

Both groups, PFSAs and PFCAs, are anionic surfactants (Stock et al. 2010). Surfactants are much used, not only for industrial processes and biological systems, but also in numerous consumer products. Examples include cosmetics, food, household items, medical applications as well as others. These types of chemicals have desired properties such as reducing surface tension of the surrounding liquid medium (Kissa 2001). The characteristics of the surfactant can be affected by the position and the extent of fluorine atoms attached to the alkyl-structure. Hence when fluorine atoms replace the hydrogen, the properties will change drastically. Fluorinated surfactants repel both oil and water (Buck et al. 2011; Kissa 2001).

## 1.2 Persistent organic pollutants in Arctic environments

In the Arctic, the area for the present study, persistent organic pollutants (POPs) are found both in the environment and the biota. POPs are described as persistent, potentially toxic, bioaccumulative and long-transported. PFAS are untraditional POPs, which will be described in section 1.2.2. Because of the few local point sources for the contaminants in arctic environments, the levels of POPs are assumed to originate from industrialized areas in lower latitudes. This is explained by the transportation by way of atmospheric and ocean circulation, large rivers, and biological agents (Barrie et al. 1992; Burkow & Kallenborn 2000). The transportation efficiency of contaminants is a function

of the chemical and physical properties of POPs, as well as the weather conditions and geographical proximity to high source areas (Barrie et al. 1992; Muir & de Wit 2010).

### *1.2.1 Persistent organic pollutants (POPs)*

As mentioned, POPs are typically characterized by persistence, being potentially toxic and bioaccumulative, and also by long-range transport (Braune et al. 2005; Jones & de Voogt 1999; Lohmann et al. 2007; Vanloon & Duffy 2011; Webster et al. 1998). Persistence means that the compound has a long half-life. POPs are known to last for years in the soil and sediment, or days in the atmosphere (Jones & de Voogt 1999). Some POPs are biodegradable. Detectable levels of POPs in remote areas such as the Arctic are a clear indicator of their persistence in the environment (Stock et al. 2007).

Most of the POPs are lipophilic/ hydrophobic, meaning that they can bioaccumulate in the lipid rich tissue of organisms. Since the metabolism in most Arctic species is very slow, the compounds persist in the body and biomagnify through the respective food chain (Jones & de Voogt 1999). Selected POPs are semi-volatile and have the ability to enter the gas-phase under higher temperatures in the environment (AMAP 2010; Muir & de Wit 2010). The global distillation theory, also termed multi-hopping, (Mackay & Wania 1995) is widely considered to be the most valid explanation for global atmospheric distribution and long-range transport of POPs (Muir & de Wit 2010).

### *1.2.2 Perfluoroalkylated substances vs. other persistent organic pollutants*

The POP classification includes different organic compounds including PFAS. Unlike other POPs, PFAS do not accumulate in lipid rich tissue. Instead, these compounds accumulate in protein rich tissue and tend to bind to blood proteins accumulating in protein-rich body liquids and organs such as kidneys, livers and bile secretions (Bonefeld-Jorgensen et al. 2011; Fei et al. 2007; Lau et al. 2007; OECD 2002). Humans are also exposed to PFAS compounds through environmental contact, in consumer goods, which contain PFAS and many occupational settings (Bonefeld-Jorgensen et al. 2011; Dimitrov et al. 2004; Giesy & Kannan 2001; Giesy et al. 2001; Martin, Jonathan W. et al. 2004; Martin, J. W. et al. 2004).

### *1.2.3 Environmental distribution*

In the Canadian Arctic, several PFAS monitoring studies have been conducted in recent years (AMAP 2010; Muir & de Wit 2010). However, there is still limited information and

knowledge from the European Arctic and other circum-polar areas (Muir & de Wit 2010; Stock et al. 2007; Young et al. 2007). If a compound is in the gas phase or sorbed to water or particles in the atmosphere, then long-range transport can occur through the air (Zhao et al. 2012). This is visualized in Figure 4. The two most important properties for assessing the potential range of transportation are water solubility and vapor pressure. The substance ability to transfer into gas phase is indicated by vapor pressure (Kaiser et al. 2005).

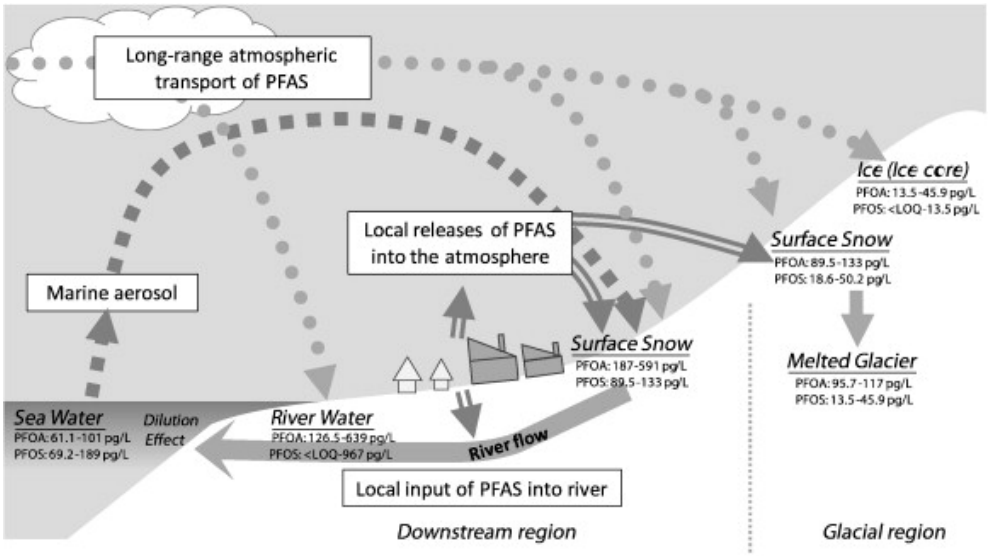



**Figure 4:** Exposure routes for PFAS
This figure describes different exposure routes and distribution of PFAS in Longyearbyen, and gives an indication on how some factors can contribute to PFAS levels in lake Linnévatnet. (License authorized by Elsevier, (Kwok et al. 2013)).

Substances of the group PFAS, that are more volatile, such as fluorotelomer alcohols (FTOH), are found to be long-range transported globally in the atmosphere and via ocean currents. It has been reported that FTOH have occurred in the Arctic atmosphere at approximately five times lower concentrations than in urban areas (Kwok et al. 2013; Stock et al. 2007). These compounds are also hypothesized precursor compounds and main source to PFOA, and its long-chained homologues, in remote areas such as the Arctic (Kwok et al. 2013; Prevedouros et al. 2006). This is consistent with other compounds including PFOS.

## 1.3 Freshwater fish in the Arctic

In most high Arctic regions, Arctic char (*Salvelinus alpinus*) is the only resident fish species in freshwaters. As indicated above, Arctic char is used as the biomonitoring species in this project. Two populations of Arctic char are distinguished on Svalbard:

anadromous and landlocked. Anadromous populations travel from freshwater to saltwater in a period of time, and are seasonal dependent. Conversely, landlocked populations remain in the freshwater bodies (Borgstrom et al. 2010; Svenning 2010). On Svalbard, the char can only travel to the ocean when the lakes are ice-free (typically between mid-July and early October). The lake Linnévatnet on Svalbard (Figure 5), the field area in this project, has a river at the north end that connects to the ocean during the snow-melt period and other times when water levels are high.


**Figure 5:** Linnévatnet
The study area was Linnévatnet on Nordenskiöld land, on Svalbard (www.npolar.no Available 13.06.2013), and license to use figure was authorized by © NPI.

Linnévatnet is typified by low species diversity, a characteristic shared by most freshwater bodies in high Arctic regions (Svenning et al. 2007). As a result, the growth-rate and period for the char is very limited, due to limited food abundance. The Arctic char lives under extreme environmental conditions. The temperatures in Linnévatnet are low (ca. 4-5°C measured in August 2008) and as mentioned the time period for the lake to be covered with ice is very long (approx. 10 months) or in some other places in the Arctic, could be permanent (Borgstrom et al. 2010). As a result, the char population remains locked in the lake during periods of ice cover. The effect of this is that spawning occurs in the lake.

The Arctic chars weight and size are affected by nutrition availability. The concentration of nutrients is affected by factors such as the lake temperature (maximum temperature of 3-6°C during summer), and light penetrating (limited in the winter by snow and ice cover). Periods, in which anadromous char experience more rapid growth, are attributed to time spent in the ocean where food is more abundant and possibly changes in diet in the lake environment. Larger resident fish (>15-20 cm in length) tend to change diet and become cannibalistic and eat smaller fish (Svenning et al. 2007). In the study by Svenning et al. (2007) the authors reported the consumption behavior of Arctic char, they concluded that the dominating diet is composed of chironomids, caddis, insect larvae, copepods, smaller fish and zooplanktons depending on the season (Isdahl 2002).

In many Arctic regions, Arctic char form an important traditional food source for the native populations as well as being an important commercial fish species. This is reported for Canada, Greenland and Iceland (Borgstrom et al. 2010).

## 1.4 Transformation and degradation

PFOS and PFOA are utilized as ingredients in PFAS based chemical products. However, they are also well identified as products of more volatile precursor compounds (FTOH, FOSE). Both have been detected in humans, surface water, in marine and freshwater biota (Giesy & Kannan 2001; Kannan et al. 2001; Martin, J. W. et al. 2004; Wang et al. 2011).

A topic that is not yet well studied and understood is the biotransformation of perfluorinated compounds and their bioaccumulation in the biosphere (Houde et al. 2006; Lau et al. 2007; Yeung et al. 2013). PFOS is potentially formed from N-ethyl 2 (N - ethyl perfluorooctane sulfonamido) ethanol (*N-EtPFOSE),* and PFOSA intermediate - as well as other perfluorinated compounds. The complex production of PFOS complicates the understand of PFOS transference through trophic levels (Tomy et al. 2004). Tomy et al (2004a) clearly identify biomagnification of PFOS through the Arctic marine food web, despite this complication.

The widespread environmental detection of compounds such as PFOA and long-chain homologues necessitates clear identification of potential sources (Dinglasan et al. 2004;

Herzke et al. 2012). Early in the 2000s, it was suggested that most likely precursors for stable PFCAs, such as PFOA, might be fluorinated telomer alcohols (FTOH). These compounds may undergo transformation in the environment that leads the formation of PFCAs - which are potentially toxic and highly bioaccumulative. The general structure of fluorotelomer alcohols is $F(CH_2)_nCH_2CH_2OH$. The $CF_2$ segments are usually in numbers of n=4. 6, 8, 10 or 12 (Stock et al. 2010). The analogues to FTOH are the fluorotelomer sulfonates (FTS) compounds, which are structurally similar and also can degrade under abiotic conditions (Wang et al. 2011). However, in order for 6:2 FTS to undergo biotransformation, the compound has to be desulfonated first.

Since FTOHs are not expected to remain in the biosphere, there is reason to believe that metabolism of FTOHs is an unlikely source for PFCAs in Arctic areas (Ellis et al. 2003). A more reasonable source is the tropospheric oxidation of FTOHs. For the atmospheric distribution processes it was explained that FTOHs have a sufficiently long half-life in the troposphere to account for the distribution of PFCAs to remote regions (Martin et al. 2005). Small amounts of PFCAs (approx. 5-10%) are produced when FTOHs go through tropospheric oxidation. Hence there is a reason to believe that nonvolatile compounds, such as PFCAs, become scavenged by wet and dry deposition in the air and transported to regions far from the source (Ellis et al. 2004; Hurley et al. 2003; Wallington et al. 2005).

## 1.5 Dietary exposure and effects of PFAS

The interest of investigating the toxicology of PFSAs and PFCAs has increased the last two decades, especially with respect to PFOS and PFOA. In this thesis, the effect of PFAS will only be described briefly. However, the PFSAs and PFCAs are of particular concern for the human health and the environment (Lau et al. 2007; Stock et al. 2010). Several studies of PFAS indicating that PFOS and PFOA show adverse health effects on experimental animals have risen the last decade because of public health concerns. The health effects that give cause for concern are immunotoxicity, hormonal effects, neurobehavioral toxicity, developmental toxicity, hepatotoxicity, lung toxicity, reproductive toxicity, carcinogenic potential and weak genotoxic potential (EFSA 2012; Eriksen et al. 2010; Lau et al. 2007; Pinkas et al. 2010). Studies from early 2000 revealed

high levels of PFAS in wildlife and the environment (Kannan et al. 2001). This was an important factor for the "phase-out" of PFOS (and its salts).

Results from multiple studies on animals (Haug et al. 2010; Kennedy et al. 2004; Lau et al. 2004; Lau et al. 2007) indicate that oral consumption is the main exposure route for absorbing PFAS. In addition these compounds are not easily eliminated as well as PFOS and PFOA are highly persistent and are not likely to undergo metabolism (Hu et al. 2003; Olsen et al. 2007; Renner 2001). PFAS do not accumulate in fatty tissue, but binds to proteins, such as β-lipoproteins, albumin and liver fatty acid-binding proteins (Jones et al. 2003), and are distributed in kidney serum and the liver. The elimination rate tends to increase with increasing perfluoroalkyl chain length, however the elimination rate in humans takes many years. PFOS, PFHxS and PFOA have half-life in humans of 5.4, 8.5 and 3.8 years, respectively (Kennedy et al. 2004; Lau et al. 2004; Lau et al. 2007; Olsen et al. 2007).

The 3M Company was previously the biggest producer of PFOS-based substances in the world. In 2000 the company announced phase-out of PFOS within the beginning of 2001 (OECD 2002). Because of the wide use of PFAS compounds, these substances have been globally distributed and entered the environment and are also found in humans (EFSA 2012). In 2010 PFOS and its salts were also included in the Annex B in the Stockholm convention as persistent organic pollutions (POPs). Now the major manufacturer is Asia and by using the electrochemical fluorination (ECF) process based on perfluorobutane (PFBS), instead of using perfluorooctane, sulfonyl chemistry, other alternative products are being produced (Carloni 2009; Olsen et al. 2009; Renner 2006).

## 1.6 Sources of PFAS

Sources for environmental PFCA emission may be classified as direct or indirect (Prevedouros et al. 2006). This classification is not without complication and definitions and examples of direct and indirect sources are discussed below.

Manufacture and use of PFCAs are direct sources, while indirect sources include chemical reaction impurities during production and degradation products when other substances degrade to PFCAs. There are four distinct synthesis routes, in which PFCAs

have been manufactured as salts: 1) electrochemical fluorination (ECF), 2) fluorotelomer iodid oxidation, 3) fluorotelomer olefin oxidation and 4) fluorotelomer iodid carboxylation. Along with routes for manufacture, PFCAs emissions also have other direct sources. These include fluorotelomer manufacturing and processing of fluoropolymers, fluoropolymer dispersion, manufacture of aqueous fire-fighting foams (AFFF) and training exercises utilizing AFFF which contain PFCAs components, and consumer and industrial products. As indirect sources, perfluoroctyl sulfonyl-based products contain impurities of PFCAs compounds after using the ECF process. Fluorotelomer-based products have also shown to contain trace levels of PFCAs. Finding trace levels of PFCAs in these production materials indicates that precursor compounds may undergo transformation and degrade to PFCAs (Prevedouros et al. 2006). This is consistent with the study by Dinglasan (2004) where it was identified that 8:2 FTOH was degraded to PFOA. The total global production of PFCAs from both direct and indirect emissions were estimated to be between 3200 and 7200 tons (Prevedouros et al. 2006). Exposure through ingestion of chemicals that have been applied to food which are in contact with paper packaging is also an indirect source for humans (D'Eon & Mabury 2007).

Direct sources of FSAs and FTOHs include spills, disposal, and releases during manufacturing processes. Not all FSAs and FTOHs become linked when producing fluorinated polymers; hence the free compounds are released into the environment directly. However it is assumed that fluorinated polymers degrade to produce FSAs and FTOHs, under environmental conditions (Dinglasan-Panlilio & Mabury 2006).

By utilizing the ECF process, the 3M Company started producing perfluorinated compounds (PFCs), such as PFOS, in 1949. Because of concerns for human exposure to these compounds and the potential toxic effects, as well as the global distribution, the 3M company was the first to announce “phase-out” of PFOS, even though 3M was the only major company that commercially synthesized salts of PFCs by using ECF (Paul et al. 2009).

## 1.7 High-Performance Liquid Chromatography Mass Spectrometry

High-resolution chromatography is a technique that operates with one phase held in place (stationary) while the other passes through (mobile) (Harris 2007). To obtain high-resolution separations, high-performance liquid chromatography (HPLC) uses pressure to force the solvent through a closed column, which contain very fine particles (Harris 2007).

High performance liquid chromatography coupled to a mass spectrometer (MS) has been used to study masses of atoms or molecules and fragments of molecules by obtaining a mass spectrum. The function of an MS is as follows: From desorbed condensed phases, samples of gaseous species are ionized. A sample containing the substance of interest is introduced into a chamber with vacuum. In this chamber the sample is vaporized and bombarded with high-energy electrons. Because of an electric field, the ions are accelerated and separated according to their mass-to-charge ratio, m/z (Harris 2007). The m/z is illustrated as a mass spectrum, showing intensity of the present ions.

To detect compounds that are not volatile, liquid chromatography (LC) is used in place of gas chromatography (Harris 2007; Hoffmann & Stroobant 2011). For instance FOSE and FTOH are volatile compounds and should be quantified by gas chromatography/mass spectrometry (GC/MS), while PFOS and PFOA are non-volatile. Liquid chromatograph connected to a tandem mass spectrometer (LC/MS-MS) is a sensitive instrument and it is therefore a suitable instrument-set up for these analyses (Hansen et al. 2001; Hoffmann & Stroobant 2011; Martin, Jonathan W. et al. 2004; Voogt & Sáez 2006).

In this thesis, ultrahigh pressure liquid chromatography triple-quadrupole (QqQ) mass-spectrometry (UHPLC-MS/MS) was used for the analyses. This method will be explained in details in the method section.

# 2 Aim

The aim of this project is to investigate levels of PFAS in fillet and liver samples from Arctic char collected from lake Linnevatnet in Svalbard (Nordenskjöldland, Spitsbergen). To gain scientific knowledge and understanding about the levels of PFAS in Arctic freshwater fish as a potential exposure route for human consumers; sampling, extraction and analytical methods were used, as well as multivariate statistics to compare the levels and to get an indication of differences in levels and patterns of PFAS from 2010 to 2013.

In this project, the focus has been on the quantification of selected PFAS compounds and their behavior, with focus on PFOS and PFOA. The main objectives are:

- Identification of PFAS in fillet and liver samples from Arctic char, from Arctic freshwater environments.
- Investigate the contribution from long-range transport atmospheric pollutants processes versus potential local sources.
- Examine the temporal distribution of PFAS levels from 2010 to 2013 in Arctic char.

Based on the phase-out of PFOS in 2003 and that short-chained PFAS are being used in the production of industrial and consumer products, the levels of PFOS are expected to be low, while short-chained PFAS to be more dominating (Paul et al. 2009; Prevedouros et al. 2006).

