Stockholm University

Fluorine mass balance in wildlife and consumer products

How much organofluorine are we missing?

Lara Schultes

Doctoral Thesis in Applied Environmental Science at Stockholm University, Sweden 2019

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Academic dissertation for the Degree of Doctor of Philosophy in Applied Environmental Science at Stockholm University to be publicly defended on Friday 17 May 2019 at 10.00 in Nordenskiöldsalen, Geovetenskapens hus, Svante Arrhenius väg 12.

Abstract

Per- and polyfluoroalkyl substances (PFASs) are a class of anthropogenic pollutants. Many PFASs are highly persistent and have been linked to adverse effects in humans. According to latest estimates, there are more than 4700 PFASs in global commerce, which poses immense challenges for environmental monitoring. This thesis aims at the development, validation and application of total fluorine (TF) and extractable organic fluorine (EOF) methods to consumer products and wildlife in order to estimate the fraction of unidentified organic fluorine in these samples via fluorine mass balance calculations.

Fluoropolymer-coated food packaging materials and reference materials were used in **paper I** to validate and compare the performance of three different TF methods. Combustion ion chromatography (CIC), particle-induced gamma ray emission spectroscopy (PIGE) and instrumental neutron activation analysis (INAA) revealed excellent analytical agreement and precision under most circumstances. PIGE and INAA had the advantage of being non-destructive, while CIC was favored due to low detection limits. Fluorine mass balance experiments indicated large amounts of unidentified EOF and non-extractable fluorine. **Paper II** investigated the occurrence of PFASs, EOF and TF in cosmetic products from the Swedish market. In addition to extremely high concentrations (up to 470 µg/g) of polyfluoroalkyl phosphate diesters (diPAPs; perfluoroalkyl acid (PFAA) precursors), unintentionally-added PFAAs were found in a number of products, together with large amounts of unidentified organic fluorine. Human exposure estimates for perfluorooctanoate (PFOA) using the latest dermal uptake coefficients revealed that PFAA exposure via cosmetics may be significant. **Paper III** evaluated time trends of PFASs, EOF and TF in Baltic cod (*Gadus morhua*) from 1981 to 2013. Increasing trends were observed for the predominant PFAS perfluorooctane sulfonate (PFOS), as well as for C9-C12 perfluoroalkyl carboxylic acids (PFCAs) at rates of up to 7.7% per year. Declining concentrations were detected for the PFOS precursor perfluorooctane sulfonamide (FOSA), the EOF and its fraction not accounted for by target PFASs, while TF did not show any significant trends. The increasing concentrations of PFAAs despite their production phase-out could be attributed to either direct exposure of cod to legacy PFAAs or to indirect exposure via PFAA-precursor metabolism. Furthermore, negative correlations of certain PFASs with liver somatic index and body length were observed, which play an important role in the under-standing of toxicological effects of PFASs on wildlife. **Paper IV** studied the distribution of PFASs, EOF and TF in tissues from a Greenland killer whale (*Orcinus orca*). The sum of target PFAS concentrations was highest in liver (339 ng/g) and lowest in blubber (9.4 ng/g), consistent with other tissue distribution studies in marine mammals. In contrast, TF and EOF concentrations were highest in blubber (1315 and 229 ng/g, respectively), suggesting the presence of high concentrations of one or more presently unidentified fluorinated compounds. With the help of high resolution mass spectrometry-based suspect screening, several PFAS homologue series and individual PFASs not included in target analysis were detected.

Keywords: *PFASs, PFOS, fluorine mass balance, organofluorine, EOF, TF, wildlife, consumer products, combustion ion chromatography, high resolution mass spectrometry, suspect screening.*

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Every act of creation is first an act of destruction.

Pablo Picasso

Table of contents

Abstract

Per- and polyfluoroalkyl substances (PFASs) are a class of anthropogenic pollutants. Many PFASs are highly persistent and have been linked to adverse effects in humans. According to latest estimates, there are more than 4700 PFASs in global commerce, which poses immense challenges for environmental monitoring. This thesis aims at the development, validation and application of total fluorine (TF) and extractable organic fluorine (EOF) methods to consumer products and wildlife in order to estimate the fraction of unidentified organic fluorine in these samples via fluorine mass balance calculations.

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Sammanfattning

Per- och polyfluorerade ämnen (PFAS) är en grupp antropogena kemikalier. Många PFASs är persistenta i miljön och har visat sig ge upphov till toxikologiska effekter i människan. Enligt de senaste uppskattningarna finns det mer än 4700 olika PFAS i global handel, vilket utgör en stor utmaning för miljöövervakning. Denna avhandling omfattar utvecklingen, valideringen och tillämpningen av metoder för mätningen av totalt fluor (TF) och extraherbart organiskt fluor (EOF) i konsumentvaror och djur. Syftet med dessa metoder är att uppskatta andelen av de fluorerade ämnena som är oidentifierade i dessa prover, med hjälp av fluor-massbalans beräkningar.

Artikel I hade för avsikt att jämföra och validera tre olika TF-metoder genom att mäta fluor halterna i referensmaterial och fluoropolymerbelagda livsmedelsförpackningar. Förbränningsjonkromatografi (CIC), partikelinducerad gammastrålningsspektroskopi (PIGE) och neutronaktiveringsanalys (INAA) visade i de flesta fall en utmärkt analytisk konsistens och precision. PIGE och INAA hade fördelen att de är icke-destruktiva, men CIC föredrogs på grund av sina låga detekteringsgränser. Fluormassbalansförsök avslöjade stora mängder oidentifierad EOF och icke-extraherbar fluorade ämnen i alla undersökta livsmedelförpackningar. Artikel II undersökte förekomsten av PFAS, EOF och TF i olika kosmetiska produkter. Förutom extremt höga koncentrationer (upp till 470 µg/g) av polyfluoralkylfosfatdiester (diPAPs; som kan brytas ner till de persistenta perfluoroalkyl syror (PFAA)) i ett antal produkter, så hittades också oavsiktligt tillsätta PFAAs och stora mängder av oidentifierat organiskt fluor. Uppskattningar av människors exponering för perfluoroktansyra (PFOA) med absorptionskoefficientern från litteraturen visade att PFAA upptag för kosmetika genom huden kan vara betydande. Artikel III handlade om tidstrender av PFASs, EOF och TF i torsk från Östersjön (*Gadus morhua*) från 1981 till 2013. Koncentrationer av perfluoroktansulfonat (PFOS) och för C9-C12-perfluoralkyl karboxylsyror (PFCAs) ökade med upp till 7,7% per år. Minskande koncentrationer upptäcktes för PFOS-prekursorn perfluoroktansulfonamid (FOSA), EOF och andelen oidentifierade EOF. Med tanke på att de flesta PFAAs har fasats ut under de sista två årtiondena, så kan deras ökande koncentrationer antingen bero på en direkt exponering för torskar av PFAAs eller på indirekt exponering via ämnen som kan metaboliseras till PFAAs. Dessutom observerade vi negativa korrelationer av vissa PFASs med lever-somatiskt index och kroppslängd, vilka båda spelar en viktig roll i förståelsen av de toxikologiska effekter PFASs har på djur. I artikel IV undersöktes fördelningen av PFASs, EOF och TF i olika vävnader av en späckhuggare (*Orcinus orca*) från östra Grönland. PFAS-koncentrationer var högst i levern (339 ng/g) och lägst i valspäck (9,4 ng/g), vilket överensstämmer med andra studier på vävnadsfördelningar i marina däggdjur. I kontrast så visade valspäck de högsta koncentrationer av TF och EOF (1315 och 229 ng/g), vilket indikerar närvaron av höga koncentrationer av en eller flera ännu inte identifierade fluorerade ämnen. Med hjälp av högupplöst masspektrometriskt baserad undersökning hittades flera olika PFASs som inte var med i den kvantitativa analysen.

