

Emerging analytical tools and strategies for PFAS discovery

Mélanie Zoé Lauria



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Abstract

Per- and polyfluoroalkyl substances (PFAS) are a diverse class of synthetic chemicals that have garnered significant attention due to their persistence, widespread occurrence, and adverse health effects. Thousands of PFAS are registered globally, occupying a wide chemical space and requiring diverse methods for their identification and quantification. Despite vast improvements in analytical coverage over the last two decades, there are increasing concerns that unknown or emerging compounds continue to be overlooked. To address these concerns, a number of new analytical strategies have emerged: one is the so-called fluorine mass balance (FMB) approach, which involves subtracting the fluorine attributed to target PFAS (Σ PFAS) from the total- or extractable organic- fluorine (TF and EOF, respectively) to deduce the quantity of unknown PFAS in a sample. This approach can be used to prioritize samples with high levels of unidentified fluorine for further interrogation. A second approach involves high resolution mass spectrometry (HRMS)-based suspect and non-target screening, which aims to identify novel PFAS in environmental samples. This thesis develops and/or applies these emerging analytical methods in order to improve our understanding of PFAS sources and occurrence in the environment, with FMB experiments applied throughout **Papers I to III**, and suspect- and nontarget screening used in **Papers II-IV**.

In **Paper I**, an FMB of different components of artificial turf (backing, filling, and blades) revealed high levels of total fluorine in all samples (ranges of 16–313, 12–310, and 24–661 μg of F/g in backing, filling, and blades, respectively), while EOF and target PFAS occurred in <42% of all samples (<200 and <1 ng of F/g, respectively). Further experiments confirmed the absence of both fluoride and perfluoroalkyl acid precursors in these samples. Collectively, these results point toward the occurrence of a polymeric organofluorine, consistent with patent literature, and shines a light on the use of fluoropolymers in plastic and rubber production which might complicate disposal of these products.

In **Paper II**, both FMB and HRMS-based suspect screening were applied to liver samples from a variety of marine mammals. As part of this work, an ionization efficiency-based model for quantification of substances lacking analytical standards was trained and validated. Thereafter, the model was used to quantify PFAS detected by suspect screening, and ultimately reduced the quantity of unidentified organofluorine from 13–70% (median: 32%) down to 0–27% (median: 17%).

Paper III delved further into FMB and non-target analysis of marine mammals, this time focusing on blubber, where unexpectedly high levels of unknown EOF were previously uncovered in the blubber of a Greenlandic killer whale. Using a combination of ion exchange solid phase extraction, gas chromatography-ion mobility-high resolution mass spectrometry (GC-IM-HRMS), and collision cross section (CCS)-based prioritization (i.e. $\text{CCS} [\text{\AA}^2] < 0.2 \text{\AA}^2 \times m/z + 100 \text{\AA}^2$) the number of plausible organofluorine peaks was reduced from several thousand down to several hundred. Structures were proposed for the most abundant on this list based on fragmentation. Five novel fluorotelomer sulfones were identified at confidence level 1 (CL 1: identified with standard) and quantified, accounting for up to 75% of the EOF in blubber.

Finally, in **Paper IV** methanol extracts of municipal wastewater treatment plant sludge, as well as sludge and dust standard reference materials (SRMs), were characterized by liquid chromatography-IM-HRMS, and the same CCS filter used in Paper III was applied, together with two additional PFAS prioritisation strategies (mass defect and mass/number of carbon atoms). A total of

Fluorine mass balance, suspect, and non-target screening are critical tools for expanding our understanding of PFAS contamination in diverse environmental and biological matrices. Integrating these advancements is essential for more comprehensive exposure assessments and informed policy decisions.