# 3 Material and methods

## 3.1 Study area

Fish samples were collected from lake Linnévatnet (78°3'N, 13°50'E), Spitsbergen (Figure 6), the largest island in the Svalbard archipelago. Linnévatnet is 4,6 $km^2$ long and is connected to the sea (Isfjorden) via a stream of about 2 km length. The lake has a maximum depth of 37 meters and is the second largest lake on Svalbard (Svenning et al. 2007). The climate has very low precipitation and low average ambient temperatures). Linnevatnet is commonly ice-free between mid-July and early October, and is classified as a cold, oligotrophic and monomictic lake. Maximum ice-thickness is typically 1.5-2 m. To the east and west, high, steep mountains surround the lake. The catchment area is ca. 55 $km^2$ and includes several glaciers (Linnébreen, Vardebreen and Vøringbreen), which add melt water and sediment into the lake system. Additional water inputs relate to groundwater sources and seasonal melting of snow. The transparency of the lake during summer is restricted (usually only a few cm) due to the high sediment concentration of glacial run off in the melt season (Borgstrom et al. 2010).

## 3.2 Field and laboratory methods

Collection of samples was conducted in March 2013. Samples for the 2010 period were obtained in September 2010, by David Huertas (CSIC Barcelona). After collection, the fish samples (*Salvelinus alpinus*) were dissected carefully in the UNIS laboratories and fillet and liver tissue was collected and stored (freezing ad -20 °C) until sample preparation. All sample preparation was performed in the chemistry laboratories at UNIS. The sample extracts were shipped to the analytical laboratories in Tromsø, Norway. The quantitative analysis of the fish samples was performed at the Norwegian Institute for Air Research (NILU) in Tromsø. The quantification and interpretation of the results was carried out at the Department of Chemistry, Biotechnology and Food Science (IKBM) at the University of Life Sciences (UMB, Ås, Norway).


**Figure 6:** Sampling site (arrow) Linnévatnet,
nearby Kapp Linné, Grønfjorden and Barentsburg, Svalbard (www.npolar.no Available 13.06.2013). The license for using the figure was authorized by © NPI.

### 3.2.1 Sampling

The sampling site for the 2013 sampling period was at Linnévatnet, located at the North end of the lake, close to the "North-hut". The sample characterization information is presented in Table 3. The fishing license was granted 8th of March 2013 and provide information about the 6 fish caught in Linnévatnet. The number of the fishing license was nr.03 and its duration was throughout the spring 2013.

In September 2010, a first sampling of Arctic char was conducted by a PhD student (David Huertas, CSIC, Barcelona) at UNIS. The 2010 sampling was performed as a collaboration program with the FP7 ArcRisk project (Prof. Dr. J. Grimalt, CSIC, Barcelona, Spain). The material was analyzed for legacy POPs (i.e. PCB, chlorinated pesticides only) and all samples have been stored at UNIS in -20 °C freezer since 2010. All fish samples have been wrapped in aluminum foil pre-cleaned with methanol (MeOH, p.a. quality) for transportation and storage. Of all 2010 Arctic char samples available, 13 have been selected for this project. Only fillet samples were available for quantitative analysis from the 2010 sample set.

**Figure 7:** Ice fishing and sample collection on lake Linnévatnet.
Here together with Tatyana Drotikova who caught the biggest fish of 120 gram (g).

In 2013 sampling was conducted during March. Ice fishing was the preferred method as sampling was conducted during the winter season. The ice thickness was greater than 1 meter; therefore a Stihl BT121 motorized drill head (STIHL PTY. LTD, Knoxfield Victoria, Australia or Stihl Inc. Headquarters, Virginia Beach, Virginia, US) with a 200 mm auger bit was used to provide access to the water surface. Commercially available ice fishing poles and ice fishing equipment were used for the fishing. Several holes were drilled close to the North hut and the outlet from the lake. In total 6 fish were caught during the 2013 period. The fish were wrapped in aluminum foil, pre-cleaned with methanol (MeOH, p.a. quality) immediately after collection and stored in the freezer (-20 °) after return to UNIS. Gloves (Nitrile gloves, Kimberly-Clara, UK) were always used in order to

avoid possible contamination. At the field site a field blank sample was also collected for quality control of the sampling and analytical procedure. Aluminum foil (pre-cleaned with MeOH) was used as a field blank and was exposed openly in the snow during sampling. The sampling positions (GPS coordinates) are provided in Table 1.

**Table 1:** Sampling site
Coordinates where the fish were caught. 5 fish (ID 03-1 to 5) were caught in hole 1 and 1 fish (ID 03-6) in hole 2.

| COORDINATES FOR SAMPLING SITE | | | | |
|---|---|---|---|---|
| Hole1 | 78.064606 | 13.774517 | 8665875.686 | 471709.6019 |
| Hole2 | 78.064654 | 13.775541 | 8665880.547 | 471733.3498 |

The fish caught in March 2013 had to be reported back to the Governors Environmental Department in Longyearbyen for administrative reasons. The fish heads had to be kept in the freezer and delivered to the Governor's Office along with the fishing license. Otoliths were examined from each fish in order to determine the age.

## 3.3 Chemicals and standards

For the quantitative analytical method (Powley et al. 2005), isotope labeled ($^{13}C$) internal standard (all PFCs listed in APPENDIX B), all compounds in 0.5 ng/µL concentration (solved in MeOH) was used, and the internal standard (ISTD) was diluted with methanol to the concentration of 0.1 ng/µL, and have a purity of >98 %. The ISTD was purchased from Sigma-Aldrich Norway AS or Wellington Laboratories Inc. (Guelph, Ontario, Canada). The standard consists of a mixture of $^{13}C$ analogues of PFAS (APPENDIX A). 0.1 ng/µl 3,7-dimethyl-branched perfluorodecanoic acid (bPFDA, IUPAC: Perfluoro-3,7-dimethyloctanoic acid) of 97% purity, dissolved in methanol, was obtained from ABCR (Karlsruhe, Germany), and used as recovery standard (RSTD). All solvents used in this project were of Lichrosolve®grade. In the extraction of the fish samples Lichrosolv methanol was used for the fillet samples and acetonitrile for the liver samples (APPENDIX B).

For the clean-up process Superclean ENVI-Carb 120/400 (Supelco 57210-U) (Supelco, PN, USA or Bellefonte, USA) was used together with glacial acetic acid (Merck, Germany).

For the HLB-water 2mM aqueous ammonium acetate $NH_4OAc$ was used, purchased from Sigma-Aldrich, St. Louis, MO, USA. (APPENDIX B). All the chemicals and standards were provided by NILU.

## 3.4 PFAS quantification

The for the quantitative determination of the target PFAS related compounds a method previously described by Powley et al. (2005) was used. This analytical method, for perfluoroalkylated substances (PFAS, ionic) in biological matrices, has been modified by Dr. Dorte Herzke at NILU (Herzke et al. 2012). Details on the analytical method can be found in (Hanssen et al. 2013) and in APPENDIX I.

All equipment used in this project was cleaned with solvents (MeOH) before usage. Glass equipment and porcelain were sterilized in a muffle furnace (450 °C for 6 h, Naberthern, Lilienthal/Bremen, Germany). The list of all equipment used in this project is provided in APPENDIX A. The fillet and liver samples from Arctic char (*Salvelinus alpinus*) were analyzed for 21 PFAS compounds (Table 2).

**Table 2:** Perfluorinated Alkylated Substances (PFAS)
PFAS analyzed in Arctic char (*Salvelinus alpinus*) fillet and liver samples from Linnévatnet on Spitsbergen, Svalbard. Denoting acronyms, analyte and chemical formula, as well as the terminology and classification are according to Buck et al. (2011).

| Polyfluorinated Alkylated Substances (PFAS): Acronym, Analyte, Chemical formula | | | | | |
|---|---|---|---|---|---|
| | **CAS.nr** | **Acronym** | **Analyte** | **Chemical formula** | **No. of Carbons** |
| **Telomers** | | | Fluorotelomer sulfonates | | |
| | 355-46-4 | 4:2 FTS | 4:2 Fluorotelomer sulfonate | $F(CF)_4CH_2CH_2SO_3^-$ | 6 |
| | 27619-97-2 | 6:2 FTS | 6:2 Fluorotelomer sulfonate | $F(CF)_6CH_2CH_2SO_3^-$ | 8 |
| | 39108-34-4 | 8:2 FTS | 8:2 Fluorotelomer sulfonate | $F(CF)_8CH_2CH_2SO_3^-$ | 10 |
| **PFSA** | | | Perfluoro sulfonates | $C_nF_{2n+1}SO_3H$ | |
| | 375-73-5 | PFBS | Perfluorobutane sulfonate | $CF_3(CF_2)_3SO_3H$ | 4 |
| | 3872-25-1 | PFPS | Perfluorpentane sulfonate | $CF_3(CF_2)_4SO_3H$ | 5 |
| | 432-50-7 | PFHxS | Perfluorohexane sulfonate | $CF_3(CF_2)_5SO_3H$ | 6 |
| | 375-92-8 | PFHpS | Perfluoroheptane sulfonate | $CF_3(CF_2)_6SO_3H$ | 7 |
| | 1763-23-1 | PFOS br/lin | Perfluorooctane sulfonate | $CF_3(CF_2)_7SO_3H$ | 8 |
| | 335-77-3 | PFDcS | Perfluorodecane sulfonate | $CF_3(CF_2)_9SO_3H$ | 10 |
| **PFCA** | | | Perfluoro carboxylates | $C_nF_{2n+1}COOH$ | |
| | 375-22-4 | PFBA | Perfluorobutanoic acid | $CF_3(CF_2)_2OOH$ | 4 |
| | 2706-90-3 | PFPA | Perfluoropentanoic acid | $CF_3(CF_2)_3OOH$ | 5 |
| | 307-24-4 | PFHxA | Perfluorohexanoic acid | $CF_3(CF_2)_4COOH$ | 6 |
| | 375-85-9 | PFHpA | Perfluoroheptanoic acid | $CF_3(CF_2)_5COOH$ | 7 |
| | 335-67-1 | PFOA | Perfluorooctanoic acid | $CF_3(CF_2)_6COOH$ | 8 |
| | 375-95-1 | PFNA | Perfluorononanoic acid | $CF_3(CF_2)_7COOH$ | 9 |
| | 335-76-2 | PFDcA | Perfluorodecanoic acid | $CF_3(CF_2)_8COOH$ | 10 |
| | 4234-23-5 | PFUnA | Perfluoroundecanoic acid | $CF_3(CF_2)_9COOH$ | 11 |
| | 307-55-1 | PFDoA | Perfluorododecanoic acid | $CF_3(CF_2)_{10}COOH$ | 12 |
| | 72629-94-8 | PFTrA | Perfluorotridecanoic acid | $CF_3(CF_2)_{11}COOH$ | 13 |
| | 376-06-7 | PFTeA | Perfluorotetradecanoic acid | $CF_3(CF_2)_{12}COOH$ | 14 |
| **FOSA** | | | Fluorooctane sulfonamides | | |
| | 1763-23-1 | PFOSA | Perfluorooctane sulfonamide | $C_8H_2F_{17}NO_2S$ | 8 |

### *3.4.1 Extraction*

Before the extraction method was carried out, the fish samples were thawed for ca. 1-2 hours in a ventilation cabinet. As mentioned above, only fillet samples (two per fish)

were available from the 13 fish collected from 2010. Two fillet samples and one to two liver samples were collected from the six fish caught in 2013. After dissecting the fish, the fillet and liver samples were homogenized in a mortar.

1 g of samples were weighed on a fine scale (Mettler Toledo, Oslo, Norway) and transferred into a 45 ml PP-centrifuge tube (Polypropylen) (VWR International AS, Oslo, Norway). The fillet and liver samples were spiked with 20 µl internal standard (ISTD) ($^{13}C$ labeled internal standard (allPFCs) 0.1 ng/µl) (Wellington Laboratories Inc. (Guelph, Ontario, Canada) using a 20 µl capillary pipette (Brand, Werthem, Germany). 8 ml (6.32 g) methanol was added to each fillet sample and 8 ml (6.288 g) of acetonitrile were added to each liver samples. The vials were capped and vortexed thoroughly with a vortex mixer (VWR International AS, Oslo, Norway).

The 45 ml centrifuge tubes were placed in an Ultrasonic bath (VWR International AS, Oslo, Norway) for three exposures of 10-minute duration. The ultrasonic bath is a cleaning device that sends high frequency waves through the sample solution. The samples were vortexed in between. For the sedimentation of the sample solution, the Centrifuge Hettich Universal 16R (Hettich, Tuttlingen, Germany) for 45 ml tubes was used for 5 min with 2000 revolutions per minute (rpm). The supernatant gained after sedimentation in PP-vials were transferred to TurboVap-glasses with Pasteur pipettes (VWR International AS, Oslo, Norway) and the TurboVap (TurboVap 500, Zymark Corporation, Hopkinton, Massachusetts, USA) started the runs. Methanol evaporates slowly and it takes approx. 20 min to gain 1 ml concentrated extract. If the solution became more concentrated than 1 ml, the supernatant extract had to be concentrated up to 1 ml with methanol for fillet samples and acetonitrile for liver samples. Usually there are 1 ml markings on the TurboVap-glasses.

### *3.4.2 Clean-up*

1.7 ml Eppendorf centrifuge tube (VWR Internaional AS, Norway) with 25 mg (= 0.0025 g) of ENVI-Carb (Superclean ENVI-Carb 120/400 (Supelco 57210-U)) (Supelco, PN, USA or Bellefonte, USA) and 50 µl glacial acetic acid (Merck, Germany) was prepared for each sample. For the glacial acetic acid 50 µl capillary pipettes was used. Approximately 0.8 ml (800 µl) supernatant extract was transferred from the TurboVap glasses with FINNPIPETTE Genex Beta 100-1000 µl (Thermo Electron Org, Vantaa, Finland) to the

eppendorf centrifuge tubes. The vials were capped and vortexed thoroughly. Further sedimentation and clean up, the Eppendorf tubes were centrifuged in Eppendorf centrifuge 5415 D (Eppendorf North America, USA) in 10 min with 10′000 rpm. After the centrifuging, 0.5 ml (500 µl) the supernatant solutions were transferred to autoinjector vials. 20 µl recovery standard (RSTD) (0.1 ng/µl RSTD (3,7 brPFDcA)) (ABCR, Karlsruhe, Germany) was added to the supernatant solutions in each vial, as a control parameter for the clean-up method.

## 3.5 Instrumental analysis of perfluorinated alkylated substances

Prior to LC-MS analysis 100 µl of the extract with RSTD was transferred to LC-vials and diluted with 100 µl of 2 mM aqueous ammonium acetate ($NH_4OAc$) in HLB-water (de-ionized water, MilliQ water rinsed with two HLB solid phase extraction columns). Then the extractions were injected in the HPLC/MS system.

The PFAS compounds were analyzed by an ultra-high pressure liquid chromatography triple-quadrupole mass spectrometer (UHPLC-MS/MS). A Thermo Scientific quaternary Accela 1250 pump (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a PAL Sample Manager (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled to a Thermo Scientific Vantage MS/MS (Vantage TSQ) (Thermo Fisher Scientific Inc., Waltham, MA, USA) were used for the analysis of PFAS. The sample separation was conducted with an injector volume of 10 µl, on a Waters Acquity UPLC HSS 3T column (2.1x100 mm, 1.8 µm) (waters Corporation, Milford, MA, USA) equipped with a Waters Van guard HSS T3 guard column (2.1x5 mm, 1.8 µm)(waters Corporation, Milford, MA, USA). A Waters XBridge $C^{18}$ column (2.1x50 mm, 5 µm) (Waters corporation, Milford, MA, USA) was installed after the pump and before the injector, in order to be able to separate the PFCAs that were leaching from the pump and the degasser (Hanssen et al. 2013).

To achieve separation, 2 mM NH4OAc in 90:10 methanol/water (A) and 2 mM NH4OAc in methanol (B) was used as the mobile phases in the column (Hanssen et al. 2013). In the study of Hanssen et al. (2013) details are provided about the analytical conditions.

### *3.5.1 Quantification by internal standard*

Quantification was conducted using internal standard (ISTD) method with isotope labeled $^{13}C$ PFAS compounds. A known amount of the ISTD was added to the samples as well as the reference solution. The chromatograms were quantified for PFAS by using software called LCquan (Version 2.6, Thermo Fisher Scientific Inc., Waltham, MA, USA). A calibration curve with concentrations ranging from 0.05-20 pg/ml was also used for quantification of PFAS.

## 3.6 Quality control

Quality assurance and method validation provide an indication that the analytical method chosen for the project is right for its purpose. Accuracy is how close a measurement is to the "true value" while precision is the results reproducibility; how the results are spread. Uncertainty pertaining to the results is a combination of these two factors (Harris 2007).

### *3.6.1 Quality assurance and method validation*

Contamination of the samples is possible in every step of the analysis. Sources for contaminations may generally be classified as instrumental, sampling and procedural or analytical (Stock et al. 2010).

Unfortunately, some of the blank samples for this project were destroyed in transit from Svalbard to the mainland. However, in order to monitor background levels and "carry-over" effects, injections of solvent, such as methanol, were also done regularly during the analysis. The quality was checked with regularly analysis of one blank sample in approximately every tenth sample. Blank samples underwent the same method, but do not contain biological matter. The purpose of blank samples is to identify contamination during sampling and sample treatment and estimate background noise.

The recoveries in each sample were monitored for every internal standard (APPENDIX F). The sample recovery (%) was calculated by using these equations:

Eq.1) $$RRF \times \frac{Amount_{13C-qstd}}{Area_{13C-qstd}} = \frac{Amount_{12C-qstd}}{Area1_{12C-qstd}}$$

Eq.2) $$Amount_{12C-spl} = \frac{Area_{12C-spl} \times Amount_{13C-spl}}{RRF \times Area_{13C-spl}}$$

Eq.3) $$\text{Recovery}(\%) = \frac{Amount_{ISTD-cal}}{Amount_{ISTD-added}} \times 100$$

In equation 1 (eq.1), the ratio between $^{13}C$ and $^{12}C$ quantification standards (qstd) constitute the basis of the relative response factor (RRF). Then, based on the amount of internal standard (ISTD) added the RRF was used to calculate the unknown amount of $^{12}C$ in all the samples (spl, eq.2). The recovery (%) was calculated by the difference between the amount ISTD calculated and amount ISTD added (eq.3), relative to the amount of Recovery standard (RSTD) added right before the quantification of the samples. This is based on the compendium "Quality control in organic trace analysis" (Oehme 2007) available for the UNIS educational program (AT-324).

The laboratory at NILU in the FRAM Centre, Tromsø, which conducted the quantitative analysis, participates regularly in interlaboratory comparison.

### *3.6.2 LOD and LOQ*

Limit of detection (LOD) was set to three times the concentration (C) of the analyte in the samples, divided by the S/N-ratio. The S/N values were related to the background noise in the chromatogram instead of concentrations in the blanks (No PFAS were detected in the blanks).

Eq.4) $$LOD = \frac{3 \times concentration}{S/N}$$

Limit of quantification was set to ten times concentration of the analyte in the samples divided by S/N.

Eq.5) $$LOQ = \frac{10 \times concentration}{S/N}$$

## 3.7 Statistics – principal component analysis (PCA)

To perform a PCA the software Minitab 16 was used. PCA is a popular multivariate statistical technique. This technique analyzes data set, which represents observations and several variables that describes the observations. The variables are dependent and in general inter-correlated. PCA represent trends and pattern of similarities of the observations and the variables. The reason for using PCA is to extract important

information from the data set. This new information is called principal components (PC) and PCs are orthogonal variables that are presented in a map. The first principal component (PC1) explain the main load of the variance in the plot (Abdi & Williams 2010). There are several goals of performing a PCA, e.g. to focus on the most important information, and compress and simplify the data set, as well as analyzing the observations and variables structure.