Zusammenfassung

Per- und polyfluorierte Alkylverbindungen (PFASs) sind eine Gruppe von anthropogenen Schadstoffen. Viele PFASs sind äußerst stabil, reichern sich in der Umwelt, Tieren und Menschen an und stehen unter anderem im Verdacht krebserregend zu sein. Nach neusten Schätzungen gibt es mehr als 4700 PFAS im globalen Handel, was eine große Herausforderung für Umweltforscher darstellt, da nur wenige von ihnen routinemäßig gemessen werden. Diese Doktorarbeit behandelt die Entwicklung und Validierung von Messmethoden für Totalfluor (TF) und extrahierbares organisches Fluor (EOF) und deren Anwendung auf Verbraucherprodukte und Wildtiere. Beim Vergleich der direkten PFAS-Analyse, EOF und TF können der Anteil unbekannter organischer Fluorverbindungen und nicht extrahierbarem Fluors abgeschätzt werden.

In **Artikel I** wurden drei verschiedene TF-Methoden (Verbrennungsionenchromatographie (CIC), partikelinduzierte Gammastrahlen-Emissionsspektroskopie (PIGE) und instrumentelle Neutronenaktivierungsanalyse (INAA)) anhand von Lebensmittelverpackungen und Referenzmaterialien verglichen und validiert. In den meisten Fällen zeigten alle drei Methoden ausgezeichnete analytische Übereinstimmung und Präzision. PIGE und INAA hatten den Vorteil zerstörungsfrei zu sein, während CIC die niedrigsten Nachweisgrenzen aufwies. Zudem wurden durch die zusätzliche Analyse von EOF und PFASs große Mengen an unbekanntem EOF und nicht extrahierbarem Fluor nachgewiesen. In **Artikel II** wurden verschiedene Kosmetika vom schwedischen Markt auf deren PFAS, EOF und TF Konzentrationen untersucht. Neben Polyfluoralkylphosphatdiestern (diPAPs), die als Inhaltsstoffe auf einigen Produkten aufgeführt waren, wurden auch unbeabsichtigt zugesetzte Perfluoralkylsäuren (PFAAs) sowie große Mengen an unbekanntem EOF in vielen Produkten nachgewiesen. Berechnungen der Aufnahme von Perfluoroctansäure (PFOA) über die Haut haben ergeben, dass die regelmäßige Anwendung bestimmter Produkte einen signifikanten Beitrag zur Gesamtbelastung des menschlichen Körpers haben kann. In **Artikel III** wurden die zeitlichen Trends (1981-2013) von PFASs, EOF und TF in Dorschlebern (*Gadus morhua*) von der Ostsee untersucht. 10 verschiedene PFASs wurden detektiert, von denen Perfluoroctansulfonsäure (PFOS) in höchsten Konzentrationen vorkam. Konzentrationen von PFOS und Perfluoralkylcarbonsäuren (C9-C12) nahmen mit bis zu 7,7% pro Jahr zu, trotz deren Produktionsstop im Jahr 2000. Konzentrationen von Perfluoroctansulfonamid, EOF und unbekanntem EOF nahmen ab, wogegen TF keine signifikanten Trends zeigte. Die ansteigenden Konzentrationen von den PFAAs können entweder auf direkte PFAA-Belastung der Dorsche oder auf indirekte Belastung durch Vorläufersubstanzen, die zu PFAAs abgebaut werden können, verursacht werden. Darüber hinaus wurden negative Korrelationen bestimmter PFASs mit dem lebersomatischen Index und der Körperlänge beobachtet, die eine wichtige Rolle beim Verständnis von toxikologischen Effekten von PFASs auf Tiere spielen. **Artikel IV** untersuchte die Verteilung von PFASs, EOF und TF zwischen verschiedenen Geweben eines Schwertwals (*Orcinus orca*) aus Grönland. Die Summe aller PFAS-Konzentrationen war am höchsten in der Leber und am niedrigsten im Walspeck, was mit anderen Gewebeverteilungsstudien bei Säugetieren übereinstimmt. Im Gegensatz dazu waren die TF- und EOF-Konzentrationen am höchsten im Walspeck, was auf das Vorhandensein von unbekannten fluorierten Verbindungen hinweist. Mit hochauflösender Massenspektrometrie wurden mehrere fluorierte Verbindungen qualitativ nachgewiesen, die nicht in der PFAS-Analyse mit einbegriffen waren.

List of publications

This doctoral thesis consists of a summary and the four articles listed below:

I Lara Schultes, Graham F. Peaslee, John D. Brockman, Ashabari Majumdar, Sean R. McGuinness, John T. Wilkinson, Oskar Sandblom, Ruth A. Ngwenyama, Jonathan P. Benskin: **Total fluorine measurements in food packaging: How do current methods perform?** *Environmental Science and Technology Letters 2019, 6 (2), 73-78. Reprinted with permission from American Chemical Society.*

II Lara Schultes, Robin Vestergren, Kristina Volkova Hellström, Emelie Westberg, Therese Jacobson, Jonathan P. Benskin: **Per- and polyfluoroalkyl substances and fluorine mass balance in cosmetic products from the Swedish market: Implications for environmental emissions and human exposure.** *Environmental Science: Processes & Impacts 2018, 20 (12), 1680-1690. Reprinted with permission from Royal Chemical Society.*

III Lara Schultes, Oskar Sandblom, Katja Broeg, Anders Bignert, Jonathan P. Benskin: **Temporal trends (1981-2013) of per- and polyfluoroalkyl substances and unidentified organofluorine in Baltic cod (*Gadus morhua*).** *Submitted Manuscript*.

IV Lara Schultes*, Carmen van Noordenburg*, Kyra Spaan, Merle Plassmann, Anna Roos, Jonathan P. Benskin: **High concentrations of unidentified organofluorine observed in blubber from a Greenland killer whale (*Orcinus orca*).** *Submitted Manuscript.*

*Shared first authorship.

Author's contributions

I Designed and coordinated the study; performed extractions, CIC and LC-MS/MS analysis and data processing; and took a lead role in writing the manuscript.

II Participated in study design; performed lab work, sample analysis and data processing; and took a lead role in writing the manuscript.

III Participated in study design; performed parts of the lab work and LC-MS/MS analysis; performed CIC analysis and data processing; and took a lead role in writing the manuscript.

IV Participated in study design, analysis and data processing; performed parts of CIC analysis; processed HRMS data; and took a lead role in writing the manuscript.

Abbreviations

General

AOF	Adsorbable organic fluorine
CIC	Combustion ion chromatography
EOF	Extractable organic fluorine
IC	Ion chromatograph/chromatography
ICP-MS	Inductively-coupled plasma mass spectrometry
IF	Inorganic fluorine
INAA	Instrumental neutron activation analysis
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LRT	Long range transport
LSI	Liver somatic index
OECD	Organization for Economic Co-operation and Development
PIGE	Particle induced gamma ray emission spectroscopy
TF	Total fluorine
(U)HRMS	(Ultra) high resolution mass spectrometry

Per- and polyfluoroalkyl substances

diPAP	Polyfluoroalkyl phosphoric acid diester
FASA	Perfluoroalkane sulfonamide
FTCA	Fluorotelomer carboxylic acid
FTOH	Fluorotelomer alcohol
monoPAP	Polyfluoroalkyl phosphoric acid monoester
PFAA	Perfluoroalkyl acid
PFAS	Per- and polyfluoroalkyl substance
PFCA	Perfluoroalkyl carboxylic acid
PFOA	Perfluorooctanoate
PFSA	Perfluoroalkane sulfonic acid
PTFE	Polytetrafluoroethylene

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To all current and former members of ITM/ACES, thanks for creating such a great atmosphere around here. What a pleasure it has been to be part of the PAX group: Tack, Mikke and Oskar for all your technical support, from finding the right screwdriver to fixing instruments. Anne-Sofie, tack att du är en så underbar person. Merle, thanks for always having a good advice, and for knowing how I truly feel about the CIC. Thanks Raed for your kind soul and always having an open ear. Anton, thanks for always spreading positivity. Robin, it has been a pleasure working with you! Bo, you are so inspirational and I'm hoping for some upcoming bike adventures. Mafalda, thanks for sweaty Mondays, long Fridays and so much more. Peanutbutter oreo-gang, Joo Hui, Ping Ping, Kyla and Vera, you were the true highlights of my conference trips. Thanks to everyone in the TESLA group for the awesome time in Aberdeen and for showing me how to appreciate whisky and rain. Big thanks to the running group, gym buddies and dance community for training your brains with me! To the power girls Kim, Melissa, Kerstin, Lisa, Cat, Berit and Maria, for sticking together, for all the fun, motivation, pancakes and for friendship! Marko, Dimi, Stathis and Li. How is it possible that I had the best officemates ever? Thanks for coping with the bikes and myriad of questions, for all the fun and conversations and for sharing the stories of your lives with me.