Keywords: *combustion ion chromatography, fluorine mass balance, suspect screening, nontarget screening, gas chromatography, liquid chromatography, marine mammals.*

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EMERGING ANALYTICAL TOOLS AND STRATEGIES FOR PFAS
DISCOVERY

Mélanie Zoé Lauria



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Abstract

Per- and polyfluoroalkyl substances (PFAS) are a diverse class of synthetic chemicals that have garnered significant attention due to their persistence, widespread occurrence, and adverse health effects. Thousands of PFAS are registered globally, occupying a wide chemical space and requiring diverse methods for their identification and quantification. Despite vast improvements in analytical coverage over the last two decades, there are increasing concerns that unknown or emerging compounds continue to be overlooked. To address these concerns, a number of new analytical strategies have emerged: one is the so-called fluorine mass balance (FMB) approach, which involves subtracting the fluorine attributed to target PFAS (Σ PFAS) from the total- or extractable organic-fluorine (TF and EOF, respectively) to deduce the quantity of unknown PFAS in a sample. This approach can be used to prioritise samples with high levels of unidentified fluorine for further interrogation. A second approach involves high resolution mass spectrometry (HRMS)-based suspect and non-target screening, which aims to identify novel PFAS in environmental samples. This thesis develops and/or applies these emerging analytical methods in order to improve our understanding of PFAS sources and occurrence in the environment, with FMB experiments applied throughout **Papers I to III**, and suspect- and nontarget screening used in **Papers II-IV**.

In **Paper I**, an FMB of different components of artificial turf (backing, filling, and blades) revealed high levels of total fluorine in all samples (ranges of 16–313, 12–310, and 24–661 μg of F/g in backing, filling, and blades, respectively), while EOF and target PFAS occurred in <42% of all samples (<200 and <1 ng of F/g, respectively). Further experiments confirmed the absence of both fluoride and perfluoroalkyl acid precursors in these samples. Collectively, these results point toward the occurrence of a polymeric organofluorine, consistent with patent literature, and shines a light on the use of fluoropolymers in plastic and rubber production which might complicate disposal of these products.

In **Paper II**, both FMB and HRMS-based suspect screening were applied to liver samples from a variety of marine mammals. As part of this work, an ionisation efficiency-based model for quantification of substances lacking analytical standards was trained and validated. Thereafter, the model was used to quantify PFAS detected by suspect screening, and ultimately reduced the quantity of unidentified organofluorine from 13-70% (median: 32%) down to 0-27% (median: 17%).

Paper III delved further into FMB and non-target analysis of marine mammals, this time focusing on blubber, where unexpectedly high levels of unknown EOF were previously uncovered in the blubber of a Greenland killer whale. Using a combination of ion exchange solid phase extraction, gas chromatography-ion mobility-high resolution mass spectrometry (GC-IM-HRMS), and collision cross section (CCS)-based prioritisation

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Finally, in **Paper IV** methanol extracts of municipal wastewater treatment plant sludge, as well as sludge and dust standard reference materials (SRMs), were characterised by liquid chromatography-IM-HRMS, and the same CCS filter used in Paper III was applied, together with two additional PFAS prioritisation strategies (mass defect and mass/number of carbon atoms). A total of 36 PFAS were identified (CL 1) or confidently annotated (CL 2b: probable structure), and 40 features were either assigned a tentative candidate (CL 3) or considered a feature of interest (CL 5), including perfluoroalkyl acids, their precursors, their branched isomers, as well as unsaturated perfluoroalkyl sulfonates and H-substituted polyfluoroalkyl sulfonates.

Fluorine mass balance, suspect, and non-target screening are critical tools for expanding our understanding of PFAS contamination in diverse environmental and biological matrices. Integrating these advancements is essential for more comprehensive exposure assessments and informed policy decisions.

Sammanfattning

Per- och polyfluoralkylsubstanser (PFAS) är en bred klass av syntetiska kemikalier som har väckt stor uppmärksamhet på grund av deras persistens, utspridd förekomst och negativa hälsoeffekter. Tusentals PFAS är registrerade globalt och täcker ett brett kemiskt spektrum, vilket kräver varierade metoder för deras identifiering och kvantifiering. Trots stora framsteg inom analytisk bevakning under de senaste två decennierna finns det stora misstankar om att okända eller nya föreningar fortfarande förbises. För att hantera detta har flera nya analytiska metoder utvecklats. En sådan metod är den så kallade fluorine mass balance (FMB), som innebär att man subtraherar den fluor som kan tillskrivas kända PFAS från total- eller extraherbar organisk fluor (total fluorine, TF, respektive extractable organic fluorine, EOF) för att uppskatta mängden okända PFAS i ett prov. Denna strategi kan användas för att prioritera prov med höga halter av oidentifierad fluor för vidare analys. En annan metod är suspect- och non-target screening genom användandet av högupplösande masspektrometri (HRMS), med målet att identifiera nya PFAS i miljöprov. Denna avhandling utvecklar och/eller tillämpar dessa nya analytiska metoder för att förbättra vår förståelse av PFAS-källor och förekomst i miljön. FMB-experiment tillämpas i **Artiklar I–III**, medan suspect- och non-target screening används i **Artiklar II–IV**.