In this project the observations are the fish samples from 2010 and 2013, and the variables are PFAS compounds from each year. The PCA was applied to visualize the relationship between the contaminants and the concentrations in samples.

# 4 Results

## 4.1 Biometric characterization

Biometric characterizations are presented in Table 3. The six fish that were caught in March 2013 were measured in the laboratory by length and weight, as well as gender and age. The age is measured in how many winters (W) the fish have survived by using a light microscope to count the "rings" on the otoliths. Unfortunately, the biometric characterizations of the 13 fish from September 2010 were not available.

**Table 3:** Biometric characterization
Fish samples collected in 2013 are presented with biometric characterizations as length (cm), weight (gram), gender and age.

| BIOMETRIC CHARACTERIZATION | | | | | | |
|---|---|---|---|---|---|---|
| **ID-nr:** | **Date:** | **Lake:** | **Length (cm):** | **Weight (gram):** | **Gender:** | **Age:** |
| 03-1 | 17.03.13 | Linnévatnet | 14.5 | 25 | Female | 8W |
| 03-2 | 17.03.13 | Linnévatnet | 22 | 62.4 | Male | 8W |
| 03-3 | 17.03.13 | Linnévatnet | 13.5 | 18.3 | Female | 7W |
| 03-4 | 17.03.13 | Linnévatnet | 24 | 110.4 | Male | 9W |
| 03-5 | 17.03.13 | Linnévatnet | 16.5 | 32.8 | Female | 7W |
| 03-6 | 17.03.13 | Linnévatnet | 25 | 120.9 | Female | 10W |

## 4.2 Contaminant levels and pattern

### *4.2.1 Levels*

Of the 21 PFAS compounds analyzed in fillet and liver samples from Arctic char, 14 compounds were detected. The PFAS that were not detected in the samples will not be considered in this thesis. Concentrations (ng/g ww) for individual PFAS from 2010 and 2013 are presented as median, minimum and maximum values in Table 4 and Table 5, respectively. The chromatograms are presented in APPENDIX H.

**Table 4:** Concentrations (ng/g ww) for individual PFAS in fillet from 2010 presented as median, minimum (min) and maximum (max) values.

| ARCTIC CHAR 2010 (n=20) | | | |
|---|---|---|---|
| | **Compound** | **Median** | **Min-Max** |
| Telomers | 4:2 FTS | <LOD* | 0.0-0.4 |
| | 6:2 FTS | 0.1 | 0.0-3.3 |
| PFSA | PFOS(lin) | <LOD* | 0.0-4.5 |
| PFCA | PFBA | 2.0 | 0.7-8.3 |
| | PFPA | 0.5 | 0.0-2.7 |
| | PFHxA | 0.03 | 0.0-0.4 |
| | PFHpA | <LOD* | 0.0-0.04 |
| | PFOA | <LOD* | 0.0-0.1 |
| | PFNA | 0.03 | 0.0-0.2 |
| | PFDcA | <LOD* | 0.0-0.4 |
| | PFUnA | 0.1 | 0.0-0.5 |
| | PFDoA | <LOD* | 0.0-0.05 |
| | PFTrA | <LOD* | 0.0-0.05 |

Blank samples were not included.

* <40% of the samples were >LOD.

**Table 5:** Concentrations (ng/g ww) for individual PFAS (fillet (F) and liver (L)) 2013, presented as median, minimum (min) and maximum (max) values - the total range including fillet and liver.

| ARCTIC CHAR (N=18) | | | | | |
|---|---|---|---|---|---|
| | **Compound** | **Median(F)** | **Min-Max(F)** | **Median(L)** | **Min-Max(L)** |
| Telomers | 6:2 FTS | <LOD* | 0.0-0.3 | <LOD* | 0.0-5.3 |
| PFSA | PFOS(br) | <LOD* | 0.0-0.0 | <LOD* | 0.0-2.9 |
| | PFOS(lin) | <LOD* | 0.0-0.09 | <LOD* | 0.0-0.2 |
| PFCA | PFBA | 0.09 | 0.02-1.5 | 0.1 | 0.03-0.4 |
| | PFPA | <LOD* | 0.0-0.03 | 0.3 | 0.09-0.8 |
| | PFHxA | 0.005 | 0.0-0.1 | 0.01 | 0.0-0.03 |
| | PFHpA | <LOD* | 0.0-0.01 | <LOD* | 0.0-0.01 |
| | PFOA | <LOD* | 0.0-0.04 | <LOD* | 0.0-0.04 |
| | PFNA | <LOD* | 0.0-0.05 | 0.2 | 0.0-1.2 |
| | PFDcA | <LOD* | 0.0-0.4 | 0.7 | 0.05-1.1 |
| | PFUnA | 0.02 | 0.0-0.2 | 1.6 | 0.3-1.8 |
| | PFDoA | <LOD* | 0.0-0.0 | 0.1 | 0.0-0.3 |
| | PFTrA | <LOD* | 0.0-0.03 | 0.2 | 0.1-0.4 |

Blank samples are not included.

* <40% of the samples were >LOD.

### *4.2.2 Pattern of perfluoroalkylated substances in fillet*

The patterns of PFAS compound distribution in the samples are presented in Figure 8 and Figure 9 for 2010. The concentrations of each compound are presented per sample. All samples are fillet samples and are denoted with for instance ID A1-1 (fish nr.1, fillet sample 1) or A2-2 (fish nr.2, fillet sample 2), etcetera. The blank samples are not included because no concentrations of PFAS were detected in the blanks.

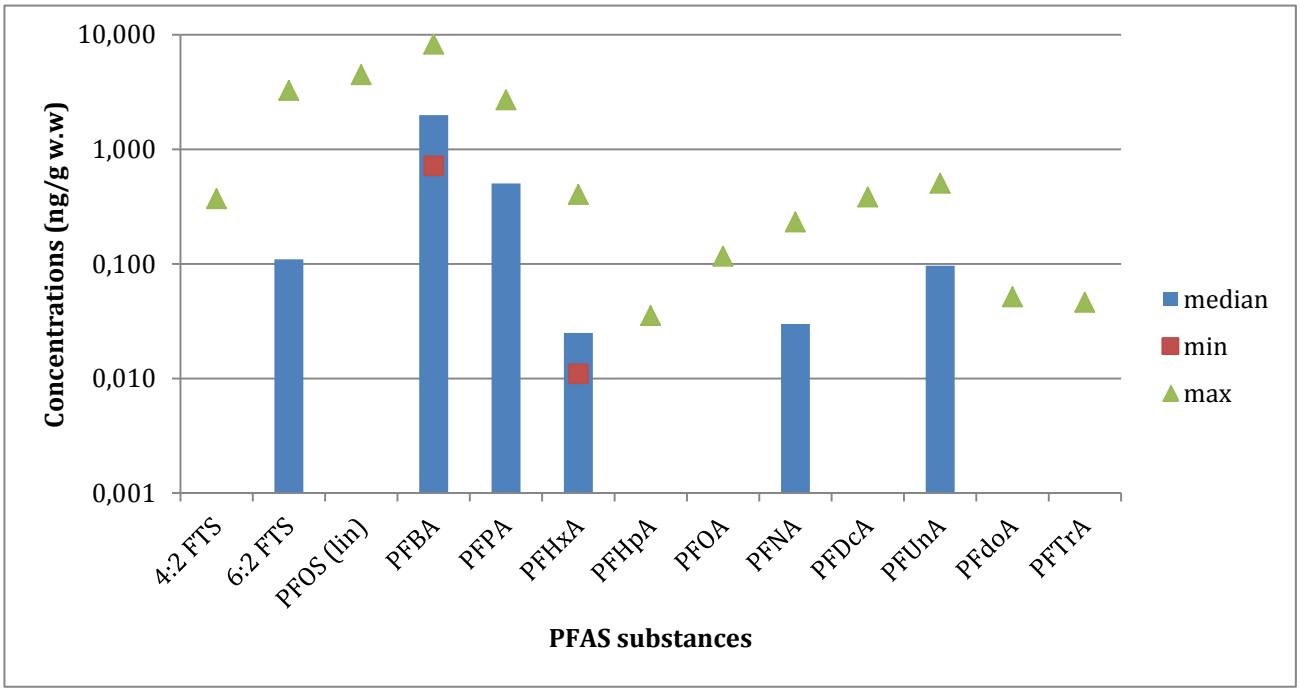



**Figure 8:** Concentrations (ng/g ww) of PFAS September 2010
PFAS detected in fillet samples from Arctic char (*Salvelinus alpinus*) from Linnévatnet. The concentrations are presented in a logarithmic scale. The results are presented as median, min and max values for each PFAS. Where median and min values are not presented, the values are <LOD.

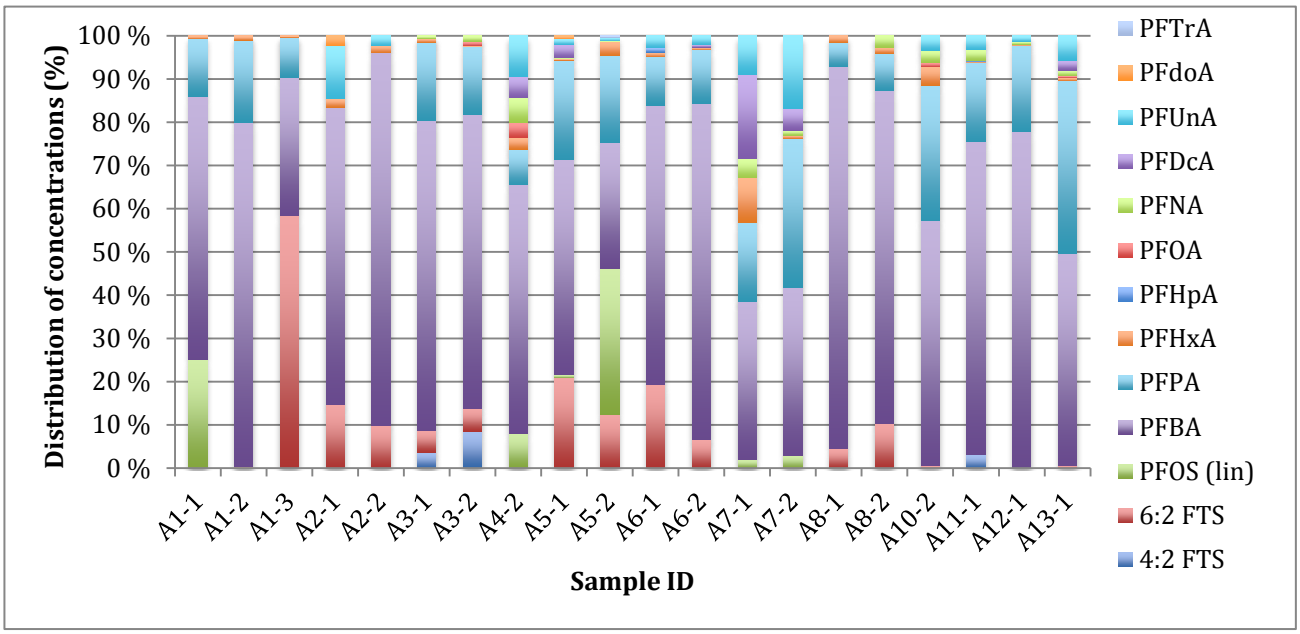



**Figure 9:** Relative distribution (%) of PFAS 2010 per sample (fillet)
The relative distribution (%) is based on the concentrations of PFAS substances within each sample (ng/g ww). Sample ID A1-1 indicates fish 1 fillet sample 1, A2-2 fish 2 fillet sample 2, etc.

The relative distributions of the concentrations of contaminants are denoted in percent (Figure 9), and are presented per sample. Of all contaminants detected in the samples, the compound that seems to contribute most to the samples was PFBA. PFHxA was also detected in all samples, but in lower concentrations. 6:2 FTS has also been detected in the majority of the samples, where it contributed more than PFBA in sample A1-3. Sample A5-2 contained 8 of the 14 PFAS detected in all of the samples in the present study, although the levels were low (APPENDIX G).

The percent distribution of the contaminants of the total concentrations of PFAS was: ∑PFBA (58.0%) > ∑PFPA (18.8%) > ∑6:2FTS (9.4%) > ∑PFOS(lin) (6.2%) > ∑PFUnA (2.6%) > ∑PFHxA (1.4%) > ∑PFDcA (1.2%) > ∑PFNA (1.2%) > ∑4:2FTS (0.9%) > ∑PFOA (0.3%) > ∑PFDoA (0.1%) > ∑PFHpA (0.1%) > ∑PFTrA (0.1%).

As mentioned, the most abundant PFAS was PFBA. PFBA and PFHxA were detected in all samples. However, PFPA was found in all samples, except for two of them. These three short-chained PFAS compounds (4-6 carbons) constitute 78 % of the total PFAS fraction. The highest concentration detected in all samples was PFBA (8.3 ng/g ww). PFOS (lin) was not the most abundant component detected in the samples, and would probably be expected to have higher levels. However, the highest PFOS concentration was 4.5 ng/g

ww. According to recent literature, PFOA would be expected to be more abundant, but all concentrations were lower than 0.1 ng/g ww. Even though the concentrations are low, the total concentration of 6:2 FTS contributes with 9.4 % of the total PFAS concentration and was detected in 14 samples. From these results, it is the short-chained PFAS compounds that contribute most to the total PFAS concentrations.

The pattern of PFAS compounds is presented in Figure 10 and Figure 11 for 2013 samples. Samples from 2013 are both fillet and liver samples, and are denoted in the same way as the samples from 2010 (Bx-1 and 2 are fillet samples, while Bx-3 and 4 are liver samples). The blank samples are not included because no levels of PFAS were detected in the blanks.

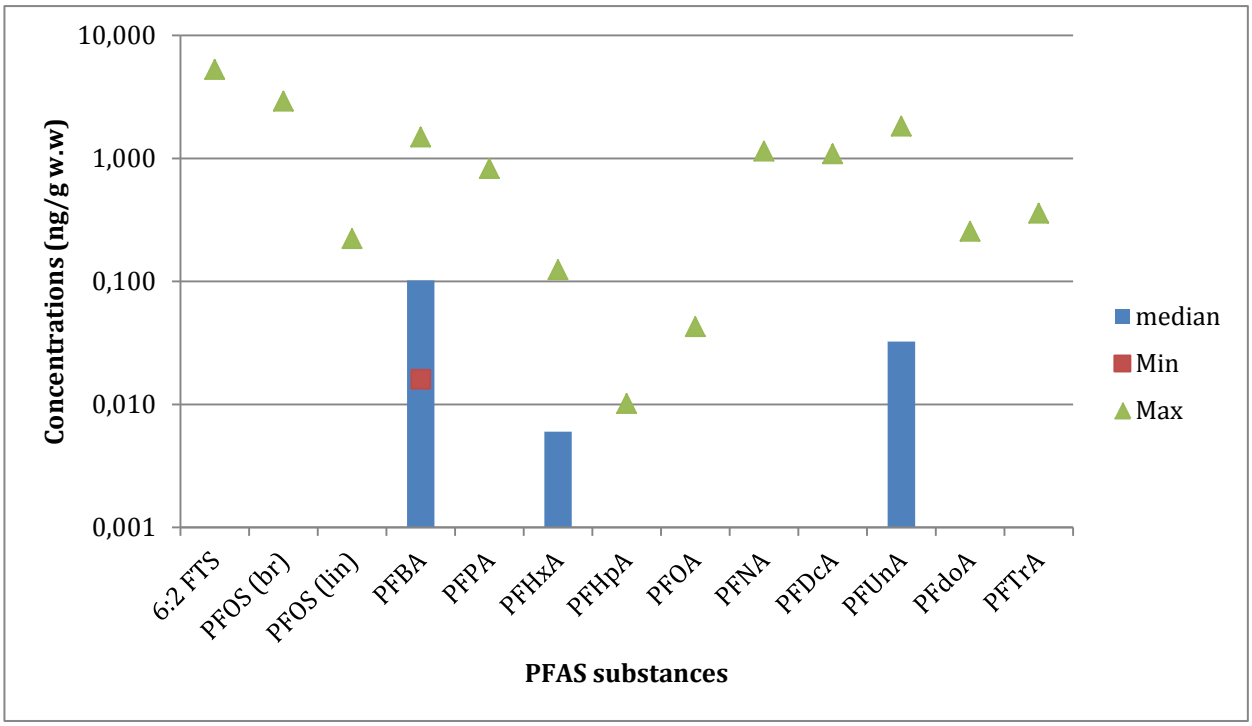



**Figure 10:** Concentrations (ng/g ww) of PFAS March 2013
PFAS detected in fillet and liver samples from Arctic char (*Salvelinus alpinus*) from Linnévatnet. The concentrations are presented in a logarithmic scale. The results are presented as median, min and max values for each PFAS. Where median and min values are not presented, the values are <LOD.

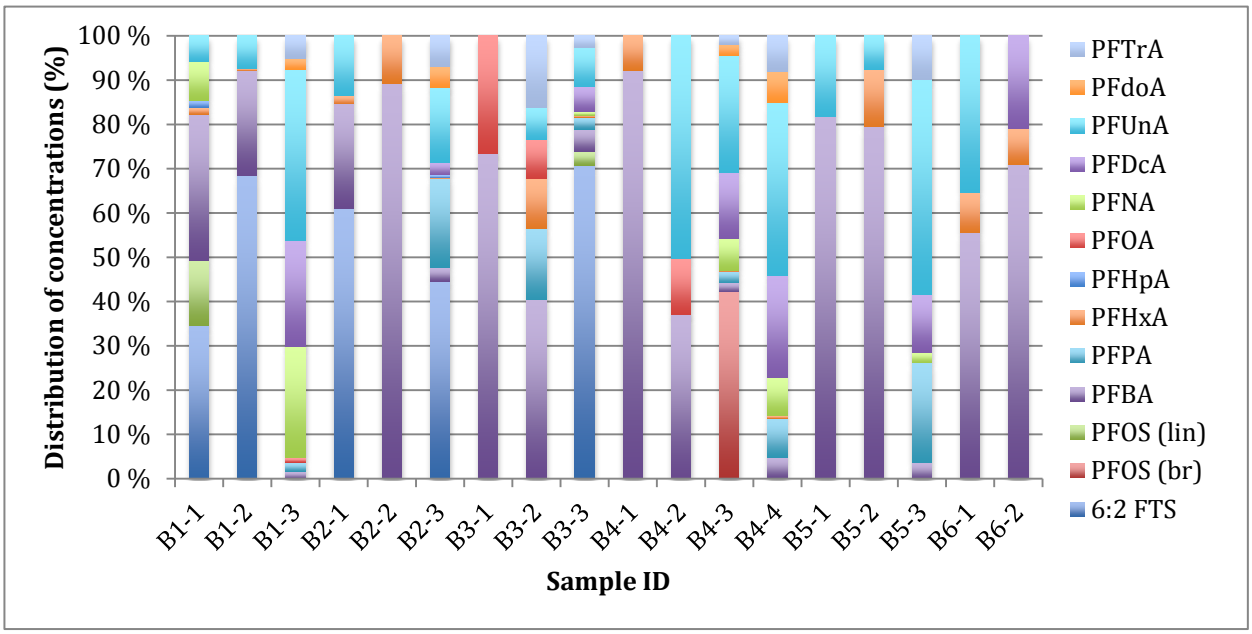



**Figure 11:** Relative distribution (%) of PFAS 2013 per fillet and liver sample.
The relative distribution (%) is based on the concentrations of PFAS compounds within each sample (ng/g ww). Sample ID B1-x indicate the fish number (Bx-1 and Bx-2 are fillet samples. Bx-3 and Bx-4 are liver samples).