This would all not have been possible without the love and support from my dearest friends and family. This page is not large enough to express my love and gratitude to you, so I will find other ways.

There are so many more of you that shared a part of this Moebius roller coaster ride with me and created unforgettable memories, and I am so thankful to have met you all.

1 Introduction

1.1 Per- and polyfluoroalkyl substances

Per- and polyfluoroalkyl substances (PFASs) comprise a unique class of anthropogenic chemicals (Figure 1). Their common feature, the perfluoroalkyl moiety C_nF_{2n+1}, imparts specific properties. Firstly, the carbon-fluorine bond has the highest bond dissociation energy of all single carbon bonds (*Lemal*, *2004*), leading to high thermal and chemical stability and thereby environmental persistence of organofluorine molecules. Secondly, as the most electronegative element, fluorine displays low polarizability and a small Van der Waals radius (*O'Hagan*, *2008*). Perfluoroalkyl chains therefore exhibit weak intermolecular dispersion forces causing simultaneous hydrophobicity and lipophobicity (*Kissa*, *2001*). This water- and oil repellency contrasts other, mainly lipophilic, polyhalogenated compounds. Lastly, PFASs possess the ability to reduce surface tension lower than hydrocarbon surfactants (*Kissa*, *2001*). Understandably, PFASs have found innumerous uses in industrial processes and consumer products, including non-stick coatings, water- and stain proof coatings of textiles and papers, pharmaceuticals, pesticides, fire-fighting foams, metal plating, cosmetics, solvents and in medical and electrical devices (*KEMI, 2015*).

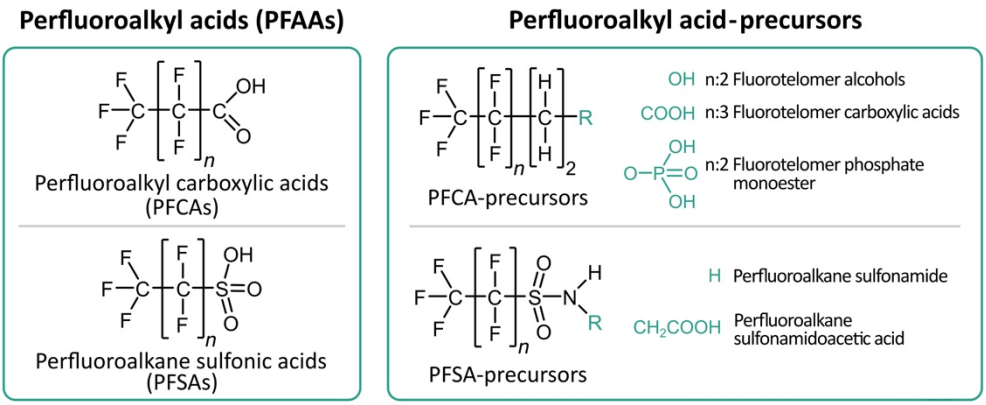

Figure 1. Chemical structures of selected per- and polyfluoroalkyl substances.

The extensive use and production of PFASs since the 1950s has led to their widespread occurrence in the environment and biota (*Giesy and Kannan*, *2001*; *Houde et al.*, *2006*). The most commonly detected are the perfluoroalkyl acids (PFAAs), which have been used commercially and are also persistent degradation products of so-called PFAA-precursors (*Wang et al.*, *2014*). PFAA-precursors, such as fluorotelomer alcohols (FTOHs) and perfluoroalkyl sulfonamides (FASAs) can degrade in the environment or be metabolized to form the ultimately persistent PFAAs (*Ellis et al.*, *2004*; *Galatius et al.*, *2013*). Since the first report on their global occurrence in wildlife (*Giesy and Kannan*, *2001*), PFAAs have been detected in humans (*Hansen et al.*, *2001*), aquatic and arctic wildlife (*Houde et al.*, *2006*; *Smithwick et al.*, *2005*), the terrestrial food web (*Müller et al.*, *2011*), and globally in seawater (*Benskin et al.*, *2012*). Besides emissions from production plants to air and water or from use of PFAS-containing fire-fighting foams to soils and groundwater, PFASs can also enter the environment via emissions from the use and disposal of products through waste water treatment plants and landfills.

There are several factors that facilitate and contribute to the global distribution of PFASs. PFASs can undergo long-range transport (LRT) via the atmosphere and the oceans (*Stock et al.*, *2007*; *Zhao et al.*, *2012*), leading to their occurrence in remote areas far from point sources (*Schiavone et al.*, *2009*). Humans and wildlife are exposed to PFAAs mainly through their diet (*Gebbink et al., 2015*), and can accumulate in biota due to their partitioning to phospholipids and binding to plasma proteins (*Han et al.*, *2003*; *Hebert and MacManus-Spencer*, *2010*). The bioaccumulation potential of PFAAs varies with species, tissue, chain-length and head group. Several adverse effects of wildlife exposure to PFASs have been reported as reviewed by Ahrens et al. (*2014*). Epidemiological studies suggest that PFASs can affect immune function, metabolic outcomes and neurodevelopment as well as lead to various forms of cancer, as reviewed by Sunderland et al. (*2019*).

Due to concerns over their adverse effects in the environment, several initiatives have been taken over the past two decades in order to reduce emissions of long-chain PFAAs. Starting in the year 2000, 3M voluntarily phased-out the production of PFOS and related substances (*3M Company, 2000*). In 2006, the United States Environmental Protection Agency launched a global stewardship program according to which several major PFASs manufacturers committed to eliminate production of PFOA and PFOA-precursors by 2015 (*US EPA, 2006*). In 2009, PFOS, its salts and perfluorooctane sulfonyl fluoride (POSF) were added to Annex B of the Stockholm Convention on Persistent Organic Pollutants to restrict their production and use (*Stockholm Convention*, *2009*). The European Chemicals Agency (ECHA) added perfluorooctanoate (PFOA) to Annex XVII of REACH in order to restrict production and use in Europe as of 2020 (*ECHA, 2013*). As a response to the reduced production of long-chain PFAAs, the fluorochemical industry has shifted production towards shorter chain homologues and replacement products featuring for example ether bonds, which are supposed to aid environmental degradation and reduce bioaccumulation potential (*Ritter*, *2011*). However, many of these fluorinated alternatives display undesirable properties, including extreme

persistence, and have been detected close to point sources and the environment (*Gomis et al., 2018*, *2015*; *Wang et al., 2013*). For example, the PFOA alternative GenX has been detected in high concentrations in river water and drinking water near a fluorochemical production plant in the Netherlands (*Gebbink et al., 2017*) and the US (*Sun et al., 2016*). Furthermore, novel classes of PFASs have been discovered in fluorinated AFFF formulations including ultra-short chain PFASs (C2-C3 PFSAs) (*Barzen-Hanson and Field*, *2015*) and zwitterionic surfactants (*Backe et al., 2013*).