I **Artikel I** användes FMB på olika komponenter i konstgräs (strån, fyllnadsmaterial och matta), vilket visade höga nivåer av totalfluor i samtliga prov (16–313, 12–310 respektive 24–661 $\mu\text{g F/g}$ i mattan, fyllningen och strån). Däremot återfanns EOF och target-PFAS i mindre än 42 % av proverna (<200 respektive <1 ng F/g). Ytterligare experiment bekräftade att varken fluorid eller PFAS prekursorer förekom i dessa prov. Tillsammans pekar resultaten mot förekomst av en polymer organofluorförening, i linje med patendlitteratur, och belyser användningen av fluorpolymerer i plast- och gummiproduktion, vilket kan försvåra avfallshanteringen av dessa produkter.

I **Artikel II** användes både FMB och HRMS-baserad suspect screening på leverprov från olika marina däggdjur. Inom detta arbete utvecklades och validerades en metod baserad på joniseringseffektivitet (ionisation efficiency) för kvantifiering av substanser utan tillgängliga standarder. Därefter användes modellen för att kvantifiera PFAS upptäckta via suspect screening, vilket minskade andelen oidentifierad organofluor från 13–70 % (median: 32 %) till 0–27 % (median: 17 %).

Artikel III fördjupade sig ytterligare i FMB och non-target screening av marina däggdjur, med fokus på späck, där oväntat höga nivåer av okänd EOF tidigare observerats i späck från en grönländsk späckhuggare. Genom en kombination av jonbytesbaserad fastfasextraktion (ion exchange SPE), gaskromatografi-kopplad till jonmobilitets--HRMS (GC-IM-HRMS), samt collision cross section (CCS)-baserad prioritering (dvs. $\text{CCS} [\text{Å}^2] < 0,2 \times m/z + 100 \text{ Å}^2$), reducerades antalet möjliga organofluor-

toppar från flera tusen till några hundra. Strukturer föreslogs baserat på fragmentering för de toppar med högst intensitet. Fem nya fluorotelomer-sulfoner identifierades med högsta säkerhet (confidence level CL 1: identifierade med standard), kvantifierades, och därmed förklarade upp till 75 % av EOF i späck.

I **Artikel IV** analyserades metanolextrakt från slam från kommunala avloppsreningsverk samt referensmaterial för slam och damm (SRMs) med LC-IM-HRMS. Samma CCS-filter som i Artikel III tillämpades, tillsammans med två ytterligare PFAS-prioriteringsstrategier (massdefekt och massan/antal kolatomer). Totalt identifierades 36 PFAS med CL 1 eller annoterades med hög säkerhet (CL 2b: trolig struktur), och 40 ytterligare toppar tilldelades antingen kategorin preliminär kandidat (CL 3) eller ansågs vara intressanta (CL 5). Dessa inkluderade perfluoralkylsyror, deras prekursorer, grenade isomerer, samt omättade perfluoralkylsulfonater och H-substituerade polyfluoralkylsulfonater.

Fluorine mass balance, suspect och non-target screening är avgörande verktyg för att öka vår förståelse av PFAS-föroreningar i olika miljö- och biologiska matriser. Att integrera dessa metoder är nödvändigt för mer heltäckande exponeringsbedömningar och för att kunna fatta välgrundade beslut i regleringssammanhang.