The relative distribution of PFAS from 2013 samples is denoted in percent (%) (Figure 11) and shows the individual PFAS contribution in each sample. PFBA was detected in all samples, however in few samples (B1-2, B2-, B2-3 and B3-3), 6:2 FTS was more abundant than PFBA. The first two samples are fillet and the last two are liver. This is also the case for PFOS (br) in sample B4-3 (liver). The long-chained contaminants (7-13 carbons) were mostly detected in liver samples, where PFUnA was the most abundant (detected in 14 samples and which was more than PFPA). In Sample B4-2 PFUnA contributes more than PFBA. The percent distribution of the contaminants contributing to the total concentration of PFAS was: ∑PFUnA (25.2%) > ∑6:2FTS (21.0%) > ∑PFDcA (12.3%) > ∑PFBA (10.9%) > ∑PFOS (br) (9.1%) > ∑PFNA (6.7%) > ∑PFPA (6.3%) > ∑PFTrA (4.3%) > ∑PFDoA (2.0%) > ∑PFOS(lin) (1.0%) > ∑PFHxA (0.9%) > ∑PFOA (0.4%) > ∑PFHpA (0.1%). This is based on both fillet and liver samples and since most of the highest concentrations are detected in liver samples, it will affect the outcome of contribution of total PFAS concentration.

The contaminant with highest concentration detected is 6:2 FTS (5.3 ng/g ww), but was only detected in six samples. However, PFBA was detected in all samples, but with concentrations <1.5 ng/g ww. PFHxA was also detected in almost all samples, but with very low values. PFUnA (1.8 ng/g ww) had higher concentration than PFBA, and was

detected in 14 of 18 samples. PFUnA contributes most to the total concentration of PFAS in the samples (∑PFUnA (25.2%)). PFOS was detected in mostly liver samples, with highest concentrations of 2.9 ng/g ww and 0.2 ng/g ww, PFOS (br) and PFOS (lin) respectively. PFOA was mostly detected in fillet samples except for one liver samples, which also contained the highest concentration (0.04 ng/g ww). In general, there are more long-chained (>6 Carbons) contaminants detected in liver samples than short-chained PFAS (APPENDIX G, Table G.2).

### *4.2.3 Combined levels of perfluoroalkylated substances*

The concentrations (ng/g ww) from both 2010 and 2013 are also presented together in Figure 12. The levels of contaminants are presented in median and min-max values.

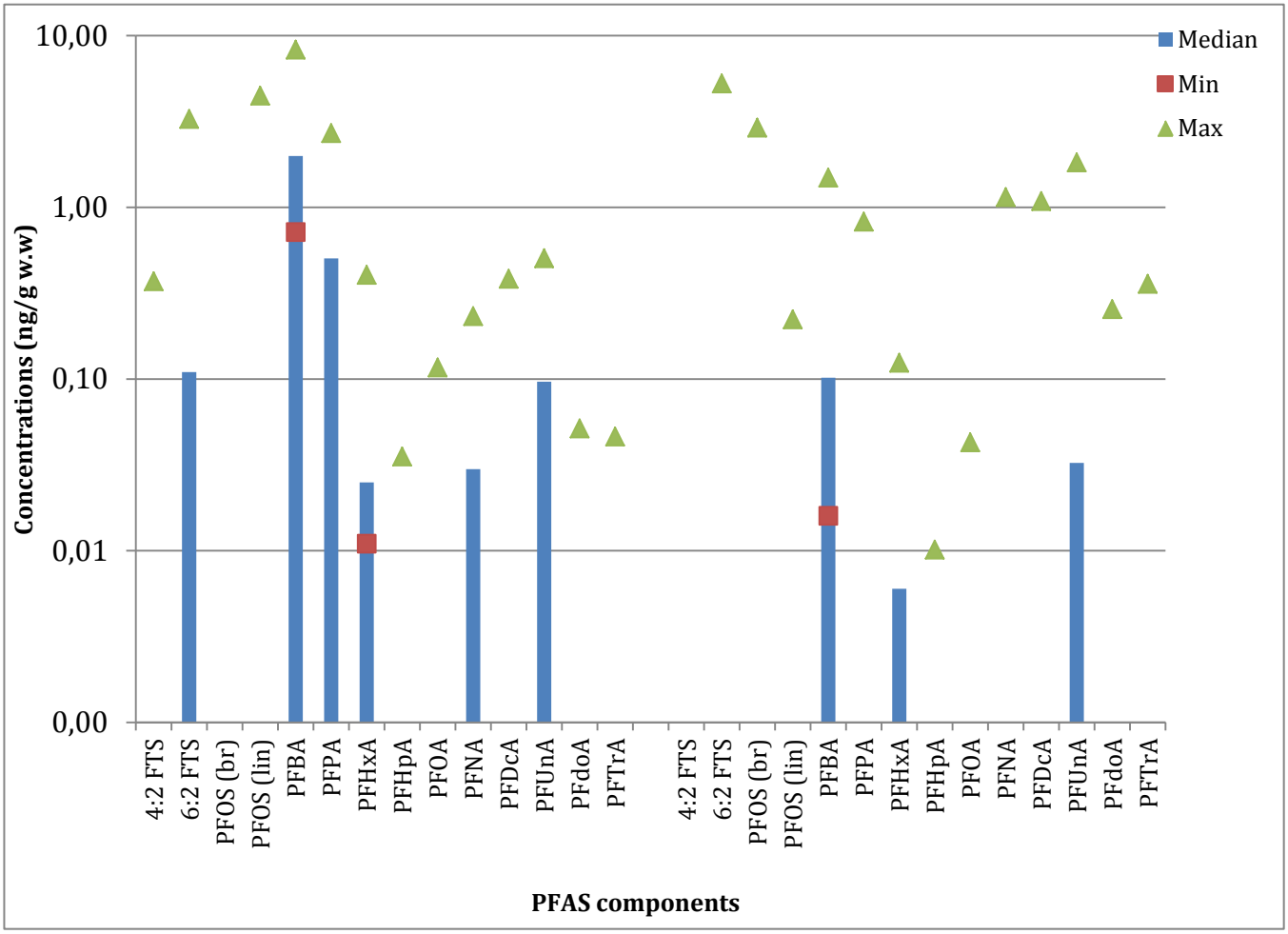



**Figure 12:** Combined diagram of levels of PFAS
The first 14 compounds are measurements from 2010, and the last 14 are from 2013. To better visualize the figure, min values that are <LOD are not shown here. In measurements where median were <LOD, only max value are shown.

Figure 12 describe the contaminants concentrations and show the comparison between both years of sampling. As mentioned already, PFBA showed the highest level detected

in the samples of 2010. The min value was also the highest detected in all samples from both years. In fish samples from 2013, 6:2FTS contribute most to the samples, though it is only detected in 6 samples where one liver sample contains (5.3 ng/g ww) and counts for most of the total concentration of 6:2FTS in the samples. 4:2FTS was not detected in samples from 2013 and PFOS (br) was not detected in samples from 2010.

## 4.3 Quality Assurance and Method Validation

### *4.3.1 Recoveries (%)*

Recoveries are presented in APPENDIX F separated by year in table F.1 and F.2. The recoveries for the internal standards (ISTD) varied between the different ISTDs. In the 2010 samples, the recoveries were lower than in 2013 (APPENDIX F). The high recoveries might have to do with more RSTD (3ng) added to the samples, than it was described in the method, and was due to personal mistake with using the pipette. There were some communication mistakes, and the nine last samples (from B4-1 to B6-2) from 2013 were not added the RSTD before being injected to the UHPLC. These samples were not used in calculating the recoveries for the method validation. However, the last nine samples were analyzed with the same method, at the same time as the other samples, and it is therefore safe to assume that the recoveries would be in the same range. The mean recoveries for all ISTDs were in an acceptable range.

### *4.3.2 LOD and LOQ*

All samples were used in the statistical analysis. LOD and LOQ were calculated for each sample and each contaminant analyzed. All samples were above LOD and individual measurements are presented in APPENDIX E, table E.1. LOQ was calculated (eq.6) and presented in APPENDIX E, table E.2. Samples <LOQ were set as 1/2LOQ for further use in statistical analysis, PCA.

### *4.3.3 Blanks*

Blank samples did not contain any traces of PFAS substances except for small amounts of PFBA and PFHxA (APPENDIX D). Hence, the blanks were not used for calculating LOD and LOQ.

In addition, one field blank was collected from the field site on lake Linnévatnet, but unfortunately was destroyed (poured out) during the transport from Svalbard to the

mainland. This happened also to the blank sample B1-bl. The rest of the method blanks were intact after transportation and used in the present study.

## 4.4 Statistics

The score plot and loading plot are presented in Figure 13 and Figure 14. Four principal components (PC) resulted from the analysis, with an eigenvalue>1. These PCs together explain 71.3 % of the variation in the dataset. However, the main load is explained by first and second principal component, respectively PC1 (31.4 %) and PC2 (18.4 %), meaning that PC1 have the highest possible variance in the data set (Abdi & Williams 2010). PC2 is orthogonal to PC1 and computed to have highest possible variance as well. The scores present different individuals from both years, 2010 and 2013. The distribution of the contaminants and the diversity are represented by the contaminant burden in the individual observations.

The score plot indicates how the different observations are related to each other. The observations from 2010 are marked with black. Red squares are not visible because all samples from 2010 were fillet. Green and blue observations are from 2013 and divided in fillet and liver, with the respectively colors. The pattern indicates a separation by PC1 between 2010 samples and 2013 liver samples, as the liver samples appear on the right side and the 2010 samples appear on the left side. However, the fillet samples from 2013 appear on the left side and are positively correlated with the samples from 2010. The liver and fillet samples within 2013, indicates a separation by PC2. PC2 contrast especially observation 28 and 12,3 and 13.

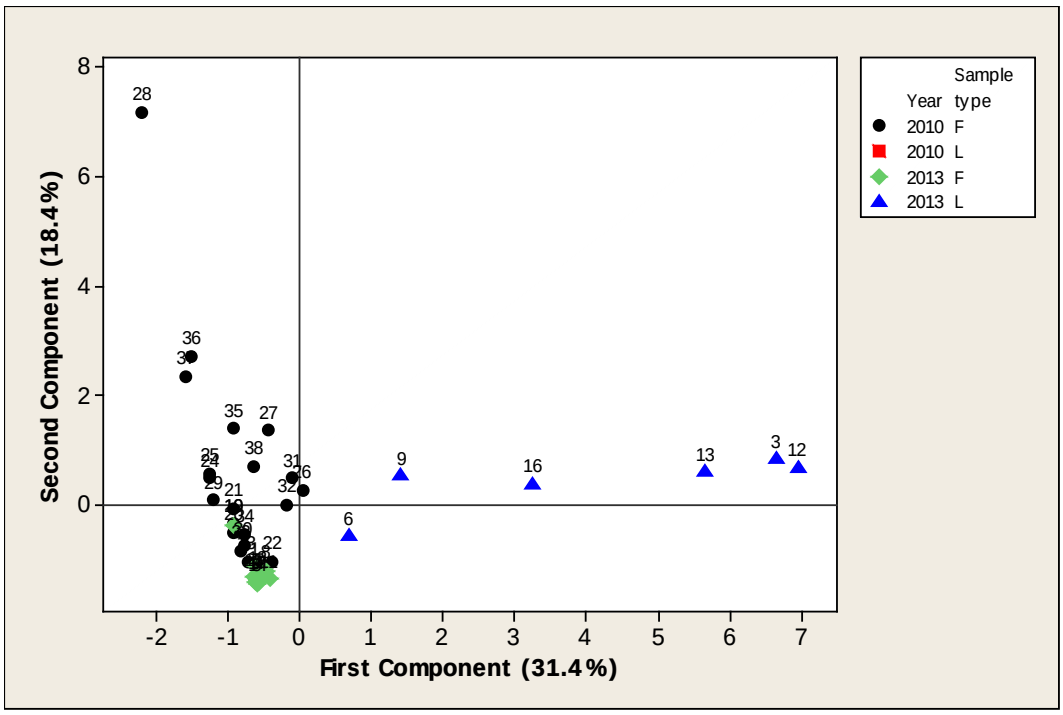



**Figure 13:** Score plot
PCA score plot of in total 38 fish samples (both fillet and liver) of Arctic char (*Salvelinus alpinus*) from Linnévatnet, in Svalbard. The observations in black are from 2010, the green and blue are from 2013, respectively fillet and liver. The labels describe which sample it is (APPENDIX G).

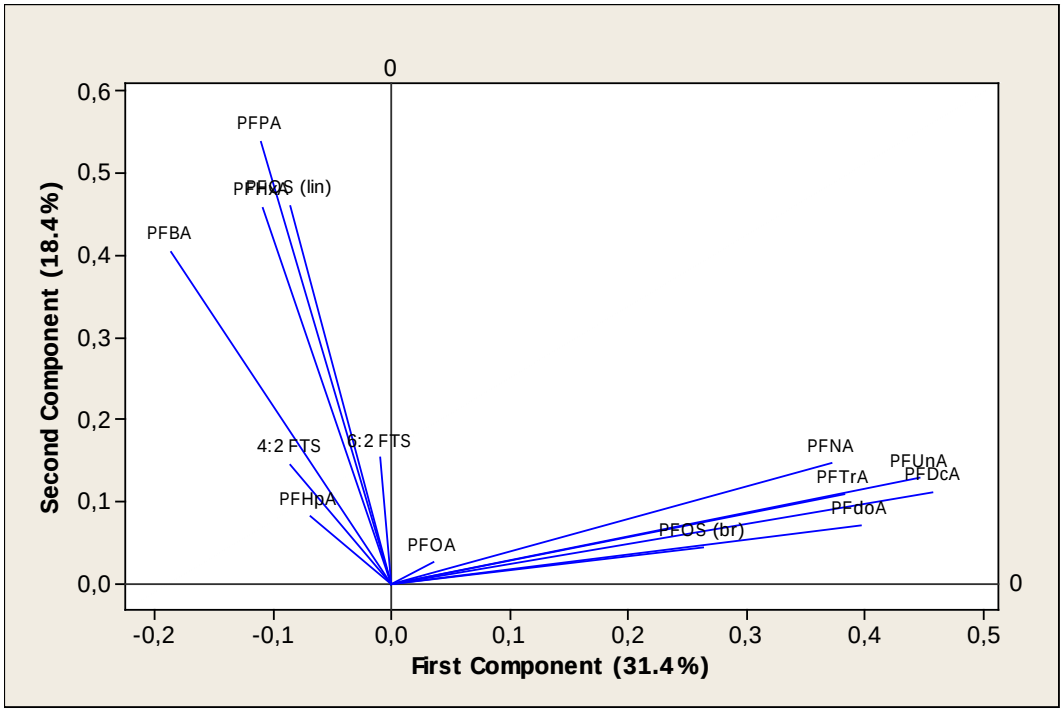



**Figure 14:** Loading plot
PCA loading plot of in total 38 fish samples (both fillet and liver) of Arctic char (*Salvelinus alpinus*) from Linnévatnet, in Svalbard. The loading plot includes the variables 4:2 FTS, 6:2 FTS, PFOS (br), PFOS (lin), PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDcA, PFUnA, PFdoA and PFTrA.

The loading plot (Figure 14) indicates how the different variables are related from each other. The loadings contribute to explain the variations in the PCs. In the loading plot, it is indicated that the variables are well separated by the PC1. The majority of the long-chained PFAS (except for PFOS (lin)) appear on the right side and the short-chained PFAS appear on the left side. The short-chained PFAS (PFBA, PFPA, PFHxA) and PFOS (lin) are separated by PC2, while in the two-dimensional space it is indicated three clusters of PFAS. PC2 contrasts especially PFPA, PFHxA and PFOS (lin) with the long-chained PFAS.

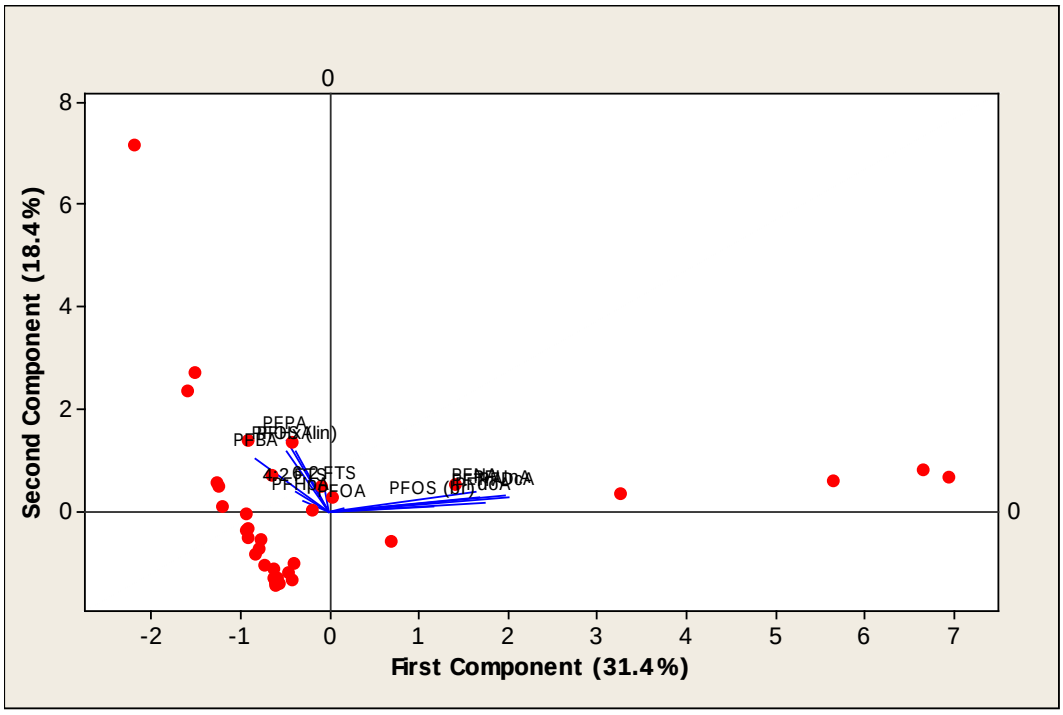



**Figure 15:** Biplot of PFAS and samples
The biplot presents the correlation between the samples from both years and the PFAS.

In a biplot, information about both variables and samples in the dataset are displayed graphically (Figure 15). The observations marked with red dots are the same shown in Figure 13. The long-chained PFAS are located on the right side together with the liver samples from 2013, while the short-chained PFAS are located on the left side together with the fillet samples from 2010, indicating that the contaminant load are separating the samples (observations). In the center of the biplot, fluorotelomer sulfides and mid-long chained PFAS (PFHpA and PFOA) are clustered together with the fillet samples from 2010 and all fillet samples from 2013.