The OECD recently released a list of PFASs in global commerce, which included 4730 individual CAS numbers (*OECD, 2018*). This immense number of structurally diverse chemicals poses a considerable challenge to the analysis of PFASs in consumer products and environmental samples. For example, the most comprehensive targeted methods are capable of measuring up to approximately 70 individual PFASs (*Yeung et al., 2016*), but more commonly around 20 are included in environmental monitoring programs. The large discrepancy between the number of PFASs being manufactured and the number that are monitored in the environment suggests that environmental exposure to PFASs may be severely underestimated. To address this concern, several analytical techniques have emerged over the last decades aimed at quantifying the total fluorine content of a given sample regardless of the organic or inorganic form of fluorine, in order to estimate the fraction of unidentified organic fluorine present in a sample.

1.2 Overview of total fluorine methods

Early approaches to measure total fluorine were mainly based on sample combustion, such as the oxygen bomb technique (*Belisle*, *1981*), the Wickbold combustion method (*Sweetser*, *1956*) or the Schöniger flask method (*Schöniger*, *1956*). Since then, more robust and sensitive methods have evolved together with increasing scientific interest in total fluorine and organofluorine measurements.

Instrumental neutron activation analysis (INAA) and particle-induced gamma ray emission spectroscopy (PIGE) are two particle beam methods that have been used for total fluorine determination for many decades (*Roelandts et al., 1986*; *van Zanten et al., 1963*), with more recent applications to human tissues (*Carioni et al., 2018*) and consumer products (*Ritter et al., 2017*), respectively. Combustion ion chromatography (CIC) has been an established method for determination of sulfur and halogens other than fluoride before its first application to total fluorine measurements in seawater in 2007 (*Miyake et al., 2007a*). CIC has since been applied to a range of environmental matrices and biota including human serum (*Miyake et al., 2007b*), fish (*Yeung & Mabury, 2013*), cetaceans (*Yeung et al., 2009*), soil (*Tan et al., 2014*), sediment (*Codling et al., 2014*; *Yeung et al., 2013*) and freshwater (*Wagner et al., 2013*). Analytical techniques that have been less frequently used for total fluorine determination are nuclear magnetic resonance (NMR) (*Ellis et al., 2000*; *Moody et al., 2001*) and continuum-source molecular absorption spectrometry (CS-MAS) (*Qin et al., 2013*, *2012*).

Other novel TF techniques have emerged in the recent years. Jamari et al. (*2018*) developed an ICP-MS method using in-source barium adduct ions to measure total fluorine. However, this method required sample extraction, and is thereby limited to measurement of extractable organic fluorine. Tokranov et al. (*2018*) reported the use of X-ray photoelectron spectroscopy (XPS) for surface measurements of fluorine. In contrast to the previously described methods, it is possible to distinguish between organic and inorganic fluorine using XPS due to their different bond energies. However, XPS has a high instrumental limit of detection (1% fluorine) and is selective for only non-volatile substances; therefore, its application is largely restricted to consumer product screening.

1.3 The fluorine mass balance concept

With the help of fluorine mass balance experiments, one can classify fluorinated compounds into different categories, as depicted in Figure 2. Total fluorine (TF) comprises the sum of all fluorinated compounds in a neat sample irrespective of their organic or inorganic nature. Dependent on the sample type, a certain fraction of the TF can be extracted using organic solvents (extractable organic fluorine, EOF). Alternatively, aqueous samples can be extracted using a sorbent, which is then subjected to fluorine analysis (adsorbable organic fluorine, AOF, not shown in Figure 2). The residues from the extraction or adsorption procedures contain the so-called non-extractable organic fluorine (NEOF) and inorganic fluorine (IF). TF, EOF, AOF and NEOF can be quantified by measuring the neat sample, extract, sorbent and sample residue respectively, using total fluorine methods as described above.

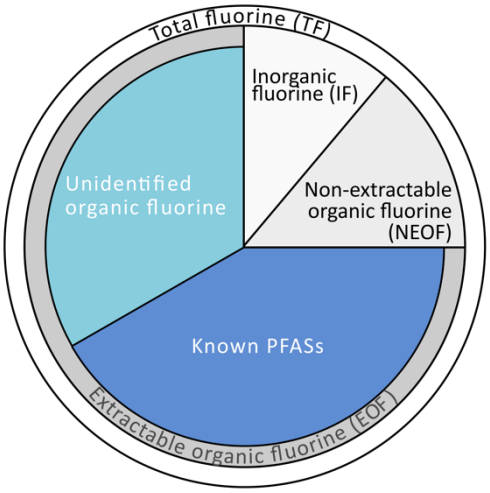

Figure 2. Fluorine mass balance concept.

The EOF (or AOF) fraction of a sample can be assumed to contain primarily anthropogenic fluorinated compounds due to the rare occurrence of natural organic fluorinated compounds (*Gribble*, *2015*). It can be further divided into the fraction of known fluorinated substances as identified and quantified by compound-specific analysis (LC-MS/MS and/or GC-MS) and the remaining fraction of unidentified organic fluorine. The composition of the latter can be tackled using non-target analysis or suspect screening using high resolution mass spectrometers.

1.4 Previous fluorine mass balance studies

Several studies have used fluorine mass balance experiments in order to estimate the amount of unidentified EOF in environmental and biotic samples, of which a selection is highlighted in the following section.

The first comprehensive fluorine mass balance studies were published by Miyake et al. with applications to water samples (*Miyake et al., 2007a*) and human blood (*Miyake et al., 2007b*). Therein, samples were subjected to ion-pair extraction and fractionation procedures, and quantified for known PFASs, EOF, TF and IF. Known PFASs accounted for less than 2% of the EOF in seawater samples and more than 30% in contaminated water; while the percentage of EOF accounted for by known PFASs in human blood was on average more than 80%. Loi et al. (*2011*), studied the trophic magnification of PFASs in a subtropical food web, including EOF and TF analysis for a subset of the samples. The analysis of PFASs and EOF in gastropods, worms and shrimps revealed that the contribution of known PFASs to EOF increased with the trophic level and the authors hypothesise that the unidentified EOF might comprise PFAS intermediates or metabolites with the potential to biotransform to PFAAs. Surface sediments analysed by Yeung et al. (*2013*) showed increasing amounts of unidentified EOF in recent years, with known PFASs accounting for 2-44% of the anionic EOF fraction.

Robel et al. (*2017*) published the first application of fluorine mass balance experiments to consumer products. Papers and textiles were analysed by PIGE for TF, LC-MS/MS and GC-MS for ionic and volatile PFASs, as well as by the total oxidizable precursor (TOP) assay. The sum of known PFASs and potential PFAA-precursors accounted for most of the EOF, which was determined indirectly by TF measurements pre- and post-extraction.

2 Hypothesis and objectives

The underlying hypothesis of this thesis is that the PFASs currently monitored in the environment only make up a small fraction of anthropogenic releases of organofluorines. Therefore, the overarching objectives of this thesis were to:

(i) Determine the occurrence of known PFASs in consumer products and selected wildlife species;
(ii) Develop and validate multi-matrix methods for total fluorine and extractable organic fluorine determination;
(iii) Quantify the fraction of unknown fluorine in all samples, which is not accounted for by target PFASs; and
(iv) Identify novel PFASs using high resolution mass spectrometry.

These objectives were addressed by papers I-IV appended to this summary. The individual aims of the four papers were as follows:

Paper I aimed to compare and validate three different total fluorine methods (CIC, PIGE and INAA), and demonstrate their advantages and limitations. To further estimate the occurrence of PFASs in food packaging materials, we carried out a fluorine mass balance combining target analysis of PFASs with TF and EOF measurements.

Paper II aimed at characterizing PFASs, and the fractions of TF and EOF accounted for by known PFASs, in a range of cosmetic products containing intentionally-added fluorinated substances. We further estimated human exposure to PFOA from applying these products to the surface of the skin.