Riassunto

Le sostanze per- e polifluoroalchiliche (PFAS) costituiscono una classe diversificata di composti chimici di sintesi, oggetto di crescente interesse per via della loro persistenza ambientale, ampia diffusione e impatti avversi sulla salute umana. A livello globale sono registrati migliaia di PFAS, caratterizzati da un'ampia varietà di proprietà chimiche e strutturali, che richiedono metodi analitici diversificati per la loro identificazione e quantificazione. Nonostante i notevoli progressi nella copertura analitica degli ultimi vent'anni, cresce la preoccupazione che composti emergenti o ancora sconosciuti continuino a sfuggire all'identificazione. Per affrontare queste problematiche, sono emerse diverse strategie analitiche innovative. Tra queste vi è il cosiddetto approccio del bilancio di massa del fluoro (*fluorine mass balance*, FMB), che consiste nel sottrarre il fluoro attribuibile ai PFAS determinati tramite analisi mirata (Σ PFAS) dal fluoro totale (*total fluorine*, TF) o dal fluoro organico estraibile (*extractable organic fluorine*, EOF), al fine di stimare la quantità di PFAS sconosciuti presenti in un campione. Questo approccio può essere utilizzato per individuare i campioni con maggiori quantità di fluoro non identificato, da destinare ad analisi più dettagliate. Un secondo approccio implica l'utilizzo della spettrometria di massa ad alta risoluzione (*high resolution mass spectrometry*, HRMS) per *suspect e non-target screening*, con l'obiettivo di identificare PFAS emergenti o sconosciuti in campioni ambientali. Questa tesi sviluppa e/o applica questi metodi analitici per migliorare la comprensione delle fonti e della presenza dei PFAS nell'ambiente. Gli esperimenti FMB sono applicati negli **Articoli I–III**, mentre il *suspect e non-target screening* sono impiegati negli **Articoli II–IV**.

Nell' **Articolo I**, un FMB su diversi componenti di campi in erba artificiale (supporto, materiale riempitivo e fili) ha rivelato alti livelli di fluoro totale in tutti i campioni (intervalli di 16–313, 12–310 e 24–661 μg di F/g in supporto, riempitivo e fili, rispettivamente), mentre EOF e Σ PFAS erano presenti in meno del 42% dei campioni (<200 e <1 ng di F/g, rispettivamente). Ulteriori analisi hanno confermato l'assenza di fluoruro e di precursori degli acidi perfluoroalchilici nei campioni. Questi risultati indicano la probabile presenza di composti polimerici fluorurati, in linea con quanto riportato nella letteratura brevettuale, e sottolineano l'impiego di fluoropolimeri nella produzione di plastica e gomma, con possibili implicazioni per la gestione a fine vita di questi materiali.

Nell' **Articolo II**, sia l'approccio FMB che il *suspect screening* basato su HRMS sono stati applicati a campioni di fegato provenienti da diverse specie di mammiferi marini. Come parte di questo lavoro, è stato sviluppato e validato un modello basato sull'efficienza di ionizzazione (*ionisation efficiency*) per la quantificazione di sostanze prive di standard analitici. Successivamente, il modello è stato utilizzato per quantificare i PFAS rilevati tramite *suspect screening*, riducendo infine la quantità di fluoro organico non identificato dal 13–70% (mediana: 32%) fino allo 0–27% (mediana: 17%).

L'Articolo III ha approfondito ulteriormente l'FMB e l'analisi *non-target* (senza a priori) nei mammiferi marini, concentrandosi questa volta sul grasso sottocutaneo (*blubber*), dove erano stati precedentemente riscontrati livelli insolitamente alti di EOF sconosciuto nel *blubber* di un'orca della Groenlandia. Utilizzando una combinazione di estrazione in fase solida a scambio ionico, cromatografia gassosa–mobilità ionica–HRMS (GC–IM–HRMS) e una strategia di prioritizzazione basata sul *collision cross section* (CCS), ovvero $CCS [\text{Å}^2] < 0.2 \times m/z + 100 \text{ Å}^2$, il numero di segnali (*features*) contenenti plausibilmente fluoro organico è stato ridotto da diverse migliaia a qualche centinaio. Le strutture dei composti più abbondanti sono state proposte in base alla frammentazione osservata. Cinque nuovi fluorotelomeri solfonati sono stati identificati con livello di confidenza 1 (CL 1: identificati con standard) e quantificati, rappresentando fino al 75% dell'EOF nel grasso.