# 5 Discussion

Levels of PFAS in Arctic char (*Salvelinus alpinus*) from Linnévatnet, Svalbard, are reported in this present study. As mentioned above, the main objectives were to identify the PFAS compounds in Arctic char, investigate whether the results can indicate contribution from long-range transport, and to elucidate trends in PFAS levels from 2010 to 2013. There are few reports on PFAS in freshwater fish, although there have been more focus on marine species (Haukas et al. 2007). However, this present study is, to our knowledge, first report identifying levels of PFAS contaminants in freshwater fish in Svalbard.

## 5.1. Biometric characteristics

Performing fieldwork in March in Svalbard can be challenging because of the logistical challenges, such as performing ice fishing, and the Arctic environmental conditions, such as the weather and short-term weather forecast. However, six fish were caught in March 2013. The characteristics of the fish from 2013 are provided in Table 3. However, the study of Svenning (2007) reported that because the fish live in such harsh conditions and that the availability of food is limited, the char are expected to be small. According to the size of the fish caught in 2013, it can be assumed that the fish remain in Linnévatnet all year. The sampling sites on the lake chosen in March were also consistent with the study of (Svenning et al. 2007), where it was reported that during winter, the fish would reside in the shallow area of the lake. Using biometric characterizations, such as age, gender and size, can be done in investigating different levels of PFAS in freshwater fish. This is important in understanding the route and fate of these contaminants, as well as investigating trends within the other biometric characteristics mentioned, than in specified tissues. In this present study there were indications that the larger fish had higher levels of PFAS, even though the levels in general were low. Since only six fish were measured for these characteristics, it is difficult to conclude any trends within the biometric variables. There was no significant statistical trend within the gender of the fish by comparing the PFAS levels.

In September 2010, a net was used to catch fish, which was a more efficient method. 13 fish from 2010 were used in this project. The biometric characteristics were not

available for these Arctic chars, and only fillet samples were available for analysis. This limits the work of further comparison of biometric variables between the two sampling years.

## 5.2 Levels and patterns of perfluorinated alkylated substances (PFAS)

In the present study, 14 of 21 PFAS compounds analyzed were detected in fillet and liver samples from Arctic char in Linnévatnet. Caution should be made concerning the PFAS, i.e. the detected concentrations may be influenced by possible contaminations, e.g. matrix effects. In accordance with recent studies (Greaves et al. 2012; Greaves & Letcher 2013; Sturm & Ahrens 2010), levels of PFAS where higher in liver than in fillet from samples analyzed. Since PFAS binds to proteins in the blood, it will bioaccumulate in tissues and organs with high protein levels, such as liver (Greaves & Letcher 2013). Hence it is expected higher levels of PFAS in liver than in fillet. However, based on the "phase-out" of PFOS-related compounds since 2000, by 3M Company (USA), and that the short-chained compounds are being used in the production of industrial and consumer products (D'Eon et al. 2006; Herzke et al. 2012; Voogt & Sáez 2006; Zhao et al. 2012), it is expected that the levels in general should be low, and especially the levels of long-chained PFAS.

In the 2010 samples (APPENDIX G), which only consist of fillet, the highest individual measurement detected was PFBA (8.3 ng/g wet weight (ww)). PFBA was the most abundant contaminant detected in all samples. However, as mentioned in the section 5.2 (above), the elevated concentrations of PFBA should be evaluated with caution due to possible contaminations. The ∑PFBA alone contributed to 58.2% of the ΣPFAS in 2010. This is not in accordance with previous studies of fish, however analyzing industrial and treated customer products, showed PFBA as the most frequently detected compound (Herzke et al. 2012). Another contaminant, which also was detected in all samples, was PFHxA, but at lower concentrations than PFBA. While the long-chained PFCAs (C7-13), also including PFOS, accounted for 11.5% together. Only the linear isomer of PFOS was detected in the samples from 2010, with a range from 0.04 to 4.5 ng/g ww. PFOA was detected in low levels, ranging from 0.02 to 0.1 ng/g ww. The most abundant long-chained PFCAs were PFUnA, followed by PFNA and the fluorotelomer sulfonate 6:2FTS. PFBA, PFHxA and PFUnA were the most dominated compounds, contradicting previous studies (Kallenborn et al. 2004; Powley et al. 2008) on polar cod (*Boreogadus saida*)

from Canada and Arctic char from the Lake á Mýranar on the Faroe Islands. Levels of PFOS were lower in polar cod (Powley et al. 2008) compared to the Arctic char in the present study (0.04-4.5 ng/g ww), although levels of PFOS in Arctic char from the Faroe Islands were higher (4.7-5.3 ng/g ww). In the study of Eriksson et al. (2013) PFOA was detected in both polar cod and farmed salmon (*Salmo salar*) from the Faroe Islands, with concentrations 0.1 ng/g ww and <0.1-0.2 ng/g ww, respectively. These levels of PFOA exceeded the levels in Arctic char from Svalbard. The majority of PFOA, in the present study, were detected in fillet samples, while PFOS was detected mainly in liver. Detecting higher levels of PFAS on the Faroe Islands than in Svalbard would be expected since the Faroe Islands are in the lower latitudes, thus closer to potential urban sources compared to Linnévatnet in Svalbard. Concentration levels of PFAS in polar cod from the Barents Sea (Haukas et al. 2007) being lower than in the present study, also confirming that background marine ecosystems have lower PFAS burdens than freshwater systems. This is supported by Berger et al (2009) among others, indicating that the levels of PFAS in fish are higher in freshwaters than in fish from the sea and in anadromous fish (Berger et al. 2009; Schuetze et al. 2010).

In 2013, the highest individual PFAS compound level was 6:2 FTS (5.3 ng/g ww), in one of the liver samples (APPENDIX G). The highest individual measurement detected in fillet samples was PFBA (1.5 ng/g ww), almost six times lower concentration than the highest concentration measured in 2010. The rest of the fillet samples had PFBA concentrations <1 ng/g ww. This is lower than the concentrations measured in 2010, where all samples except for two had PFBA concentrations >1 ng/g ww. In 2013, ∑6:2 FTS account for 21 % of the ∑PFAS in all samples, while ∑PFBA had decreased to 10.7%. Both branched and linear PFOS were detected, mainly in the liver samples. The highest concentration was found for PFOS (br) (2.9 ng/g ww) in liver. PFOA ranged from 0.02 to 0.04 ng/g ww in fillet samples, as well as detected in one liver sample (0.04 ng/g ww), although it was no different than the highest level detected in fillet. The highest PFOA level was three times lower than the highest concentration in 2010. However, the long-chained PFCAs (C7-13), together, account for 56.9%. The findings of PFOA and 6:2FTS can be explained by being more hydrophilic than the other PFAS and partitioning directly with the surrounding water. Since there are only liver samples from 2013, temporal trends in this present study are only to be discussed in accordance to the fillet samples analyzed for both years. Even though the levels are low, the results indicate a

decrease in PFAS levels from 2010 to 2013. The PFAS pattern show similarities between the sampling years, with the short-chained PFAS dominating. The samples from 2013 show levels of the same PFAS (except for 4:2 FTS), though the majority of the levels detected are in liver samples. Temporal trend studies of fish and marine mammals are rare. Nevertheless, a study of water samples, from 2009-2010 comparing with studies from 2007, indicated a decline in ∑PFAS in the North Atlantic Ocean (Zhao et al. 2012). Huber et al (2012) reported an increase in long-chained PFAS (C9-13) in liver samples from harbor porpoise (*Phocoena phocoena*) from Baltic and North Sea (year 1991-2008).

Of the long-chained PFAS detected in Arctic char from Svalbard, PFUnA was the most abundant in 2010 and 2013. PFUnA was found in 14 of 18 samples from 2013, with highest concentrations in liver samples (1.8 ng/g ww). In 2010, PFUnA was detected in 13 of 20 samples with concentrations <1 ng/g ww. According to Martin et al. (2004) PFUnA have probably the highest bioaccumulation and biomagnification potential among the PFAS compounds, which suggest higher concentrations in liver (Conder et al. 2008) than in fillet, as well as bioaccumulation potential seem to increase with the perfluoro alkyl chain length (Martin et al. 2003). This is consistent with the long-chained PFAS in the present study being detected more frequently in the samples from 2013 than 2010, as well as the contaminants are detected in the majority of the liver samples. This is supported by Powley (2008), as PFUnA was the second dominating compound detected in polar cod. High concentrations of PFUnA in Asian tuna liver indicated a distinctive source that comes from the Asian industries in the East (Sturm & Ahrens 2010). Given the high concentrations of PFUnA, and also detected in the European Arctic indicate long-range transport and is also consistent with a variety of PFAS related compounds still being produced in Asia (Paul et al. 2009; Prevedouros et al. 2006). Even though Asia and especially China is the main source for these contaminants transported to the Arctic, inputs from North America and Western Europe should not be neglected in the assessment of potential sources despite the reduced inputs the last decade.

However, there are only few reports on PFAS in the freshwater ecosystems, and the majority of the published reports are from the Canadian Arctic. Butt et al (2010) reported levels of PFAS in land-locked Arctic char from various lakes in the Canadian Arctic; Amituk lake, Char lake and Resolute lake. Fillet tissue was analyzed, and the major PFAS detected were PFOS, PFOSA and PFNA. Concentrations of PFHxS and PFHpS

were also detected in the Canadian lakes, but PFHxS and PFHpS were not detected in the samples of Arctic char in the here presented study. Also the PFOS levels were higher in the fish from Canada compared to the Arctic char from Linnévatnet. PFOSA was not detected in the Arctic char from Svalbard. This feature is in accordance with previous studies (Berger et al. 2009; Holmstrom et al. 2005; Verreault et al. 2005). This indicated that PFOSA possibly biotransform to PFOS via abiotic processes or in the fish via metabolism (D'Eon et al. 2006; Tomy et al. 2004). PFNA was detected in 50% of the samples from both years, 2010-2013, but with a slight decrease from 2010 to 2013. Though in 2013 the majority of the PFNA concentrations was detected in liver samples, and can be explained by strong persistence (Haukas et al. 2007). The comparisons of the results, from the study in the Canadian Arctic and from Svalbard, reveal significant differences. Higher levels of PFAS in the Arctic char from Canada indicate that the Canadian Arctic is more polluted than the European Arctic.

The physical and chemical properties of PFAS do not make these contaminants typical candidates for long-range atmospheric transport (Martin, J. W. et al. 2004; Martin et al. 2005), and Arctic is not considered the main source for PFAS compounds. However, due to more volatile compounds degrading to PFCAs and PFSAs, Arctic regions become "sinks" for globally emitted contaminants (D'Eon et al. 2006; Ellis et al. 2004; Greaves et al. 2012; Young et al. 2007). However, detection of PFAS in the Arctic char of Linnévatnet supports this suggestion of long-range atmospheric transport, especially when considering observed levels of PFOS and PFOA, which are considered as persistent and not degradable. However, the influence of local contamination of PFAS cannot be excluded as levels of PFAS are found in the nearby settlement, Barentsburg (Miljeteig & Gabrielsen 2009) and Lonyearbyen (Kwok et al. 2013).

Linnévatnet is located in an area where there is no settlement, except for Isfjord Radio, which is a hotel, used only in the tourist season. The lake is located upstream from the hotel, and the hotel activities are not considered as a local point source for the lake. However, during the winter season, guided trips travel to Kapp Linné, Isfjord Radio, by snowmobiles, which might contribute to the contamination to the lake, when the snow and ice melts during summer. High proportions of long-chained PFAS >8 carbons were found in the surface snow in Sweden (Nilsson et al. 2010), which is in accordance with studies from Longyearbyen. The results from both studies were suggested to be caused

by skiing activities where ski wax containing PFCAs have been used. Since the lake is located approximately 80 km from Longyearbyen, snowmobiles (eg. Gore-Tex in scooter suites) are expected to contribute more than skiers to the PFAS contamination, but skiing activities cannot be excluded as a minor, but potentially source.

The lake is surrounded by glaciers in the South and steep mountains in East and West (Borgstrom et al. 2010). Runoff from the glaciers during melting periods is suggested to contribute to PFAS levels in Linnévatnet. The precipitation measured during 1996 at Linnévatnet was approximately 350 mm, although during winter (January-May) only 80 mm fell as snow (Svenning et al. 2007). There are reasons to assume this could have changed over the years, however the precipitation is low and could explain the contribution of low levels of PFAS. Long-range atmospheric transport of PFAS was found to be the major transport pathway to the glaciers (Borgstrom et al. 2010; Kwok et al. 2013). In the study of Kwok (2013), ice cores, snow and water samples were collected from the Longyear glacier (close to Longyearbyen, Svalbard) for analysis of PFAS. PFOA was the major PFAS compound detected in snow and surface water, while the ice cores had the lowest concentration of PFAS with PFBA, PFOA and PFNA dominating the total PFAS burden. Concentrations of C8-C12 PFAS compounds were much lower than levels in Canadian ice cores, indicating that the levels of PFAS are lower in the European Arctic (Fei et al. 2007).

It has been suggested that the main exposure route for PFAS in the human population is through the diet, and the major contributors are fish and seafood (EFSA 2012), also supported by Berger et al (2009). Though levels of PFAS have also been reported more frequently within other food groups. Tolerable daily intake (TDI) was set for PFOS as 150 ng/kg body weight (bw.) per day and 1500 ng/kg bw. per day for PFOA. The estimation of daily dietary intake of PFOS was 60 ng/kg bw. per day for the average consumers in Europe and for the high consumers of fish, the estimation was 200 ng/kg bw. per day. The estimations for PFOA were 2 and 6 ng/kg bw. per day, respectively. The establishment of TDI was done even though the Scientific Panel on Contaminants in the Food Chain (CONTAM) noted that the general daily dietary intake was lower than the TDI (Cornelis et al. 2012; EFSA 2008; Haug et al. 2010; Kärrman et al. 2009). However, food can be contaminated not only in contact with the environment and by accumulation in the environment but also from the cookware that contain PFAS, such as teflon, and

material of food packages (Trier et al. 2011). There are reasons to assume that the contribution of PFAS from eating Arctic char of Linnévatnet will not affect the human consumers, due to low concentrations and the amount that have to be consumed to exceed the TDIs. However, monitoring levels of PFAS in fish is important to obtain information about the potential exposure pathways to humans and especially humans at high risk (Haug et al. 2010) in certain areas.

### 5.3 Principal component analysis

The PCA plot (Figure 15) indicates concentration pattern differences between the contaminant loads in the samples. The PCA multivariate statistical analysis confirmed a more dominated burden of long-chained PFAS (C8-13) in the liver samples, while short and mid-long chained PFAS (C4-7) contributed most to ΣPFAS in the fillet samples. The different isomers of PFOS were clearly separated in the loading plot (Figure 14), where the linear isomer was clustered with the short-chained PFAS and the branched isomer clustered together with long-chained PFAS. This suggests that the isomers distribution of PFOS might be tissue specific perhaps due to protein affinities and differences in interaction with proteins and metabolism in the body (Greaves & Letcher 2013). In addition, there was also a clear separation between 2010 and 2013, in which 2010 showed a relationship to fillet samples, while 2013 with the liver samples. This pattern could have been different if liver samples had been available from 2010. This can also further explain that the PCA biplot presenting branched PFOS in relation to year 2013, might not be consistent with studies providing information about phase-out of ECF process and the use of telomerization process (Lindstrom et al. 2011).

In the study of Greaves (2013) different tissue samples from polar bears from Greenland were studied, and the results showed that PFOS (lin) was detected in all tissues, while branched isomers were only detected in liver and brain. The distribution of PFOS isomers is in accordance with results from this present study, although the amount of different sample types, in the present study, was limited, especially liver samples. PFOS (lin) was detected in both liver and fillet, while PFOS (br) was only detected in liver. PFOS (lin) was also the more abundant isomer. Powley et al (2008) found that polar cod from the Canadian Arctic showed high percentage of branched PFOS in the liver (50%). There are few studies on wildlife that have examined the pattern of PFOS isomers in different tissues. In rat studies (Benskin et al. 2007; De Silva & Mabury 2004) linear

isomers were more abundant than branched, which also indicated that branched PFOS was eliminated faster than linear PFOS (PFOS (br) has shorter half-life compared to PFOS (lin)). This might be in accordance with the present study since branched PFOS was only detected in one sample, while linear PFOS was detected in eight samples in total from both years. Higher percentage of linear compared to branched PFOS can also be due to the phased-out of the ECF process (explained in the introduction) in 2003, and that the telomerization process now dominates the production, exclusively producing linear compounds (Lindstrom et al. 2011).

In the study of Greaves et al (2012), tissue-specific distribution and pattern of PFAS were investigated in polar bears from East Greenland. In the comparison of the different PFAS the results showed that the concentrations were highest in the liver, and that liver and brain contained more of the short/mid-long chained PFAS (C6-11), while the fillet and muscles contained more of the long-chained PFAS (C13-15). However, the findings might be due to the long-chained compounds' affinity to bioaccumulate in lipid rich tissues, and that less lipophilic PFAS accumulate in protein rich tissues, such as the liver. These findings from previous studies are not in accordance with the present study as the PCA biplot (Figure 15) indicated that the long-chained PFAS were the dominating burden in the liver samples than in the fillet samples, which is consistent with the individual measurements in the present study. Long-chained PFAS dominated in the liver samples, indicating that there is a relationship between chain length and lipophilic behavior (Jing et al. 2009). In addition, PFCAs with low lipophilicity accumulate in liver, and PFCAs with high lipophilicity accumulate in brain. This might be explained not by lipid content in the tissues, but distribution of different proteins binding to different PFAS chain lengths.

# 6 Conclusion and future perspectives

Arctic char was caught in Linnévatnet in western Svalbard. Thirteen fish from 2010 and six fish from 2013 were used for analysis of PFAS, in this pilot study. Only fillet was used from 2010 samples, and both fillet and liver were used from 2013 samples.

The present study indicated a temporal trend in which concentrations of PFAS decreased from 2010 to 2013 within the fillet samples. PFBA, PFHxA and PFUnA were the most frequently detected compounds both years, showing a similar pattern. PFBA was the most dominated compound in 2010 and 2013. In 2013 the long-chained PFAS were detected mainly in liver samples, and detected more frequently than in 2010. However, levels of PFOS and PFOA were low, suggesting a different PFAS pattern than presented in previous studies. Although levels of PFAS were higher in the liver samples than in fillet samples, as expected. The levels in Arctic char were assumed to be lower than TDI. PFAS is assumed to have toxicological effects and is of concern for the human health, and the total diet should be taken into account when the TDIs are being discussed. With the low levels detected, and a low-frequent intake, Arctic char from Linnévatnet is not expected to give a major contribution to the PFAS exposure of Svalbard inhabitants.

Finding levels of PFAS in Arctic char in Svalbard and long-range atmospheric transport being the dominant pathway for PFAS to the European Arctic, give reasons for further investigation and monitoring studies, as well as for studies on temporal trends. Investigating differences in PFAS levels in various lakes in Svalbard, and comparing with marine fish or conducting samples closer to settlements to investigate local contribution of PFAS, is important in understanding the route and fate of the PFAS. It could also be interesting to investigate differences between other biometric variables (such as age and gender), and also between tissues as PFAS are likely distributed in tissues in accordance to protein affinity. For statistical use a Pearson correlation test could be utilized in further studies, to investigate and confirm or invalidate the indications from the PCA plot. This is, to our knowledge, the first report on levels of PFAS in freshwater fish in Svalbard, and there is still need for further work and to study PFAS long-range transport and deposition in the Arctic.