The aim of Paper III was to analyze temporal trends of PFASs, EOF and TF in Baltic Cod (*Gadus morhua*) from 1981-2013 to assess the effects of the manufacturing phase-out on both known and unknown organofluorine. We further aimed to examine correlations between PFAS concentrations and biological variables.

Paper IV aimed at assessing whether the tissue distribution of TF and EOF matched that of known PFASs in a killer whale (*Orcinus orca*) from Greenland. HRMS-based non-target and suspect screening was also included in order to elucidate the structure of PFASs not included in targeted analysis.

3 Experimental section

3.1 Selection of samples

The sample selection strategy aimed to cover a broad spectrum of sample types, from consumer products containing intentionally-added PFASs (**papers I-II**), to predators exposed to PFASs occurring in the environment (**papers III-IV**).

For **paper I** a range of food packaging materials suspected to contain fluorinated coatings (microwave popcorn bags and French fry containers) from local fast food restaurants and grocery stores were selected. Food packaging materials have received regulatory attention and a TF-based guidance value has been introduced by the Danish Veterinary and Food Administration (*Ministry of Environment and Food of Denmark*, *2018*). Therefore, it is crucial to demonstrate the accuracy and comparability of different TF methods.

Despite the fact that cosmetic products have been known to contain PFASs (*KEMI, 2015*), their occurrence and dermal absorption has been scarcely studied. Therefore, we selected a variety of products from different product categories (creams, foundations, powders, pencil and shaving foam), with and without PFASs as listed ingredients for analysis in **paper II**. Sample selection was based on a database containing about 3000 cosmetic products from the Swedish market compiled by the Swedish Society for Nature Conservation (SSNC).

For **paper III**, Baltic cod liver samples were obtained from the Environmental Specimen Bank of the Swedish Museum of Natural History (NRM). The time span of the samples (1981-2013) covers the period during which important regulatory actions were taken to reduce PFAS emissions, such as the voluntary phase-out of PFOS by 3M in 2000 and the addition of PFOS to the Stockholm Convention in 2009.

Samples for **paper IV** were obtained in collaboration with the Greenland Institute of Natural Resources, who carried out subsampling of eight different tissues (liver, blood, kidney, lung, ovary, blubber, skin and muscle) of a female killer whale from East Greenland in 2017. Killer whales are top predators and therefore sentinel species of arctic contamination.

3.2 Sample preparation

The sample preparation procedures for **papers I-IV** followed a general scheme, where each sample was treated in three different ways (Figure 3). The following paragraphs describe each sample preparation method, which varied depending on the matrix and associated instrumental platform.

The extraction methods for *target PFAS analysis by LC-MS/MS*, were based on published methods (*Gebbink et al., 2016, 2013*) with small modifications. Briefly, each sample aliquot was fortified with a mixture of mass-labelled internal standards to account for losses during the extraction procedure and for matrix effects during quantification. PFASs were extracted using methanol and stirring (**paper I**), methanol and ultra-sonic bath (**paper II**) or acetonitrile and bead blending (**papers III-IV**). After repeated extraction, the combined extracts were concentrated and cleaned using ENVI-Carb (**papers I-II, IV**) or a weak anion exchange solid-phase extraction (**paper III**). Prior to injection on the LC, recovery standards were added to the extract for determination of internal standard recovery. The same extracts were used for LC-HRMS analysis in **paper IV**.

For analysis of *EOF using CIC*, samples were extracted in a similar manner to that above. However, addition of recovery and internal standards was omitted at all stages, as these would contribute to the fluorine signal measured by CIC. Instead, extracts were weighed for the purpose of quantification.

No sample preparation was required for *TF analysis using CIC*, therefore sample materials were weighed in directly onto the analysis boats.

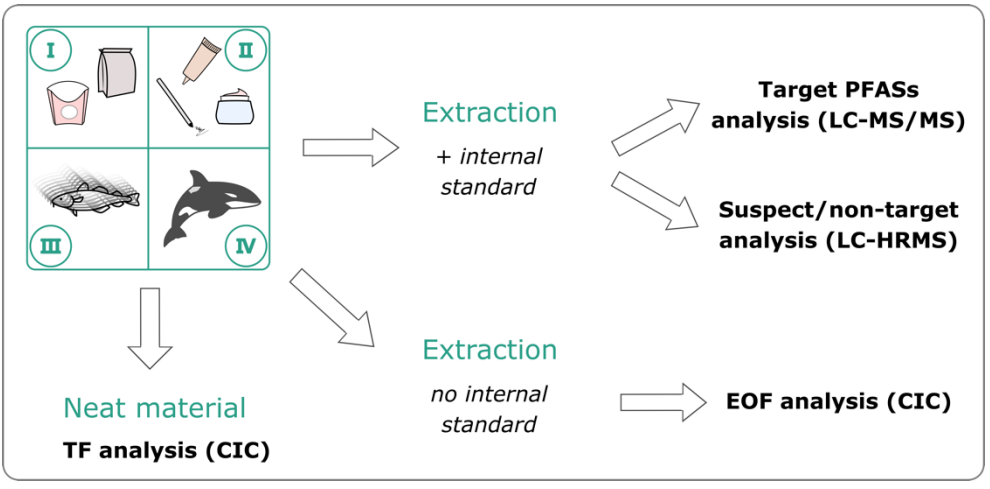

Figure 3. General overview of sample preparation and analysis.

3.3 Sample analysis

Target PFASs were measured in **papers I-IV** using LC-MS/MS (Acquity UPLC coupled to Xevo TQS, both Waters) operated in negative electrospray ionization (ESI) multiple reaction monitoring mode, according to methods described in Gebbink et al. (*2013, 2015*) and Nyberg et al. (*2018*). A reversed phase stationary phase (C_{18} Bridged Ethylene Hybrid (BEH)) was used for all analyses, together with polar mobile phases (either water and methanol, or water and acetonitrile) together with 1-methylpiperidine or ammonium acetate as additives for improved chromatography.

EOF and TF analysis methods using CIC (combustion oven HF-210 and adsorption unit GA-210, both Mitsubishi; coupled to Dionex Integrion HPIC, Thermo Fisher Scientific, Figure 4) were developed and validated as part of the thesis (**paper I**), and applied with modifications dependent on the type of matrix and expected concentration to **papers II-IV**. In general, samples (100-200 µl for liquids, 5-300 mg for solids) were placed onto ceramic combustion boats containing quartz glass wool for better dispersion of fluids. Using argon and water vapor as carrier gas and oxygen as combustion gas, samples were combusted slowly in a quartz glass tube heated to 1100°C. Complete combustion was ensured by monitoring oxygen levels, whereby the length of the combustion program was adjusted to matrix type and sample size. All adsorption gases were bubbled through water, which, after complete combustion, was filled up with water to a constant level (10 or 18 ml). An aliquot of the absorption solution was then transferred to the ion chromatograph (IC). The IC injection volume was adjusted depending on the expected concentration or desired instrumental limit of detection. Large volume injections (2000 µl) were loaded onto a pre-concentration column, which was subsequently eluted in reverse flow onto the analytical column using an external pump (**paper III**); while smaller volumes (20 µl for **paper I-II** and 200 µl for **paper II&IV**) were injected directly onto the analytical column. The anion exchange analytical and guard columns featured an alkanol quaternary ammonium as solid phase (AS19 7.5 µm, Thermo Fisher Scientific) maintained at 30°C. Chromatographic separation of fluoride from other ions was achieved by running a gradient of electrolytically generated potassium hydroxide mobile phase ramping from 8 to 100 mM at a flow rate of 0.25 ml/min. Post chromatographic

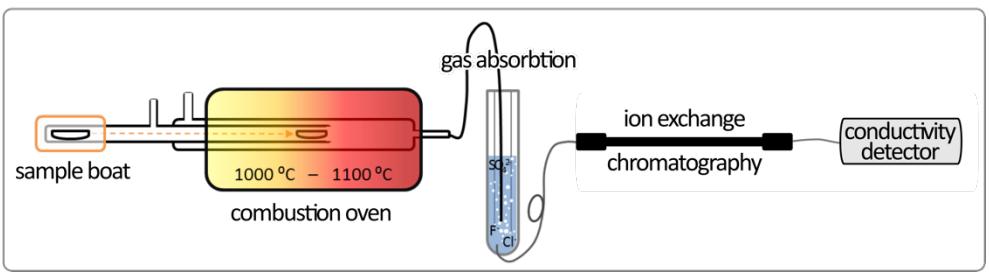

Figure 4. Schematic drawing of the combustion ion chromatograph used for EOF and TF analysis.

separation and prior to conductivity detection, the mobile phase was passed through to an electrolytic suppressor for eluent regeneration. High purity gases (5.0) and water (MilliQ, 18 MΩ cm, 3 ppb TOC) were used to minimize background contamination. Samples for paper I were sent to the University of Notre Dame (Indiana, US) and the University of Missouri (Missouri, US) for *TF analysis by PIGE and INAA*, respectively.