Infine, nell'Articolo IV, estratti metanolici di fanghi di impianti municipali di trattamento delle acque reflue, nonché materiali di riferimento standard (SRMs) di fango e polvere, sono stati analizzati mediante cromatografia liquida–IM–HRMS. È stato applicato lo stesso filtro CCS utilizzato nell'Articolo III, insieme ad altre due strategie di prioritizzazione per i PFAS (difetto di massa e rapporto massa/numero di atomi di carbonio). In totale, 36 PFAS sono stati identificati (CL 1) o annotati con fiducia (CL 2b: struttura probabile), mentre a 40 segnali sono state assegnati candidati provvisori (CL 3) o considerate segnali interessanti (CL 5), includendo per esempio acidi perfluoroalchilici, i loro precursori, isomeri ramificati, oltre a solfonati perfluoroalchilici insaturi e solfonati polifluoroalchilici con sostituente H.

L'FMB, il *suspect* e il *non-target screening* rappresentano strumenti fondamentali per ampliare la nostra comprensione della contaminazione da PFAS in matrici ambientali e biologiche eterogenee. Integrare questi sviluppi è essenziale per valutazioni di esposizione più complete e per decisioni politiche basate su solide evidenze.

List of publications

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

- I Mélanie Z. Lauria, Ayman Naim, Merle Plassmann, Jenny Fäldt, Roxana Sühling, Jonathan P. Benskin; **Widespread Occurrence of Non-Extractable Fluorine in Artificial Turfs from Stockholm, Sweden.** *Environmental Science and Technology Letters* 2022, 9 (8), 666-672.
- II Mélanie Z. Lauria*, Helen Sepman*, Thomas Ledbetter, Merle Plassmann, Anna M. Roos, Malene Simon, Jonathan P. Benskin, and Anneli Krueve; **Closing the Organofluorine Mass Balance in Marine Mammals Using Suspect Screening and Machine Learning-Based Quantification.** *Environmental Science and Technology* 2024, 58 (5), 2458-2467.
*shared first authorship
- III Mélanie Z. Lauria, Xiaodi Shi, Faiz Haque, Merle Plassmann, Anna M. Roos, Malene Simon, Jonathan P. Benskin, Karl J. Jobst; **Discovery of Fluorotelomer Sulfones in the Blubber of Greenland Killer Whales (*Orcinus Orca*).** *Submitted manuscript.*
- IV Kyra Spaan, Mélanie Z. Lauria, Xiaodi Shi, Cynthia A. De Wit, Jonathan P. Benskin; **Closing the organohalogen mass balance in municipal wastewater treatment plant sludge using ion mobility-high resolution mass spectrometry.** *Manuscript.*

Author's contributions

- I Designed and implemented confirmatory experiments to deduce the nature of the fluorine in the artificial turf; carried out inventory calculations; took the lead in writing the manuscript.
 - II Participated in study design, extractions, instrumental analyses and data processing; assisted in modelling; took a lead role in writing the manuscript.
 - III Participated in study design; performed extractions, instrumental analyses and data processing; took the lead in writing the manuscript.
 - IV A previous version of this manuscript focusing strictly on GC-HRMS analysis of organochlorine and -bromine substances was included in the PhD thesis of K.M. Spaan. In the current thesis, this manuscript was modified via addition of new data on organofluorine substances, along with verification of organochlorine and -bromine substances using analytical standards. I was responsible for carrying out all extractions, LC-based instrumental analyses and data processing for organofluorine substances, as well as verification of putative identifications by GC-HRMS. Further, I took a lead role in updating all relevant section of the manuscript, including addition and/or modification of the graphical TOC, Figure 1, Table 1 and 2, and the supporting information.
- Kappa Wrote the first draft. ChatGPT was used to polish the text using the prompts "Check for spelling and grammar mistakes and improve fluency if possible".