# 7 References


3MCompany. (1999). Fluorochemical use, distribution and release overview. .

3MCompany. (2000a). Phase-out Plan for POSF-Based Products.

3MCompany. (2000b). Sulfonated Perfluorochemicals in the Environment: Sources, Dispersion, Fate and Effects.

Abdi, H. & Williams, L. J. (2010). Principal component analysis. *Wiley Interdisciplinary Reviews: Computational Statistics*, 2 (4): 433-459.

AMAP. (2010). AMAP Assessment 2009 - Persistent Organic Pollutants (POPs) in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), P.O. Box 8100 Dep, N-0032 Oslo, Norway (www.amap.no).

Barrie, L. A., Gregor, D., Hargrave, B., Lake, R., Muir, D., Shearer, R., Tracey, B. & Bidleman, T. (1992). Arctic Contaminants - Sources, Occurrence and Pathways. *Science of the Total Environment*, 122 (1-2): 1-74.

Benskin, J. P., Bataineh, M. & Martin, J. W. (2007). Simultaneous characterization of perfluoroalkyl carboxylate, sulfonate, and sulfonamide isomers by liquid chromatography-tandem mass spectrometry. *Anal Chem*, 79 (17): 6455-6464.

Benskin, J. P., Ahrens, L., Muir, D. C. G., Scott, B. F., Spencer, C., Rosenberg, B., Tomy, G., Kylin, H., Lohmann, R. & Martin, J. W. (2011). Manufacturing Origin of Perfluorooctanoate (PFOA) in Atlantic and Canadian Arctic Seawater. *Environmental Science & Technology*, 46 (2): 677-685.

Berger, U., Glynn, A., Holmstrom, K. E., Berglund, M., Ankarberg, E. H. & Tornkvist, A. (2009). Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden - Analysis of edible fish from Lake Vattern and the Baltic Sea. *Chemosphere*, 76 (6): 799-804.

Bonefeld-Jorgensen, E. C., Long, M., Bossi, R., Ayotte, P., Asmund, G., Kruger, T., Ghisari, M., Mulvad, G., Kern, P., Nzulumiki, P., et al. (2011). Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: A case control study. *Environmental Health*, 10.

Borgstrom, R., Ebne, I. & Svenning, M. A. (2010). High lacustrine gillnet catchability of anadromous Arctic charr. *Hydrobiologia*, 650 (1): 203-212.

Braune, B. M., Outridge, P. M., Fisk, A. T., Muir, D. C. G., Helm, P. A., Hobbs, K., Hoekstra, P. F., Kuzyk, Z. A., Kwan, M., Letcher, R. J., et al. (2005). Persistent organic pollutants and mercury in marine biota of the Canadian Arctic: An overview of spatial and temporal trends. *Science of the Total Environment*, 351: 4-56.

Buck, R. C., Franklin, J., Berger, U., Conder, J. M., Cousins, I. T., de Voogt, P., Jensen, A. A., Kannan, K., Mabury, S. A. & van Leeuwen, S. P. J. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integrated Environmental Assessment and Management*, 7 (4): 513-541.

Burkow, I. C. & Kallenborn, R. (2000). Sources and transport of persistent pollutants to the Arctic. *Toxicol Lett*, 112: 87-92.

Butt, C. M., Berger, U., Bossi, R. & Tomy, G. T. (2010). Levels and trends of poly- and perfluorinated compounds in the arctic environment. *Science of the Total Environment*, 408 (15): 2936-2965.

Carloni, D. (2009). Perfluorooctane Sulfonate (PFOS) Production and Use: Past and Current Evidence. Research Fellow UNIDO Regional Office in China.

ChemIDplus. (Downloaded 25.09.2013). http://chem.sis.nlm.nih.gov/chemidplus/.

Clara, M., Scheffknecht, C., Scharf, S., Weiss, S. & Gans, O. (2008). Emissions of perfluorinated alkylated substances (PFAS) from point sources - identification of relevant branches. *Water Science and Technology*, 58 (1): 59-66.

Conder, J. M., Hoke, R. A., De Wolf, W., Russell, M. H. & Buck, R. C. (2008). Are PFCAs bioaccumulative? A critical review and comparison with regulatory lipophilic compounds. *Environmental Science & Technology*, 42 (4): 995-1003.

Cornelis, C., D'Hollander, W., Roosens, L., Covaci, A., Smolders, R., Van Den Heuvel, R., Govarts, E., Van Campenhout, K., Reynders, H. & Bervoets, L. (2012). First assessment of population exposure to perfluorinated compounds in Flanders, Belgium. *Chemosphere*, 86 (3): 308-314.

D'Eon, J. C., Hurley, M. D., Wallington, T. J. & Mabury, S. A. (2006). Atmospheric Chemistry of N-methyl Perfluorobutane Sulfonamidoethanol, C4F9SO2N(CH3)CH2CH2OH: Kinetics and Mechanism of Reaction with OH. *Environmental Science & Technology*, 40 (6): 1862-1868.

D'Eon, J. C. & Mabury, S. A. (2007). Production of Perfluorinated Carboxylic Acids (PFCAs) from the Biotransformation of Polyfluoroalkyl Phosphate Surfactants (PAPS): Exploring Routes of Human Contamination. *Environmental Science & Technology*, 41 (13): 4799-4805.

De Silva, A. O. & Mabury, S. A. (2004). Isolating Isomers of Perfluorocarboxylates in Polar Bears (Ursus maritimus) from Two Geographical Locations. *Environmental Science & Technology*, 38 (24): 6538-6545.

Dimitrov, S., Kamenska, V., Walker, J. D., Windle, W., Purdy, R., Lewis, M. & Mekenyan, O. (2004). Predicting the biodegradation products of perfluorinated chemicals using CATABOL. *SAR and QSAR in Environmental Research*, 15 (1): 69-82.

Dinglasan, M. J. A., Ye, Y., Edwards, E. A. & Mabury, S. A. (2004). Fluorotelomer alcohol biodegradation yields poly- and perfluorinated acids. *Environmental Science & Technology*, 38 (10): 2857-2864.

Dinglasan-Panlilio, M. J. A. & Mabury, S. A. (2006). Significant Residual Fluorinated Alcohols Present in Various Fluorinated Materials. *Environmental Science & Technology*, 40 (5): 1447-1453.

EFSA. (2008). (European Food Safety Authority), Opinion of the Scientific Panel on Contaminants in the Food chain on Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. . *The EFSA Journal 653:1-131*.

EFSA. (2012). (European Food Safety Authority), Perfluoroalkylated substances in food: occurrence and dietary exposure. *EFSA Journal 2012;10(6):2743*.

Ellis, D. A., Martin, J. W., Mabury, S. A., Hurley, M. D., Andersen, M. P. S. & Wallington, T. J. (2003). Atmospheric lifetime of fluorotelomer alcohols. *Environmental Science & Technology*, 37 (17): 3816-3820.

Ellis, D. A., Martin, J. W., De Silva, A. O., Mabury, S. A., Hurley, M. D., Andersen, M. P. S. & Wallington, T. J. (2004). Degradation of fluorotelomer alcohols: A likely atmospheric source of perfluorinated carboxylic acids. *Environmental Science & Technology*, 38 (12): 3316-3321.

Eriksen, K. T., Raaschou-Nielsen, O., Sørensen, M., Roursgaard, M., Loft, S. & Møller, P. (2010). Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 700 (1–2): 39-43.

Eriksson, U., Kärrman, A., Rotander, A., Mikkelsen, B. & Dam, M. (2013). Perfluoroalkyl substances (PFASs) in food and water from Faroe Islands. *Environmental Science and Pollution Research*, 20 (11): 7940-7948.

Fei, C. Y., McLaughlin, J. K., Tarone, R. E. & Olsen, J. (2007). Perfluorinated chemicals and fetal growth: A study within the Danish National Birth Cohort. *Environ Health Perspect*, 115 (11): 1677-1682.

Giesy, J. P. & Kannan, K. (2001). Global distribution of perfluorooctane sulfonate in wildlife. *Environmental Science & Technology*, 35 (7): 1339-1342.

Giesy, J. P., Kannan, K. & Jones, P. D. (2001). Global Biomonitoring of Perfluorinated Organics. *ScientificWorldJournal*, 1: 627-629.

Greaves, A. K., Letcher, R. J., Sonne, C., Dietz, R. & Born, E. W. (2012). Tissue-Specific Concentrations and Patterns of Perfluoroalkyl Carboxylates and Sulfonates in East Greenland Polar Bears. *Environmental Science & Technology*, 46 (21): 11575-11583.

Greaves, A. K. & Letcher, R. J. (2013). Linear and branched perfluorooctane sulfonate (PFOS) isomer patterns differ among several tissues and blood of polar bears. *Chemosphere*, 93 (3): 574-580.

Hansen, K. J., Clemen, L. A., Ellefson, M. E. & Johnson, H. O. (2001). Compound-specific, quantitative characterization of organic: Fluorochemicals in biological matrices. *Environmental Science & Technology*, 35 (4): 766-770.

Hanssen, L., Dudarev, A. A., Huber, S., Odland, J. Ø., Nieboer, E. & Sandanger, T. M. (2013). Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. *Science of The Total Environment*, 447 (0): 430-437.

Harris, D. C. (2007). *Quantitative chemical analysis*. 7th utg. New York: W. H. Freeman and Company.

Haug, L. S., Thomsen, C., Brantsæter, A. L., Kvalem, H. E., Haugen, M., Becher, G., Alexander, J., Meltzer, H. M. & Knutsen, H. K. (2010). Diet and particularly seafood are major sources of perfluorinated compounds in humans. *Environment International*, 36 (7): 772-778.

Haukas, M., Berger, U., Hop, H., Gulliksen, B. & Gabrielsen, G. W. (2007). Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. *Environmental Pollution*, 148 (1): 360-371.

Herzke, D., Olsson, E. & Posner, S. (2012). Perfluoroalkyl and polyfluoroalkyl substances (PFASs) in consumer products in Norway - A pilot study. *Chemosphere*, 88 (8): 980-987.

Hoffmann, E. d. & Stroobant, V. (2011). *Mass Spectrometry, Principles and Applications*. 3rd utg. West Sussex, England: John Wiley & Sons Ltd. 489 s.

Holmstrom, K. E., Jarnberg, U. & Bignert, A. (2005). Temporal trends of PFOS and PFOA in guillemot eggs from the Baltic Sea, 1968-2003. *Environmental Science & Technology*, 39 (1): 80-84.

Houde, M., Martin, J. W., Letcher, R. J., Solomon, K. R. & Muir, D. C. G. (2006). Biological monitoring of polyfluoroalkyl substances: A review. *Environmental Science & Technology*, 40 (11): 3463-3473.

Hu, W. y., Jones, P. D., DeCoen, W., King, L., Fraker, P., Newsted, J. & Giesy, J. P. (2003). Alterations in cell membrane properties caused by perfluorinated compounds. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 135 (1): 77-88.

Huber, S., Ahrens, L., Bardsen, B. J., Siebert, U., Bustnes, J. O., Vikingsson, G. A., Ebinghaus, R. & Herzke, D. (2012). Temporal trends and spatial differences of perfluoroalkylated substances in livers of harbor porpoise (Phocoena phocoena) populations from Northern Europe, 1991-2008. *Science of the Total Environment*, 419: 216-224.

Hurley, M. D., Sulbaek Andersen, M. P., Wallington, T. J., Ellis, D. A., Martin, J. W. & Mabury, S. A. (2003). Atmospheric Chemistry of Perfluorinated Carboxylic Acids: Reaction with OH Radicals and Atmospheric Lifetimes. *The Journal of Physical Chemistry A*, 108 (4): 615-620.

Isdahl, T. (2002). *Populasjonsdynamikk hos røye, Salvelinus alpinus, i Nordre Borgfiskdam, Svalbard. Hovedoppgave i fiskeforvaltning*. Ås: Norges Lansbrukshøgskole, Institutt for biologi og naturforvaltning.

Jing, P., Rodgers, P. J. & Amemiya, S. (2009). High Lipophilicity of Perfluoroalkyl Carboxylate and Sulfonate: Implications for Their Membrane Permeability. *Journal of the American Chemical Society*, 131 (6): 2290-2296.

Jones, K. C. & de Voogt, P. (1999). Persistent organic pollutants (POPs): state of the science. *Environmental Pollution*, 100 (1-3): 209-221.

Jones, P. D., Hu, W., De Coen, W., Newsted, J. L. & Giesy, J. P. (2003). Binding of perfluorinated fatty acids to serum proteins. *Environmental Toxicology and Chemistry*, 22 (11): 2639-2649.

Kaiser, M. A., Larsen, B. S., Kao, C.-P. C. & Buck, R. C. (2005). Vapor Pressures of Perfluorooctanoic, -nonanoic, -decanoic, -undecanoic, and -dodecanoic Acids. *Journal of Chemical & Engineering Data*, 50 (6): 1841-1843.

Kallenborn, R., Berger, U. & Järnberg, U. (2004). Perfluorinated Alkylated Substances (PFAS) in the Nordic Environments. Nordic Counsil of Ministers, Copenhagen 2004.

Kannan, K., Koistinen, J., Beckmen, K., Evans, T., Gorzelany, J. F., Hansen, K. J., Jones, P. D., Helle, E., Nyman, M. & Giesy, J. P. (2001). Accumulation of perfluorooctane sulfonate in marine mammals. *Environmental Science & Technology*, 35 (8): 1593-1598.

Kelly, B. C., Ikonomou, M. G., Blair, J. D., Surridge, B., Hoover, D., Grace, R. & Gobas, F. A. P. C. (2009). Perfluoroalkyl Contaminants in an Arctic Marine Food Web: Trophic Magnification and Wildlife Exposure. *Environmental Science & Technology*, 43 (11): 4037-4043.

Kennedy, G. L., Butenhoff, J. L., Olsen, G. W., O'Connor, J. C., Seacat, A. M., Perkins, R. G., Biegel, L. B., Murphy, S. R. & Farrar, D. G. (2004). The Toxicology of Perfluorooctanoate. *Critical Reviews in Toxicology*, 34 (4): 351-384.

Key, B. D., Howell, R. D. & Criddle, C. S. (1997). Fluorinated organics in the biosphere. *Environmental Science & Technology*, 31 (9): 2445-2454.

Kissa, E. (1994). *Fluorinated Surfactants; Synthesis, Properties, Applications*. Surfactant science series, b. 50. New York: Marcel Dekker, Inc. 469 s.

Kissa, E. (2001). *Fluorinated surfactants and repellents*. 2nd, Revised and expanded utg. Surfactant science series, b. 97. New York, USA: Marcel Dekker, Inc. 615 s.

Kwok, K. Y., Yamazaki, E., Yamashita, N., Taniyasu, S., Murphy, M. B., Horii, Y., Petrick, G., Kallerborn, R., Kannan, K., Murano, K., et al. (2013). Transport of Perfluoroalkyl substances (PFAS) from an arctic glacier to downstream locations: Implications for sources. *Science of The Total Environment*, 447 (0): 46-55.

Kärrman, A., Harada, K. H., Inoue, K., Takasuga, T., Ohi, E. & Koizumi, A. (2009). Relationship between dietary exposure and serum perfluorochemical (PFC) levels--a case study. *Environment international*, 35 (4): 712-717.

Lau, C., Butenhoff, J. L. & Rogers, J. M. (2004). The developmental toxicity of perfluoroalkyl acids and their derivatives. *Toxicology and Applied Pharmacology*, 198 (2): 231-241.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A. & Seed, J. (2007). Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicological Sciences*, 99 (2): 366-394.

Lehmler, H.-J. (2005). Synthesis of environmentally relevant fluorinated surfactants—a review. *Chemosphere*, 58 (11): 1471-1496.

Lindstrom, A. B., Strynar, M. J. & Libelo, E. L. (2011). Polyfluorinated Compounds: Past, Present, and Future. *Environmental Science & Technology*, 45 (19): 7954-7961.

Lohmann, R., Breivik, K., Dachs, J. & Muir, D. (2007). Global fate of POPs: Current and future research directions. *Environmental Pollution*, 150 (1): 150-165.

Mackay, D. & Wania, F. (1995). Transport of Contaminants to the Arctic - Partitioning, Processes and Models. *Science of the Total Environment*, 160-61: 25-38.

Martin, J. W., Mabury, S. A., Solomon, K. R. & Muir, D. C. G. (2003). Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). *Environmental Toxicology and Chemistry*, 22 (1): 196-204.

Martin, J. W., Kannan, K., Berger, U., Voogt, P. D., Field, J., Franklin, J., Giesy, J. P., Harner, T., Muir, D. C. G., Scott, B., et al. (2004). Peer Reviewed: Analytical Challenges Hamper Perfluoroalkyl Research. *Environmental Science & Technology*, 38 (13): 248A-255A.

Martin, J. W., Smithwick, M. M., Braune, B. M., Hoekstra, P. F., Muir, D. C. G. & Mabury, S. A. (2004). Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environmental Science & Technology*, 38 (2): 373-380.

Martin, J. W., Ellis, D. A., Mabury, S. A., Hurley, M. D. & Wallington, T. J. (2005). Atmospheric Chemistry of Perfluoroalkanesulfonamides: Kinetic and Product Studies of the OH Radical and Cl Atom Initiated Oxidation of N-Ethyl Perfluorobutanesulfonamide. *Environmental Science & Technology*, 40 (3): 864-872.

Miljeteig, C. & Gabrielsen, G. W. (2009). Contaminants in black-legged kittiwake eggs form Kongsfjorden, Barentsburg and Pyramiden. *Kortrapport / Brief report series No.14*. Norsk Polarinstitutt, Tromsø.

Muir, D. C. G. & de Wit, C. A. (2010). Trends of legacy and new persistent organic pollutants in the circumpolar arctic: Overview, conclusions, and recommendations. *Science of the Total Environment*, 408 (15): 3044-3051.

Nilsson, H., Kärrman, A., Westberg, H., Rotander, A., van Bavel, B. & Lindström, G. (2010). A Time Trend Study of Significantly Elevated Perfluorocarboxylate Levels in Humans after Using Fluorinated Ski Wax. *Environmental Science & Technology*, 44 (6): 2150-2155.

OECD. (2002). *Joint meeting of the chemicals committee and the working party on chemicals, pesticides and biotechnology*. I: ENV/JM/RD(2002)17/FINAL (red.). CO-OPERATION ON EXISTING CHEMICALS HAZARD ASSESSMENT OF PERFLUOROOCTANE SULFONATE (PFOS) AND ITS SALTS. Tilgjengelig fra: http://www.oecd.org/ dataoecd/23/18/2382880.pdf.

Oehme, M. (2007). *Quality control in organic trace analysis*. Applied Analytical Chemistry. CH-4144 Arlesheim and University of Basel, CH-4056 Basel.

Olsen, G. W., Burris, J. M., Ehresman, D. J., Froehlich, J. W., Seacat, A. M., Butenhoff, J. L. & Zobel, L. R. (2007). Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environmental Health Perspectives*, 115 (9): 1298-1305.