Suspect screening was carried out on a UHPLC-UHRMS (Dionex Ultimate 3000 coupled to Q-Exactive HF Orbitrap, both Thermo Fisher Scientific). The LC was operated under similar conditions to those described above for target analysis. The mass spectrometer was operated in full scan mode (resolution of 120000 at *m/z* 200) with data dependent (~500 PFASs, based on mass list S9 from the Norman suspect list exchange portal) MS2 acquisition. Suspect screening was based on three lists (S9, S14 and S25 (*NORMAN database*)) and further compounds reported in recent literature (*Liu et al., 2018b*, *2018a*; *Wang et al., 2018*; *Yu et al., 2018*).

3.4 Fluorine mass balance calculations

The following section presents equations used for fluorine mass balance calculations. Total fluorine (C_{F_TF}), extractable organic fluorine (C_{F_EOF}) and the sum of all individual PFASs concentrations (ΣC_{PFAS}) are the concentrations obtained by CIC and LC-MS/MS analysis. While C_{F_TF} and C_{F_EOF} reflect concentrations in equivalents of fluorine, individual PFAS concentrations (C_{PFAS}) are given in equivalents of molecular weight, as denoted by the absence of the subscript F in C_{PFAS}. Therefore, a unit conversion is necessary for comparison. Individual PFAS concentrations (C_{PFAS}) are converted to units of fluorine equivalents (C_{F_PFAS}) using the following equation:

$$C_{F\_PFAS} = n_F \times A_F / MW_{PFAS} \times C_{PFAS} \quad \text{(Eq.1)}$$

where n_F is the number of fluorine atoms, MW_{PFAS} the molecular weight of the respective PFAS and A_F the atomic weight of fluorine (both g/mol). The total known extractable fluorine concentration (ΣC_{F_PFAS}, ng F/g) is obtained by summing all individual C_{F_PFAS}. Now we can calculate the fluorine mass balance, and express for example the total concentration of *unidentified* extractable organic fluorine ($C_{F\_extr.unknown}$, ng F/g) according to equation 2:

$$C_{F\_extr.unknown} = C_{F\_EOF} - \Sigma C_{F\_PFAS} \quad \text{(Eq.2)}$$

In addition, we can calculate the total non-extractable fluorine concentration ($C_{F\_non.extr}$, ng F/g) as shown in equation 3:

$$C_{F\_non.extr} = C_{F\_TF} - C_{F\_EOF} \quad \text{(Eq.3)}$$

4 Results and Discussion

4.1 Comparison of TF methods and application to food packaging (Paper I)

As outlined in the Introduction, there is growing interest in, and an increasing number of available techniques for determining TF and EOF. However, no inter-laboratory or inter-method comparison had been carried out to demonstrate consistency of measurements or advantages/disadvantages of different techniques. Paper I addressed this knowledge gap by comparing three TF methods (CIC, PIGE and INAA).

To demonstrate accuracy of the methods, a certified reference material (CRM, BCR-461, fluorine in clay) was analyzed. Precision was assessed by replicate analysis (n=5). Statistical analysis (individual t-tests) revealed that the concentrations measured by CIC and PIGE were not significantly different (at α=0.05) from one another or to the reference concentrations. The CRM could not be analyzed with INAA due to aluminum present in the clay which causes interferences with the fluorine signal. Therefore, replicate sets of in-house standard materials (PFOA-spiked filters at five different concentrations) were prepared at the University of Notre Dame and measured by all three methods. Statistical analysis (repeated t-tests with Bonferroni correction) revealed no significant differences between all three methods.

A set of nine food packaging materials (microwave popcorn bags and French fry containers) served as a further comparison between CIC, PIGE and INAA. With the exception of a thick paperboard, all other samples were paper materials. While the paper materials showed excellent agreement between methods (ANOVA), concentrations in the paperboard reported by PIGE were much higher than those by CIC and INAA, due to the limited penetration depth of the PIGE particle beam. Therefore, PIGE is suitable for distinguishing between coated and uncoated sides of a sample, while INAA and CIC return bulk concentrations. A comparison of method detection limits revealed CIC to be lowest (0.8 μg/g) compared to INAA and PIGE (20 and 38 μg/g, respectively) assuming a 10 mg sample size.

The last objective of paper I was to apply the validated in-house CIC method to carry out a fluorine mass balance in the food packaging samples. Target analysis revealed the

presence of mainly PFCAs in the sample extracts, but also PFSAs and mono- and diPAPs. However, total PFASs concentrations accounted for negligible parts of the EOF (a maximum of 0.28%). Similarly, compared to TF concentrations, EOF concentrations were low, accounting for maximum 5.5% of the TF. These results suggest the presence of large amounts of unidentified extractable and non-extractable fluorinated substances in these materials.

4.2 Fluorine mass balance in cosmetics and implications for human exposure (Paper II)

Cosmetic products are known to contain fluorinated substances as additives (*Henricsson*, *2017*; *KEMI*, *2015*); however, to the best of our knowledge, only one study had measured PFASs in cosmetics (PFCAs only) at the time of publication of paper II, which focused on a range of samples from the Japanese Market (*Fujii et al.*, *2013*). In paper II, we investigated the occurrence of 39 PFASs in 31 cosmetic samples (creams, foundations, powders and eye shadows, pencil and shaving foams) listing eight different fluorinated ingredients, including PAPs.

Most foundation and powder products contained PFCAs, despite listing other fluorinated substances as ingredients such as PTFE, PAPs or perfluorooctyl triethoxysilane. The PFCA patterns were dominated by the C8 and C6 homologues, but compounds of chain lengths from C4 up to C14 were detected as well. In contrast, four other products that also listed PFTE, did not contain any detectable levels of target PFASs. The three products that listed undefined mixtures of PAPs showed high Σ_{14}PAP concentrations of up to 0.47 mg/g, yet large amounts (72-90%) of the EOF were still unaccounted for. In all other samples, the percentage of EOF accounted for by target PFASs was relatively low (0-1.3%), demonstrating the presence of unidentified organofluorine. While in most cases the analysis of the intentionally-added ingredients was not carried out due to the lack of available analytical methods, presumably unintentionally-added PFASs were present in most samples. TF concentrations were highest (up to 19 mg/g) in samples that listed polymers. EOF concentrations were comparably low in samples listing PTFE (0-0.5%) and polyperfluoromethylisopropyl (0%), but higher for 7-28% for polyperfluoro-ethoxymethoxy difluoroethyl PEG phosphate. This suggests that methanol was not suitable for extracting fluorinated polymers from the products. No target PFASs were detected in creams, pencil and shaving foam. The seven samples which did not list fluorinated ingredients were indeed below the limit of detection for all target PFASs. However, TF levels were relatively high in two of these products, which could comprise polymeric or inorganic fluorine (e.g. the mineral fluorphlogopite).