Abbreviations

AOF	Adsorbable organic fluorine
APCI	Atmospheric pressure chemical ionisation
AT	Artificial Turf
CCS	Collision cross section
CIC	Combustion ion chromatography
ECHA	European chemical agency
EFSA	European food safety authority
EI	Electron ionisation
EOF	Extractable organic fluorine
EPA	Environmental protection agency
ESI	Electrospray ionisation
EU	European Union
FMB	Fluorine mass balance
GC	Gas chromatography
HRMS	High resolution mass spectrometry
<i>IE</i>	Ionisation efficiency
IF	Inorganic fluorine
IM	Ion mobility
KMD	Kendrick mass defect
LC	Liquid chromatography
MD	Mass defect
NTS	Non target screening
OECD	Organisation for economic cooperation and development
PFAS	Per-and polyfluoroalkyl substances
PFAAs	Perfluoroalkyl acids

PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
TF	Total fluorine
TFA	Trifluoroacetic acid
TOPA	Total oxidisable precursor assay

1. Introduction

1.1 Per- and polyfluoroalkyl substances

Per- and polyfluoroalkyl substances (PFAS), a class of fluorinated organic compounds, have garnered significant scientific and political attention over the last two decades. Many PFAS have unique thermal and chemical stability as well as hydrophobic and lipophobic properties, which has led to their use in a wide range of products and technical processes (Glüge et al., 2020). However, their extreme persistence in the environment, a common trait of many PFAS or of their final transformation products, poses a considerable threat to human and ecosystem health (Cousins et al., 2020).

According to the latest definition from the Organisation for Economic Co-operation and Development (OECD), all substances containing a $-CF_2-$ or $-CF_3$ moiety (with no H, Cl, Br or I attached to it), are considered PFAS (OECD, 2021). This large class of >7 million compounds (Schymanski et al., 2023), spans relatively low molecular weight (i.e. <1000 Da) substances such as the short-chain perfluoroalkyl acid (PFAA) trifluoroacetic acid (TFA; Figure 1), or long-chain PFAAs perfluorooctanoic acid (PFOA; Figure 1) and perfluorooctane sulfonic acid (PFOS), to high molecular weight (i.e. >1000 Da) polymers, such as polytetrafluoroethylene (PTFE; Figure 1). PFAAs are the most-studied PFAS sub-class, and are known to accumulate in organisms by interacting with phospholipids and proteins, and biomagnifying through food webs (De Silva et al., 2021). Humans are primarily exposed to PFAS through food and drinking water and several PFAS have multiple adverse health effects, particularly impacting the liver, kidneys, thyroid, and immune system (Fenton et al., 2021). Based on the association between PFAS exposure and decreased immune response to vaccination, the European Food Safety Authority (EFSA) recently established a tolerable weekly intake of only 4.4 ng/kg body weight for the sum of four representative PFAS: PFOA, PFOS, perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS; Schrenk et al., 2020).

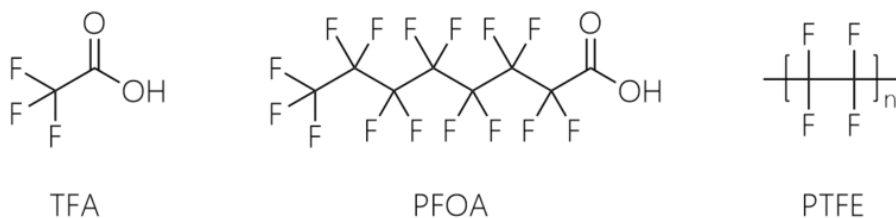


Figure 1 – Structures of TFA and PFOA, two perfluoroalkyl acids, and PTFE, a fluoropolymer of several thousands repeating monomers (where n is typically 5000 - 100 000 (Pompe et al., 2002)).

PFAS find applications in a diverse range of industries, including manufacturing of plastic, textiles, automotive vehicles and electronics, and products such as fire-fighting foams, coatings, paints and lubricants (Glüge et al., 2020). Due to their widespread use, PFAS can end up in various waste streams, making recycling (e.g. of plastic, paper, and textiles) or reuse (e.g. sludge) a potential pathway for their reintroduction into consumer products and food. While recycling or reuse of materials is often seen as a sustainable solution to reduce waste and promote a circular economy, the presence of persistent pollutants like PFAS complicates this approach since this contamination undermines the safety of recycled materials and challenges the European Union's vision of a circular economy.