Olsen, G. W., Chang, S.-C., Noker, P. E., Gorman, G. S., Ehresman, D. J., Lieder, P. H. & Butenhoff, J. L. (2009). A comparison of the pharmacokinetics of

perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans. *Toxicology*, 256 (1–2): 65-74.
Parsons, J. R., Saez, M., Dolfing, J. & de Voogt, P. (2008). Biodegradation of Perfluorinated Compounds. *Reviews of Environmental Contamination and Toxicology, Vol 196*, 196: 53-71.
Paul, A. G., Jones, K. C. & Sweetman, A. J. (2009). A First Global Production, Emission, And Environmental Inventory For Perfluorooctane Sulfonate. *Environmental Science & Technology*, 43 (2): 386-392.
Pinkas, A., Slotkin, T. A., Brick-Turin, Y., Van der Zee, E. A. & Yanai, J. (2010). Neurobehavioral teratogenicity of perfluorinated alkyls in an avian model. *Neurotoxicology and Teratology*, 32 (2): 182-186.
Powley, C. R., George, S. W., Ryan, T. W. & Buck, R. C. (2005). Matrix Effect-Free Analytical Methods for Determination of Perfluorinated Carboxylic Acids in Environmental Matrixes. *Analytical Chemistry*, 77 (19): 6353-6358.
Powley, C. R., George, S. W., Russell, M. H., Hoke, R. A. & Buck, R. C. (2008). Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. *Chemosphere*, 70 (4): 664-672.
Prevedouros, K., Cousins, I. T., Buck, R. C. & Korzeniowski, S. H. (2006). Sources, fate and transport of perfluorocarboxylates. *Environ Sci Technol*, 40 (1): 32-44.
Renner, R. (2001). Growing Concern Over Perfluorinated Chemicals. *Environmental Science & Technology*, 35 (7): 154A-160A.
Renner, R. (2006). The long and the short of perfluorinated replacements. *Environmental Science & Technology*, 40 (1): 12-13.
Schenker, U., Scheringer, M., MacLeod, M., Martin, J. W., Cousins, I. T. & Hungerbühler, K. (2008). Contribution of Volatile Precursor Substances to the Flux of Perfluorooctanoate to the Arctic. *Environmental Science & Technology*, 42 (10): 3710-3716.
Schuetze, A., Heberer, T., Effkemann, S. & Juergensen, S. (2010). Occurrence and assessment of perfluorinated chemicals in wild fish from Northern Germany. *Chemosphere*, 78 (6): 647-652.
Sinclair, E., Mayack, D. T., Roblee, K., Yamashita, N. & Kannan, K. (2006). Occurrence of perfluoroalkyl surfactants in water, fish, and birds from New York State. *Archives of Environmental Contamination and Toxicology*, 50 (3): 398-410.
Smart, B. E. (2001). Fluorine substituent effects (on bioactivity). *Journal of Fluorine Chemistry*, 109 (1): 3-11.
Stock, N. L., Furdui, V. I., Muir, D. C. G. & Mabury, S. A. (2007). Perfluoroalkyl Contaminants in the Canadian Arctic: Evidence of Atmospheric Transport and Local Contamination. *Environmental Science & Technology*, 41 (10): 3529-3536.
Stock, N. L., Muir, D. C. G. & Mabury, S. (2010). *Persistent Organic Pollutants*. United Kingdom: John Wiley and Sons, Ltd, Publication and Blackwell Publishing Ltd. 277 s.
Sturm, R. & Ahrens, L. (2010). Trends of polyfluoroalkyl compounds in marine biota and in humans. *Environmental Chemistry*, 7 (6): 457-484.
Svenning, M. A., Klemetsen, A. & Olsen, T. (2007). Habitat and food choice of Arctic charr in Linnévatn on Spitsbergen, Svalbard: the first year-round investigation in a High Arctic lake. *Ecology of Freshwater Fish*, 16 (1): 70-77.
Svenning, M. A. (2010). Metodikk for prøvefiske etter røye på Svalbard - NINA Rapport 645. 30 s. Tromsø: Norsk institutt for naturforskning.
Tomy, G. T., Tittlemier, S. A., Palace, V. P., Budakowski, W. R., Braekevelt, E., Brinkworth, L. & Friesen, K. (2004). Biotransformation of N-ethyl perfluorooctanesulfonamide

by rainbow trout (Onchorhynchus mykiss) liver microsomes. *Environmental Science & Technology*, 38 (3): 758-762.
Trier, X., Granby, K. & Christensen, J. (2011). Polyfluorinated surfactants (PFS) in paper and board coatings for food packaging. *Environmental Science and Pollution Research*, 18 (7): 1108-1120.
Vanloon, G. W. & Duffy, S. J. (2011). *Environmental Chemistry, A global perspective*. 3. utg. England: Oxford University Press. 484-485 s.
Verreault, J., Houde, M., Gabrielsen, G. W., Berger, U., Haukas, M., Letcher, R. J. & Muir, D. C. G. (2005). Perfluorinated alkyl substances in plasma, liver, brain, and eggs of glaucous gulls (Larus hyperboreus) from the Norwegian Arctic. *Environmental Science & Technology*, 39 (19): 7439-7445.
Voogt, P. d. & Sáez, M. (2006). Analytical chemistry of perfluoroalkylated substances. *TrAC Trends in Analytical Chemistry*, 25 (4): 326-342.
Wallington, T. J., Hurley, M. D., Xia, J., Wuebbles, D. J., Sillman, S., Ito, A., Penner, J. E., Ellis, D. A., Martin, J., Mabury, S. A., et al. (2005). Formation of C7F15COOH (PFOA) and Other Perfluorocarboxylic Acids during the Atmospheric Oxidation of 8:2 Fluorotelomer Alcohol. *Environmental Science & Technology*, 40 (3): 924-930.
Wang, N., Liu, J. X., Buck, R. C., Korzeniowski, S. H., Wolstenholme, B. W., Folsom, P. W. & Sulecki, L. M. (2011). 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere*, 82 (6): 853-858.
Webster, E., Mackay, D. & Wania, F. (1998). Evaluating environmental persistence. *Environmental Toxicology and Chemistry*, 17 (11): 2148-2158.
www.npolar.no. (Available 13.06.2013). The Norwegian Polar Institute.
Yeung, L. W. Y., De Silva, A. O., Loi, E. I. H., Marvin, C. H., Taniyasu, S., Yamashita, N., Mabury, S. A., Muir, D. C. G. & Lam, P. K. S. (2013). Perfluoroalkyl substances and extractable organic fluorine in surface sediments and cores from Lake Ontario. *Environment International*, 59 (0): 389-397.
Young, C. J., Furdui, V. I., Franklin, J., Koerner, R. M., Muir, D. C. G. & Mabury, S. A. (2007). Perfluorinated Acids in Arctic Snow: New Evidence for Atmospheric Formation. *Environmental Science & Technology*, 41 (10): 3455-3461.
Zhao, Z., Xie, Z., Möller, A., Sturm, R., Tang, J., Zhang, G. & Ebinghaus, R. (2012). Distribution and long-range transport of polyfluoroalkyl substances in the Arctic, Atlantic Ocean and Antarctic coast. *Environmental Pollution*, 170 (0): 71-77.

# APPENDIX A: INTERNAL STANDARD PFAS

**Table A.1:** Constituents in the internal standard added during contamination analysis of PFAS in fillet and liver samples from Arctic char (*Salvelinus alpinus*). 20 µl internal standard (ISTD) (13 C labeled internal standard (allPFCs) 0.1 ng/µl) was used.

| **$^{13}$C PFAS** |
|---|
| 13CPFBA |
| 13CPFPA |
| 13CPFHxA |
| 13CPFHxS |
| 13CPFOA |
| 13CPFOS |
| 13CPFNA |
| 13CPFDcA |
| 13CPFUnA |
| 13CPFOSA |
| 13CPFDoA |

# APPENDIX B: CHEMICALS, ISTD, RSTD AND SOLVENTS

**Table B.1:** Chemicals and solvents used in the extraction and clean-up method, and contamination analysis on UHPLC-MS/MS.

| CAS-number | Full name: | Supplier/Firm/Country | Quantity/purity |
|---|---|---|---|
| - | $^{13}C$ marked Internal Standard (allPFCs) | Sigma- Aldrich (Norway) AS or Wellington Laboratories Inc. (Guelph, Ontario, Canada) | 1 mL 0.5 ng/µL<br>5 mL 0.1 ng/µL<br>>98 % purity |
| - | 3,7-dimethyl-branched perfluorodecanoic acid (bPFDA, Perfluoro-3,7-dimethyloctanoic acid | ABCR (Karlsruhe, Germany) | 0.1 ng/µL<br>97 % purity |
| 64-19-7 | Glacial acetic acid | Merck /Germany | 50 µL pr. sample<br>For analysis, ≥99.85 % purity |
| 67-56-1 | Lichrosolv methanol | Merck / Germany | 8 mL pr sample and for washing equipments<br>For liquid chromatography |
| 75-05-8 | Acetonitrile | Merck / Germany | 8 mL pr liver sample<br>For gas chromatography, 99.9 % purity |
| 67-64-1 | Acetone | Merck Germany | Used only for washing/cleaning, but also for gas chromatography |
| 110-54-3 | n-Hexane | Merck Germany | Used only for washing/cleaning, but also for gas chromatography |
| 1333-86-4 | Superclean ENVI-Carb 120/400 (Supelco 57210-U) | Sigma-Aldrich, USA, or Bellefonte, PA, USA or Supelco, PN, USA | 0.025g Superclean |
| 631-61-8 | HLB-water 2mM $NH_4OAc$ 99% | Sigma-Aldrich, St. Louis, MO, USA | 100µl<br>≥99 % purity |

# APPENDIX C: EQUIPMENTS FOR FIELDWORK AND LABORATORY

**Table C.1:** The complete list of equipment used for the fieldwork and in the laboratory, full name, supplier and country where it is possible to purchase the equipment.

| **Full name:** | **Supplier/Company:** | **City/State/Country:** |
|---|---|---|
| Ultrahigh pressure liquid chromatography triple quadrupole tandem mass-spectrometry, UHPLC-MS/MS | Thermo Fisher Scientific Inc. | Waltham, MA, USA |
| Thermo Scientific Vantage MS/MS, | Thermo Fisher Scientific Inc. | Waltham, MA, USA |
| Thermo Scientific quaternary Accela 1250 pump | Thermo Fisher Scientific Inc. | Waltham, MA, USA |
| PAL Sample Manager | Thermo Fisher Scientific Inc. | Waltham, MA, USA |
| Waters Acquity UPLC HSS T3 column (2.1×100 mm, 1,8 µm) | Waters Corporation | Milford, MA, USA |
| Waters Van guard HSS T3 guard column (2.1 × 5 mm, 1.8 µm) | Waters Corporation | Milford, MA, USA |
| Waters XBridge $C_{18}$ column (2.1×50 mm, 5 µm) | Waters Corporation | Milford, MA, USA |
| LCQuan software (Version 2.6) | Thermo Fisher Scientific Inc. | Waltham, MA, USA |
| TurboVap 500 | Zymark Corporation | Hopkinton, Massachusetts, USA |
| Ultrasonic Cleaner | VWR International AS | Oslo, Norway |
| Eppendorf centrifuge 5415 D | Eppendorf North America or VWR International AS | Hauppauge, NY, USA or Oslo, Norway |
| Centrifuge Hettich Universal 16R | Hettich or Nerliens Kemisk-Tekniske AS | Tuttlingen, Germany or Oslo, Norway |
| Vortex mixer | VWR International AS | Oslo, Norway |
| Fine Scale | Mettler Toledo | Oslo, Norway |
| Muffle furnace | Nabertherm | Lilienthal/ Bremen, Germany |

**Table C.2:** The complete list of equipment used for the fieldwork and in the laboratory, full name, supplier and country where it is possible to purchase the equipment.

| Full name: | Supplier/Company: | City/State/Country: |
|---|---|---|
| Plastic vials (PP) | VWR International AS | Oslo, Norway |
| Eppendorf (1,7ml) tubes, or micro-centrifuge tubes (2ml) | VWR International AS | Oslo, Norway |
| TurboVap glasses | VWR International AS | Oslo, Norway |
| Sthil (BT121) drill head with drill (250 mm) (used in field) | STIHL PTY. LTD / Sthil Inc. Headquarters | Knoxfield Victoria, Australia / Virginia Beach, Virginia, US |
| Fishing equipments (seasonal dependent and used in field) | (General sports/outdoor activities-shops) | (Any country that has these shops) |
| Gloves (purple) Nitrile | Kimberly- Clark KIMTECH Science Brand | Reigate, Surrey, UK |
| Gloves (blue) Nitrile | VWR International AS | Oslo, Norway or Geldenaaksebaan Lauven, Norway |
| Cappilar pipettes | Brand | Werthem, Germany |
| Pasteur Pipettes | VWR International AS | Oslo, Norway |
| Finnpipette | Thermo Electron Org | Vantaa, Finland |
| Plastic bags | VWR International AS, Grippie | Oslo, Norway |
| Aluminum foil | VWR International AS, Labor | Oslo, Norway |

# APPENDIX D: BLANK SAMPLES

**Table D.1:** Blank samples did not contain any traces of PFAS substances except for PFBA and PFHxA. The levels were low and were not used to calculate LOQ.

| ID: | PFBA (pg) | S/N | PFHxA (pg) | S/N |
|---|---|---|---|---|
| A1-bl | 18.5 | 15.1 | 18.5 | 12.1 |
| A2-bl | 4.2 | 19.3 | 4.2 | 7.4 |
| A3-bl | 19.9 | 11.3 | 19.9 | 34.2 |
| B2-bl | 4.1 | NA | 4.1 | 5.7 |

* NA = not available

# APPENDIX E: LOD and LOQ FOR PFAS

**Table E.1:** Limit of detection (LOD, ng/g ww) calculated for analysis of PFAS in samples of fillet and liver from Arctic char (*Salvelinus alpinus*), from 2010 and 2013. LOD was calculated with 3xsignal to noise (S/N). S/N is the average of all S/N within each analyzed substance.

| Compound | LOD (ng/g ww) 2010 | LOD (ng/g ww) 2013 |
|---|---|---|
| 4:2 FTS | 0.04 | - |
| 6:2 FTS | 0.02 | 0.04 |
| PFOS(br) | - | 0.6 |
| PFOS(lin) | 0.2 | - |
| PFBA | 0.2 | 0.05 |
| PFPA | 0.08 | 0.02 |
| PFHxA | 0.02 | 0.005 |
| PFHpA | NA* | NA* |
| PFOA | NA* | NA* |
| PFNA | 0.005 | 0.01 |
| PFDcA | 0.03 | 0.04 |
| PFUnA | 0.009 | 0.02 |
| PFDoA | 0.005 | 0.005 |
| PFTrA | 0.004 | 0.01 |

-: Compound not detected in samples
* NA: S/N not available and therefore not calculated.

**Table E.2**: Limit of quantification (LOQ) was calculated 10xsignal-to-noise (S/N). S/N is the average of all S/N within each analyzed substance.

| Compound | LOQ (ng/g ww) 2010 | LOQ (ng/g ww) 2013 |
|---|---|---|
| 4:2 FTS | 0.1 | - |
| 6:2 FTS | 0.06 | 0.1 |
| PFOS(br) | - | 2.0 |
| PFOS(lin) | 0.6 | - |
| PFBA | 0.6 | 0.2 |
| PFPA | 0.3 | 0.07 |
| PFHxA | 0.07 | 0.02 |
| PFHpA | NA* | NA* |
| PFOA | NA* | NA* |
| PFNA | 0.02 | 0.04 |
| PFDcA | 0.1 | 0.1 |
| PFUnA | 0.03 | 0.07 |
| PFDoA | 0.02 | 0.02 |
| PFTrA | 0.01 | 0.03 |

-: Compound not detected in samples
* NA: S/N not available and therefore not calculated.

# APPENDIX F: RECOVERIES PFAS

**Table F.1:** Recoveries (%) measured for every internal standard added during the analysis of PFAS, in 2010 samples. Presented with Mean ± SD and Min-Max.

| Compound | Mean ± SD | Min-Max |
|---|---|---|
| 13C PFHxS | 60.1 ± 17.8 | 35.4-104.8 |
| 13C PFOS(lin) | 42.6 ± 14.0 | 13.5-70.4 |
| 13C PFBA | 34.8 ± 13.2 | 15.3-72.0 |
| 13C PFPA | 34.8 ± 13.2 | 15.3-72.0 |
| 13C PFHxA | 34.8 ± 13.2 | 15.3-72.0 |
| 13C PFOA | 47.7 ± 11.5 | 25.3-75.1 |
| 13C PFNA | 48.9 ± 27.0 | 14.2-107.3 |
| 13C PFDcA | 41.8 ± 8.6 | 22.3-56.2 |
| 13C PFUnA | 36.9 ± 12.1 | 18.4-68.7 |
| 13C PFDoA | 31.2 ± 12.4 | 12.3-58.8 |
| 13C PFOSA | 27.2 ± 22 | 6.6-84.3 |

**Table F.2:** Recoveries (%) measured for every internal standard added during the analysis of PFAS, in 2013 samples. Presented with Mean ± SD and Min-Max.

| Compound | Mean ± SD | Min-Max |
|---|---|---|
| 13C PFHxS | 67.2 ± 30.4 | 32.5-118.2 |
| 13C PFOS(lin) | 66.8 ± 30.4 | 38.5-136.7 |
| 13C PFBA | 85.4 ± 35.4 | 50.4-150.5 |
| 13C PFPA | 85.4 ± 35.4 | 50.4-150.5 |
| 13C PFHxA | 85.4 ± 35.4 | 50.4-150.5 |
| 13C PFOA | 67.0 ± 24.8 | 41.5-124.8 |
| 13C PFNA | 80.3 ± 44.8 | 26.5-169.0 |
| 13C PFDcA | 67.8 ± 35.0 | 28.6-137.0 |
| 13C PFUnA | 65.1 ± 36.0 | 24.0-138.4 |
| 13C PFDoA | 64.1 ± 33.7 | 35.9-131.7 |
| 13C PFOSA | 57.2 ± 17.8 | 29.7-91.9 |

# APPENDIX G: INDIVIDUAL PFAS CONCENTRATIONS

**Table G.1:** Individual PFAS concentrations (ng/g ww) measured in fillet samples from Arctic char (*Salvelinus alpinus*), September 2010. There were no PFAS compounds detected in the blank samples and therefore are not included in the table.