According to recent human exposure assessment studies, dermal absorption of PFASs is considered to have a negligible contribution compared to uptake of PFASs from

food, drinking water and ingested dust (*Gebbink et al., 2015; Trudel et al., 2008*). However, these studies did not take into account the contribution of dermal exposure through cosmetic products. Such calculations are further impeded by the lack of dermal absorption coefficients for all PFASs but PFOA (*Franko et al.*, *2012*). We estimated that the daily uptake of PFOA via regular use of certain cosmetic products can exceed daily intakes of PFOA via diet for the Swedish population.

4.3 Temporal trends of PFASs and TF in Baltic cod (Paper III)

Human activities have impacted the Baltic Sea and contributed to eutrophication and over-fishing. In addition, several classes of POPs have been detected in the Baltic Sea food web (*Naturvårdsverket*, *2009*) including PFASs (*Kratzer et al.*, *2011*). The Baltic cod is a predatory fish and therefore susceptible to biomagnification of POPs. A total of ten different PFASs were observed in the Baltic cod liver samples analyzed in this study, of which PFOS was dominant. Time trend analysis revealed increasing PFOS concentrations at a rate of 3.5% per year over the entire monitored time period (1981-2013). Steeper increasing trends were seen for long-chain PFCAs (C9-C12), with rates of up to 7.7% per year. FOSA was the only compound with a decreasing trend (-4.4% per year) over the monitored time period. Further compounds that were detected in the samples were PFOA, which was detected in relatively low concentrations and did not show a significant trend, as well as 6:2 FTSA and PFTrDA, which were detected at frequencies that were too low for time trend analysis. Change-point detection trend analysis was tested for all compounds, but no significant changes in slopes were detected. While the declining trends for FOSA could be attributed to a shift in production and use, no apparent effect of the phase-out of PFOS taken during 2000-2002 was observed.

Total fluorine concentrations in the liver of the Baltic cod ranged from 102±52 to 368±460 ng/g (geometric means), but did not show any significant temporal trends or correlations with biological variables. However, TF concentration in earlier years (1981, 1990) were higher than in later years (2000, 2007 and 2013) (t-test, $p<0.05$). EOF concentrations accounted for 10±7% (2013) up to 27±76% (1981) of the TF. The non-extractable fluorine (of up 1611 ng/g) is likely comprised of mainly inorganic fluoride, which has been reported in similar concentrations in cod liver from the North Sea (*Wright and Davison*, *1974*). EOF concentrations decreased significantly over the monitored time period at a rate of -1.8% per year. Furthermore, the fraction of EOF unaccounted for by ΣC_{F_PFAS} decreased at an even faster rate of -3.3% per year.

We also investigated associations between PFAS concentrations and various health endpoints in Baltic cod. PFCAs (C8-C12) and FOSA were negatively correlated with liver somatic index (LSI), while only PFOA was negatively correlated with the condition factor. Body length was positively correlated with PFDoDA and FOSA, as well as negatively

correlated with PFOA and PFNA. While PFASs have been shown to cause increases in liver weights of rodents (*Berthiaume and Wallace*, *2002*; *Lau et al.*, *2003*), the opposite effect is commonly observed in fish, consistent with our results (*Oakes et al.*, *2005*). Overall, these data indicate that health effects of PFAS on fish remain poorly understood.

4.4 Known and unknown PFASs in killer whale tissues (Paper IV)

PFASs are hypothesized to bind to plasma proteins and partition to phospholipids, and therefore to accumulate mainly in protein-rich tissues such as liver and blood (*Dassuncao et al.*, *2019*). Several PFAS tissue distribution studies in wildlife have been carried out which consistently report a similar pattern of highest ΣPFAS concentrations in liver and blood (*Aas et al.*, *2014*; *Ahrens et al.*, *2009*; *Greaves et al.*, *2012*). No studies have investigated whether EOF concentrations follow the same tissue distribution pattern.

The results of the target PFAS analysis in the eight killer whale tissues align well with previous tissue distribution studies, with the highest ΣPFAS concentrations occurring in liver and blood, and lowest in muscle, skin and blubber. PFAS profiles were dominated by long-chain PFAAs (PFOS, PFUnDA and PFTrDA), but a considerable amount (up to 23%) of FOSA was detected as well. Low ratios of PFOS to FOSA in cetaceans as compared to other marine mammals have been previously reported by Galatius et al. (*2013*) and Letcher et al. (*2014*), who hypothesized that they originate from the lack of cytochrome P450 enzymes necessary to biotransform PFAA-precursors. Remarkably, 7:3 fluorotelomer carboxylic acid (FTCA), which is a biotransformation product of the volatile 8:2 fluorotelomer alcohol (FTOH), was detected in liver and blood tissues. To date, only a few studies have reported the environmental occurrence of this compound, and mostly at lower concentrations than observed in the present study (*Loi et al.*, *2011*; *Nilsson et al.*, *2013*; *Persson*, *2017*; *Powley et al.*, *2008*).

Interestingly, EOF and TF displayed distinctively different distribution patterns than ΣPFAS concentrations. The highest EOF concentrations were detected in blubber, for which ΣPFASs were lowest of all tissues. The commonly known and most studied PFASs – PFAAs and PFAA precursors – are lipophobic chemicals and therefore not expected to accumulate in tissues rich in non-polar storage lipids (e.g. triglycerides, wax esters and cholesterol) such as blubber. The large gap in identified EOF in blubber indicates the presence of high concentrations of one or several presently unidentified fluorinated compounds. In contrast, ΣC_{F_PFAS} accounted for more than 91 % of the EOF in liver, blood, lung and kidney; essentially closing the mass balance between ΣC_{F_PFAS} and C_{F_EOF}. Blubber also revealed the highest TF concentrations, followed by lung and kidney; and with the exception of liver, large differences between EOF and TF concentrations were observed. The composition of these large fractions of non-extractable fluorine remains

unidentified but can possibly be explained by inorganic fluoride or non-polar compounds that are not captured by the acetonitrile extraction procedure.

All sample extracts were analyzed using LC-HRMS in an effort to characterize the unidentified compounds. For the more than 8000 indexed peaks, 348 matched with masses in the suspect PFAS list. MS/MS data was available for 43 of those, of which 20 were identified with reference standards (target PFASs). Several substances were identified my means of their exact mass and MS/MS data, including two chlorinated polyfluorinated ether sulfonates, long-chain PFCAs perfluorohexadecanoate, perfluorooctadecanoate and PFOS-precursor N-ethyl-perfluorooctane sulfonamide. Using homologue series mining, 5 PFASs classes (including unsaturated PFCAs, unsaturated perfluoroalcohols and hydrogen-substituted PFCAs) were identified at a low confidence level. However, based on the comparison of peak areas, none of these identified PFASs appeared high enough to explain the large amount of unidentified EOF in blubber.

4.5 Summary of fluorine mass balance results from papers I-IV

Figure 5 summarizes the fluorine mass balance results (C_{F_TF}, C_{F_EOF} and ΣC_{F_PFAS}) of papers I-IV. Fluorine concentrations are plotted on a logarithmic scale, due to the large range of concentrations. While the consumer products analyzed in papers I-II displayed the highest TF and EOF concentrations due to the presence of intentionally-added PFASs, they also showed much higher variation among the samples compared to the wildlife samples analyzed in papers III-IV. It is pertinent to note that samples for papers I-II were selected based on the suspected or known presence of fluorinated substances (with the exception of the control samples in paper II) and are therefore not representative of their entire product class. Nevertheless, the observation of a large gap in C_{F_EOF} and ΣC_{F_PFAS} in both food packaging and cosmetics indicates that the identity of PFASs used in consumer products is poorly characterized (Figure 5). This ultimately hinders estimates of environmental and human health risks associated with use of these products.

Another notable finding from this work was that that the gap between C_{F_EOF} and ΣC_{F_PFAS} is considerably smaller for the wildlife species compared to consumer products. One plausible explanation for this is that various PFAA precursors which make up the unidentified EOF of consumer products are transformed in the environment to form ultimately-persistent PFAAs. This hypothesis is supported by the finding that the killer whale, which occupies a higher trophic level compared to the cod, contained the lowest amounts of unidentified EOF in liver. Alternatively, the intentionally-added compounds in consumer products may be less persistent or bioaccumulative which would ultimately reduce their accumulation higher up in the food chain. Finally, we cannot overlook the role of inter-species variability in PFAS accumulation and PFAA-precursor transformation

as well as differences in sampling location (in terms of proximity to source), both of which are likely to be important determinants of the PFAS burden in wildlife. Further efforts to identify the structure of the unknown organofluorine will undoubtedly help to explain these observed differences.

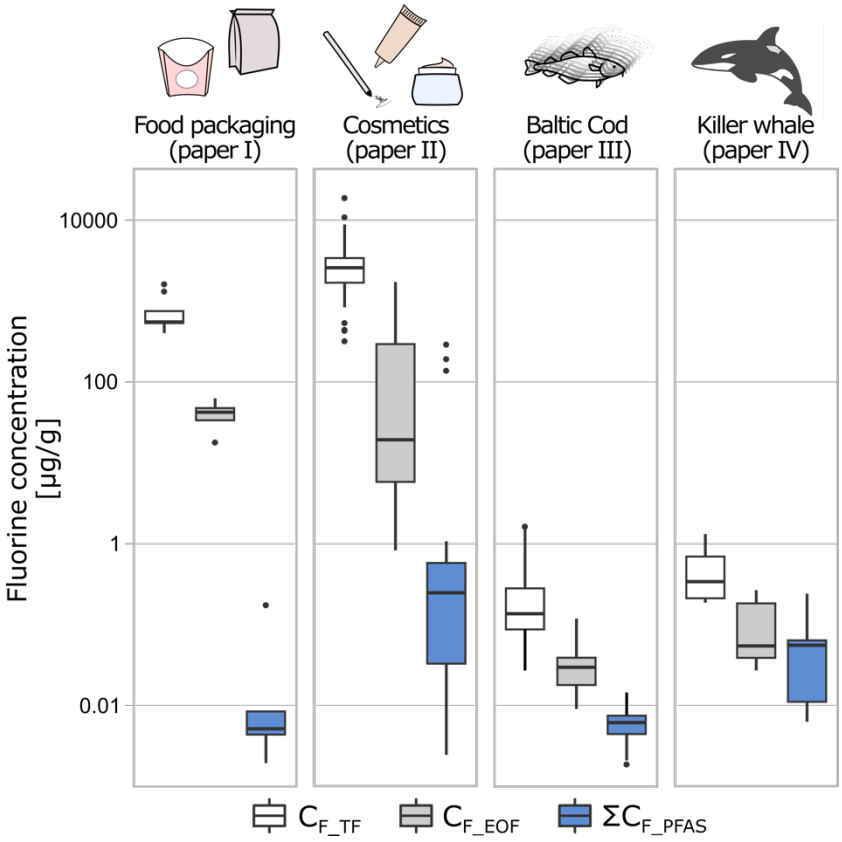

Figure 5. Comparison of total fluorine (C_{F_TF}), extractable organic fluorine (C_{F_EOF}) and total PFAS concentrations (ΣC_{F_PFAS}) (in µg of fluorine per g, logarithmic scale) in samples from papers I-IV. Box divider line represents the median, the end of the box shows the upper and lower quartiles, the extreme lines shows the highest and lowest value excluding outliers, which are plotted as dots.

5 Conclusions and future perspectives

Findings from all four papers supported the initial hypothesis of this thesis, that the commonly analyzed PFASs only represent a fraction of anthropogenic organofluorine found in consumer products and wildlife.

In **paper I**, a successful first comparison and validation of several total fluorine methods was carried out. The advantages and disadvantages of each technique were highlighted in order to assist the selection of these instruments for future applications. For example, CIC was most suitable for applications where low limits of detection are required, whereas PIGE and INAA were deemed more suitable for rapid screening purposes. Ideally, a larger inter-laboratory comparison should be carried out comparing a wider variety of matrices with additional available TF methods to gain a more comprehensive understanding of the possibilities and limitations of TF methods. An inter-laboratory comparison of EOF is also needed. In addition, we demonstrated that food packaging samples contained unidentified extractable organic fluorine which, as opposed to non-extractable fluorine, may contribute to human exposure to PFASs. Future work should aim to characterize this unidentified EOF and assess its potential to migrate to food.

Paper II presented the first analysis of PFASs and mass balance of fluorine in cosmetic products from the Swedish market. Large mass balance gaps were observed in all samples, for many due to the lack of analysis methods for the intentionally-added ingredients. However, even for products in which the listed ingredients have been quantified (e.g. PAPs), large portions of unidentified organofluorine remain. The occurrence of PFAAs, including PFOA, in several samples despite their absence from ingredient lists, points to the need for improved regulation of consumer products. Furthermore, the human exposure estimates via dermal absorption of PFASs contained in foundations and powders remain uncertain due to the lack of absorption coefficients for compounds other than PFOA. Clearly, further research is needed on dermal absorption of different PFASs including precursors such as PAPs, which are often found in high concentrations in products that come into contact with skin.

The time trends investigated in Baltic cod as part of **paper III** indicated increasing concentrations for most long-chain PFAAs and decreasing concentrations for the PFAA precursor FOSA, as well as for EOF and unidentified EOF. Together, these trends can be interpreted in two ways. Our first hypothesis is that the unidentified EOF is comprised mainly of PFAA-precursors, which contribute to PFAA levels in Baltic cod through

metabolism. Under this scenario, PFAS inputs to the Baltic may be declining, but transformation of precursors to PFAAs would cause an apparent delay in the reduction of PFAA concentrations. This hypothesis is supported by the fact that levels of both FOSA, which has been phased-out in 2000, and EOF, declined, yet PFOS continues to increase. Additional experiments could be used to confirm this hypothesis. For example, the amount of PFAA-precursors present in the EOF could be determined via the total oxidizable precursor (TOP) assay (*Houtz et al.*, *2016*). Our second hypothesis is based on the assumption that the unidentified EOF is mainly comprised of non-PFAA precursors, such as fluorinated pharmaceuticals or pesticides. Under this scenario the increasing concentrations of long-chain PFAAs observed in cod liver would be attributed to ongoing input of PFAAs into the Baltic and direct exposure of Baltic cod. In both cases, identification of the unknown fraction of EOF would help to confirm or refute these hypotheses. The observed correlations of certain PFASs with health variables call for further ecotoxicological studies for a better understanding of health outcomes associated with individual PFASs in wildlife. Furthermore, cumulative effects associated with exposure to multiple PFASs as well as mixture effects of PFASs with other environmental contaminants in cod should be investigated.

Paper IV led to the main conclusion that wildlife exposure to PFASs may be heavily underestimated by conventional analysis of known PFASs in liver and/or blood tissues. Although PFASs are presumed to be lipophobic, the large amounts of unidentified EOF detected in blubber indicated the presence of lipophilic fluorinated contaminants. However, the identity of these substances remains unclear. An exploration of non-polar extraction solvents combined with more in-depth LC- and GC-based non-target analysis of polar and non-polar sample extracts is needed in order to shed light on the identity of the emerging fluorinated contaminants in blubber. The fluorine mass balance experiments further demonstrated that in tissues which are known to accumulate legacy PFASs (mainly liver and blood, but also lung and kidney), the persistent long-chain PFAAs, together with the stable metabolites and major PFSA- and PFCA-precursors FOSA and 7:3 FTCA, account for virtually the entire EOF. Furthermore, the observation of 7:3 FTCA in liver and blood of the killer whale demonstrates the need for further research on exposure pathways PFAS precursors.

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