| PCA nr: | ID: | 4:2 FTS | 6:2 FTS | PFOS (lin) | PFBA | PFPA | PFHxA | PFHpA | PFOA | PFNA | PFDcA | PFUnA | PFdoA | PFTrA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | A1-1 | - | - | 0.6 | 1.6 | 0.3 | **0.02 | - | - | - | - | - | - | - |
| 20 | A1-2 | - | 0.008 | - | 2.0 | 0.5 | **0.02 | - | - | - | - | - | - | - |
| 21 | A1-3 | - | 3.3 | - | 1.8 | 0.5 | **0.02 | - | - | - | - | - | - | - |
| 22 | A2-1 | - | 0.2 | - | 0.8 | - | **0.03 | - | - | - | - | 0.2 | 0.03 | - |
| 23 | A2-2 | - | 0.1 | - | 1.2 | - | **0.02 | - | - | - | - | 0.03 | - | - |
| 24 | A3-1 | 0.2 | 0.2 | - | 3.2 | 0.8 | **0.04 | - | - | 0.03 | - | - | - | - |
| 25 | A3-2 | 0.4 | 0.2 | - | 2.9 | 0.7 | **0.02 | - | 0.05 | 0.05 | - | - | - | - |
| 26 | A4-2 | - | - | 0.3 | 1.9 | 0.3 | **0.03 | - | 0.1 | 0.2 | 0.2 | 0.3 | - | - |
| 27 | A5-1 | - | 1.6 | 0.06 | 3.8 | 1.8 | **0.03 | - | - | 0.02 | 0.2 | 0.1 | 0.05 | - |
| 28 | A5-2 | - | 1.7 | 4.5 | 3.9 | 2.7 | 0.4 | - | - | 0.04 | - | 0.1 | - | 0.05 |
| 29 | A6-1 | - | 0.7 | - | 2.2 | 0.4 | **0.04 | 0.04 | - | - | - | 0.09 | - | - |
| 30 | A6-2 | - | 0.2 | - | 1.7 | 0.3 | **0.01 | - | - | - | 0.01 | 0.04 | - | - |
| 31 | A7-1 | - | - | 0.04 | 0.8 | 0.4 | **0.05 | - | - | 0.09 | 0.4 | 0.2 | - | - |
| 32 | A7-2 | - | - | 0.09 | 1.2 | 1.0 | **0.01 | - | - | 0.04 | 0.1 | 0.5 | - | - |
| 33 | A8-1 | - | 0.09 | - | 1.7 | 0.1 | **0.02 | - | - | - | - | - | - | - |
| 34 | A8-2 | - | 0.3 | - | 2.0 | 0.2 | **0.03 | - | - | 0.07 | - | - | - | - |
| 35 | A10-2 | - | 0.02 | - | 2.4 | 1.3 | **0.1 | - | 0.05 | 0.1 | - | 0.1 | - | - |
| 36 | A11-1 | 0.3 | - | - | 7.1 | 1.8 | **0.04 | 0.03 | - | 0.23 | - | 0.3 | - | - |
| 37 | A12-1 | - | - | - | 8.3 | 2.1 | **0.02 | - | - | 0.06 | - | 0.1 | - | - |
| 38 | A13-1 | - | 0.03 | - | 2.1 | 1.7 | **0.03 | - | 0.02 | 0.06 | 0.1 | 0.3 | - | - |

*All samples are fillet. * -: Not detected

**: Concentrations detected <LOQ is set to 1/2LOQ, for further use in statistics.

**Table G.2:** Individual PFAS concentrations (ng/g ww) measured in fillet and liver samples from Arctic char (*Salvelinus alpinus*), March 2013. There were no PFAS compounds detected in the blank samples and therefore are not included in the table.

| PCA nr: | ID: | 6:2 FTS | PFOS (br) | PFOS (lin) | PFBA | PFPA | PFHxA | PFHpA | PFOA | PFNA | PFDcA | PFUnA | PFdoA | PFTrA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | B1-1 (F) | 0.2 | - | 0.09 | 0.2 | - | 0.008 | 0.01 | - | 0.05 | - | 0.03 | - | - |
| 2 | B1-2 (F) | 0.3 | - | - | 0.1 | - | **0.002 | - | - | - | - | 0.03 | - | - |
| 3 | B1-3 (L) | - | - | - | **0.06 | 0.09 | **0.003 | - | 0.04 | 1.2 | 1.1 | 1.8 | 0.1 | 0.2 |
| 4 | B2-1 (F) | 0.2 | - | - | 0.07 | - | **0.003 | - | - | - | - | 0.04 | - | - |
| 5 | B2-2 (F) | - | - | - | **0.02 | - | **0.002 | - | - | - | - | - | - | - |
| 6 | B2-3 (L) | 0.8 | - | - | **0.03 | 0.4 | 0.004 | 0.01 | - | - | 0.05 | 0.3 | 0.08 | 0.1 |
| 7 | B3-1 (F) | - | - | - | 0.09 | - | - | - | 0.03 | - | - | - | - | - |
| 8 | B3-2 (F) | - | - | - | 0.08 | 0.03 | 0.02 | - | 0.02 | - | - | 0.02 | - | 0.03 |
| 9 | B3-3 (L) | 5.3 | - | 0.2 | 0.4 | 0.2 | 0.03 | - | - | 0.06 | 0.4 | 0.7 | - | 0.2 |
| 10 | B4-1 (F) | - | - | - | 1.5 | - | 0.1 | - | - | - | - | - | - | - |
| 11 | B4-2 (F) | - | - | - | 0.1 | - | - | - | 0.04 | - | - | 0.2 | - | - |
| 12 | B4-3 (L) | - | 2.9 | - | 0.1 | 0.2 | 0.01 | - | - | 0.5 | 1.0 | 1.8 | 0.2 | 0.1 |
| 13 | B4-4 (L) | 0.005 | - | - | 0.2 | 0.3 | 0.02 | - | - | 0.3 | 0.9 | 1.4 | 0.3 | 0.3 |
| 14 | B5-1 (F) | - | - | - | **0.04 | - | - | - | - | - | - | 0.01 | - | - |
| 15 | B5-2 (F) | - | - | - | 0.1 | - | 0.02 | - | - | - | - | 0.01 | - | - |
| 16 | B5-3 (L) | - | - | - | 0.1 | 0.8 | **0.004 | - | - | 0.09 | 0.5 | 1.8 | - | 0.4 |
| 17 | B6-1 (F) | - | - | - | 0.05 | - | 0.008 | - | - | - | - | 0.03 | - | - |
| 18 | B6-2 (F) | - | - | - | 0.1 | - | 0.02 | - | - | - | 0.04 | - | - | - |

*F: Fillet *L: Liver * -: Not detected

**: Concentrations detected <LOQ is set to 1/2LOQ, for further use in statistics.

# APPENDIX H: PFAS CHROMATOGRAMS

The chromatograms are presented in figures H.1 to 5. The first figure shows the standards with concentration 2pg/µl, and the other figures show two samples from 2010.

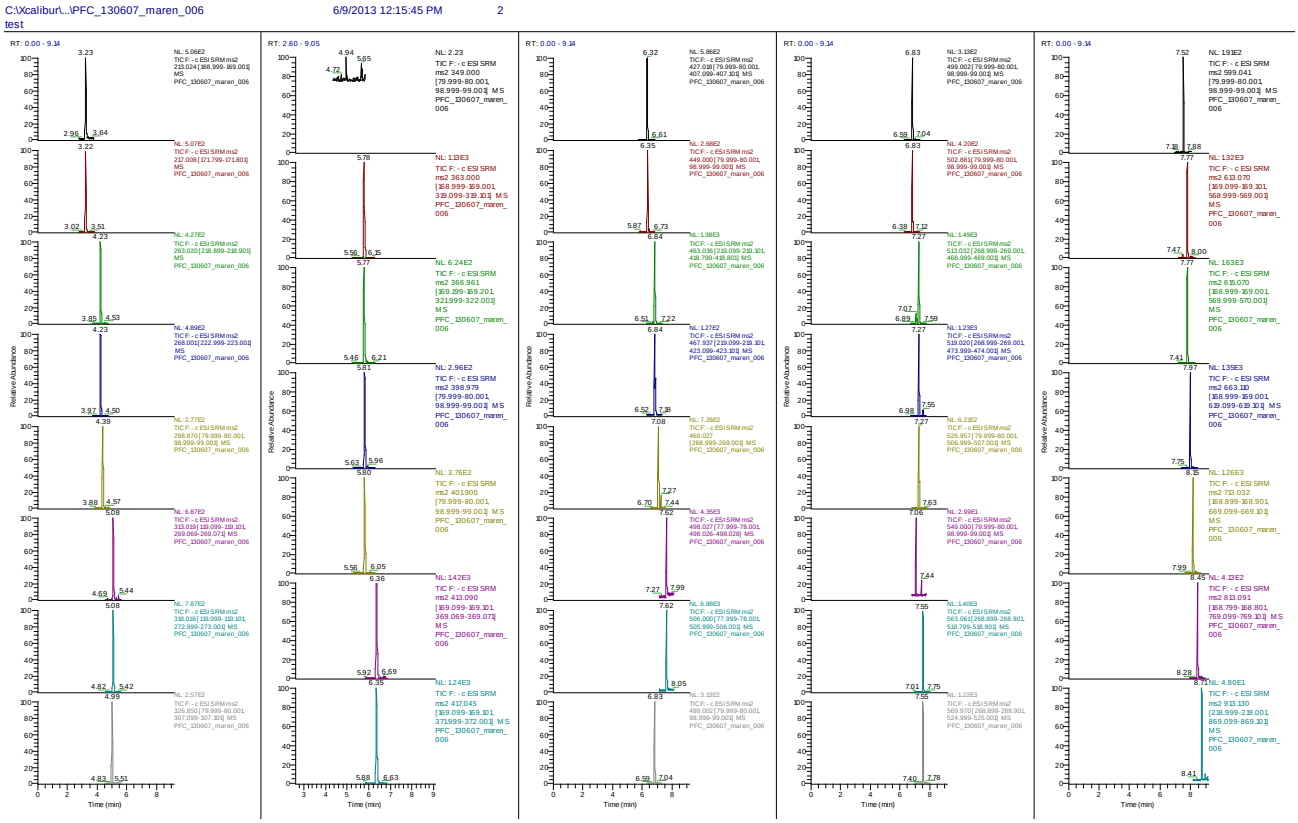


**Figure H.1:** Standards 2pg/µl

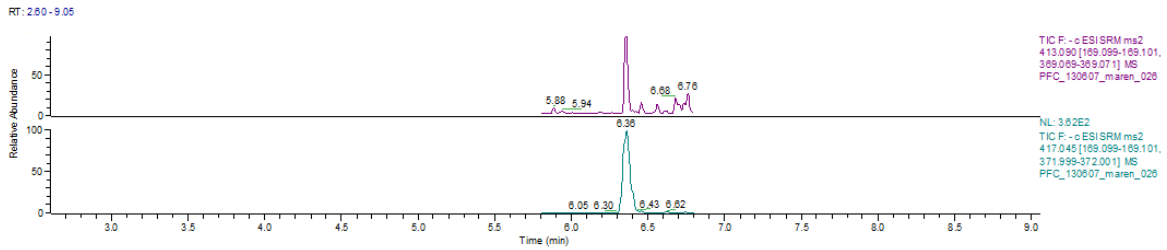


**Figure H.2:** Sample A4-2 presents mother ion of respectively $^{12}C$ PFOA and $^{13}C$ PFOA.

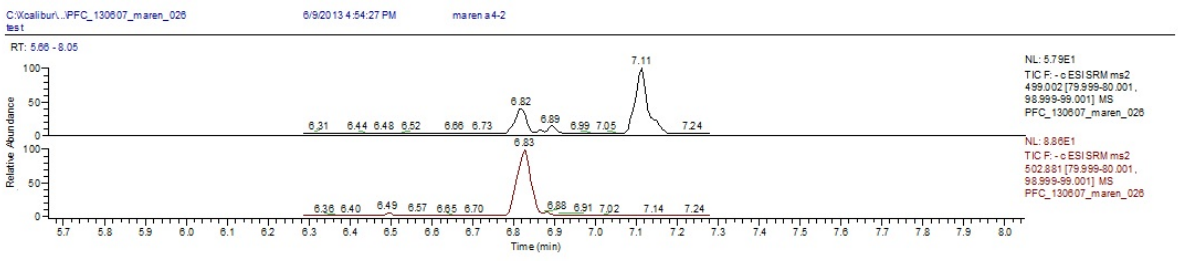


**Figure H.3:** Sample A4-2 presents mother ion of respectively $^{12}C$ PFOS and $^{13}C$ PFOS.

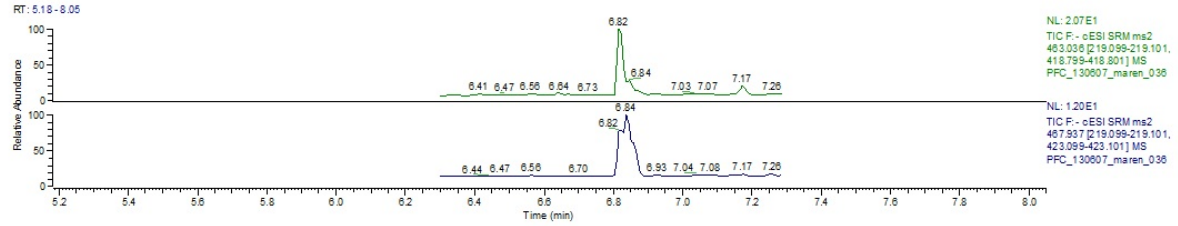



**Figure H.4:** Sample A10-2 presents mother ion of respectively $^{12}C$ PFNA and $^{13}C$ PFNA.

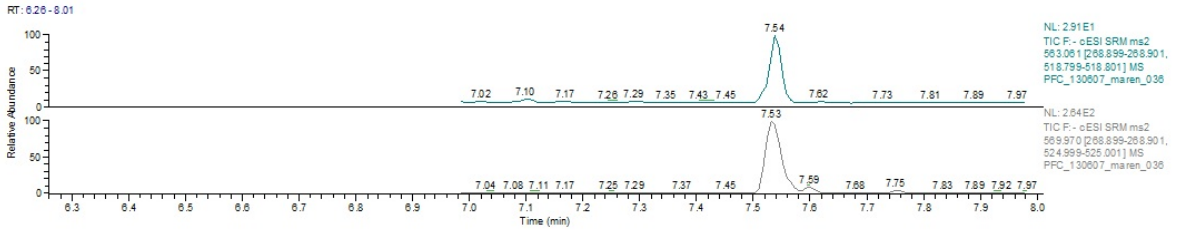


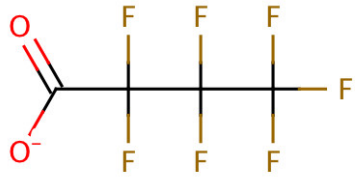

**Figure H.5:** Sample A10-2 presents mother ion of respectively $^{12}C$ PFUnA and $^{13}C$ PFUnA.

# APPENDIX I: DETAILS FOR UHPLC-MS/MS

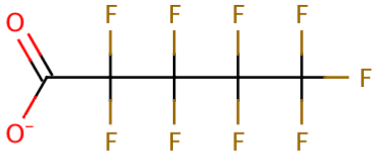
**Table I.1:** Presents an overview of parent ions, transitions, collision energies and S-lens conditions for the UHPLC-MS/MS. Q1 is the quantifier ion, and Q2 the qualifier ion (Hanssen et al. 2013).

| Analyte | Parent ion (m/z) | Transition 1 (m/z) (Q1) | Transition 2 (m/z) (Q2) | Collision energy (V) | S-lens (V) |
|---|---|---|---|---|---|
| **PFBA** | 213.02 | 169 | - | 11 | 39 |
| **PFPeA** | 263.02 | 218.9 | - | 7 | 43 |
| **PFBS** | 298.87 | 80 | 99 | 44 | 85 |
| **PFPS** | 349.00 | 80 | 99 | 44 | 85 |
| **PFHxA** | 313.02 | 269.07 | 119.10 | 25 | 43 |
| **PFHpA** | 363.00 | 319.1 | 169 | 18 | 43 |
| **PFHxS** | 398.98 | 80 | 99 | 45 | 86 |
| **PFOA** | 413.09 | 369.07 | 169.10 | 18 | 55 |
| **PFHpS** | 449.00 | 99 | 80 | 48 | 95 |
| **FOSA** | 498.03 | 78 | 498.03 | 43 | 124 |
| **PFOS** | 499.00 | 80 | 99 | 50 | 103 |
| **PFNA** | 463.04 | 418.80 | 219.10 | 18 | 68 |
| **PFDA** | 513.03 | 469 | 269 | 19 | 68 |
| **PFDcS** | 599.04 | 80 | 99 | 59 | 120 |
| **PFUnDA** | 563.06 | 518.80 | 268.90 | 18 | 78 |
| **PFDoDA** | 613.07 | 569 | 169.10 | 25 | 73 |
| **PFTrDA** | 663.11 | 619.1 | 169.0 | 28 | 85 |
| **PFTeDA** | 713.03 | 669.1 | 168.9 | 30 | 85 |
| | | | | | |
| **13C PFBA** | 217.01 | 171.80 | - | 11 | 39 |
| **13C PFHxA** | 315.02 | 273 | 119.10 | 25 | 43 |
| **13C PFHxS** | 402.98 | 102.98 | 83.90 | 45 | 86 |
| **13C PFOA** | 417.05 | 372 | 169.10 | 18 | 55 |
| **13C PFNA** | 467.94 | 423.10 | 219.10 | 6 | 68 |
| **13C PFOS** | 502.88 | 99 | 80 | 50 | 119 |
| **13C FOSA** | 506 | 78 | 506 | 43 | 124 |
| **13C PFDA** | 515.02 | 470 | 269 | 19 | 68 |
| **13C PFUnA** | 564.97 | 519.80 | 268.90 | 18 | 78 |
| **13C2 PFDoA** | 615.07 | 570 | 169 | 25 | 73 |

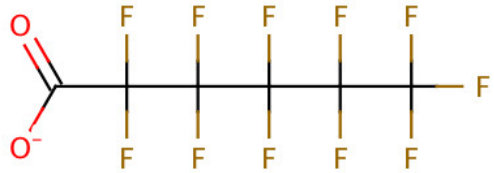
# APPENDIX J: PFAS CHEMICAL STRUCTURES



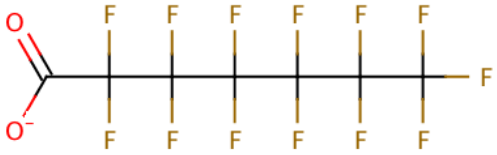
PFBA



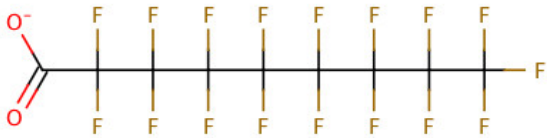
PFPA



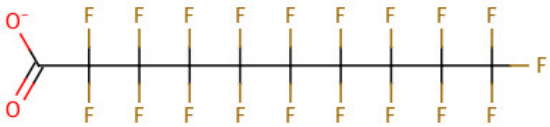
PFHxA



PFHpA



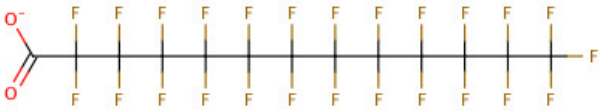
PFNA



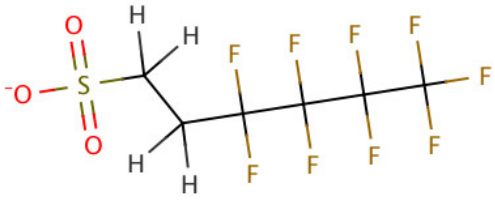
PFDcA

PFUnA

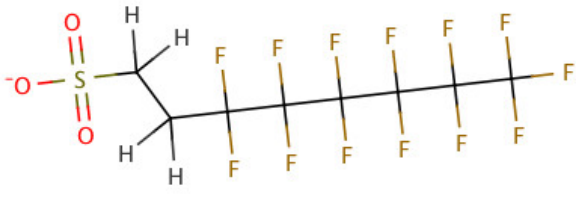
PFDoA



PFTrA



4:2 FTS



6:2 FTS