In the 2000s, mounting concerns over the persistence, bioaccumulation, and potential health risks of PFOS and PFOA prompted phase out of usage and production of these substances: 3M, the only U.S. manufacturer of PFOS, announced its phase out and substitution in May 2000 (U.S. EPA, 2000); in 2006, the U.S. Environmental Protection Agency (EPA) together with leading PFAS manufacturers committed to a stewardship programme to drastically reduce PFOA emission by 2010 and eliminate it by 2015 (U.S. EPA, 2006). PFOS was added to the Stockholm Convention on persistent organic pollutants (POPs) in 2009, followed by PFOA in 2019, and PFHxS in 2022 (Stockholm Convention on POPs, n.d.). These global restrictions marked significant milestones in efforts to mitigate PFAS pollution, but also drove the development of novel PFAS chemistries, with increasing structural diversity and complexity (e.g. short-chain PFAS, perfluoroethers). This shift presents new challenges for environmental monitoring since traditional analytical methods may not fully capture emerging PFAS. Recognising that all PFAS or their final degradation products display a common property of extreme persistence, and in order to reduce the risk of regrettable substitutions, in 2023 five European Union member states proposed a comprehensive restriction on all PFAS to the European Chemicals Agency (ECHA), under the EU's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation (ECHA, 2021). The aim of this initiative is to drastically reduce emissions of PFAS across their entire life cycle, preventing further contamination and exposure. Nevertheless, the extent of contamination is already considerable, with the cost of clean-up estimated to be as high as a €100 billion euros per year for Europe if no restriction is implemented (The forever pollution project, 2025).

Despite the large number and structural diversity of PFAS, analytical methods typically only include a limited suite of PFAAs, PFAA-precursors, and alternatives, raising questions surrounding the number of PFAS which may be overlooked. A recent review of PFAS standards revealed availability of only 6% of the 10 000 PFAS that ECHA intends to regulate (Trier et al., 2025). To address this gap, analytical methods have evolved along two paths. The first involves so-called "sum parameter" measurements, which typically use (extractable organic) fluorine as a proxy for PFAS (e.g. "total fluorine"-based approaches) or provide an indirect measurement of PFAS that degrade to PFAAs (e.g. by the total

oxidisable precursors assay (TOPA; Houtz & Sedlak, 2012) or by photocatalysis (photoTOP; Zweigle et al., 2022)). These methods are non-specific, allowing for a wide range of known and unknown PFAS to be captured and the results can be compared to target PFAS in what is called the fluorine mass balance (FMB). The second approach involves non-target and suspect screening using high resolution mass spectrometry (HRMS). Here the aim is to identify novel or emerging PFAS. Both FMB and suspect/non-target approaches are described in further detail in the following sections.

1.2 Fluorine mass balance

FMB experiments involve measuring fluorine in a neat sample (i.e. total fluorine; TF), in an extract (i.e. extractable organic fluorine; EOF), or alternatively in an adsorbent material (i.e. adsorbable organic fluorine; AOF) and comparing these measurements to the fluorine content of known fluorinated compounds measured in the same sample by targeted analysis (Σ PFAS; Figure 2). The difference between EOF and known PFAS reveals the quantity of unknown EOF, while the gap between TF and EOF can be due to inorganic fluorine (IF) and non-extractable organic fluorine (NEOF).

While many analytical approaches exist for the determination of TF, such as particle induced gamma-ray emission spectroscopy (PIGE) and instrumental neutron activation analysis (INAA), combustion ion chromatography (CIC) is the most widely used in FMB studies given its low detection limits and potential to handle both solid and liquid samples, as well as the possibility to measure both TF and EOF (Schultes et al., 2019). Combustion at sufficiently high temperatures (>1000 °C) breaks down organofluorines to form hydrogen fluoride gas (Aguilar et al., 2025), which is captured in an absorption solution and then fluoride is measured by ion chromatography (IC). Since CIC does not differentiate between inorganic and organic fluorine, it is crucial to remove inorganic fluorine during sample preparation for EOF analysis. Furthermore, variations in extraction method and clean-up can affect

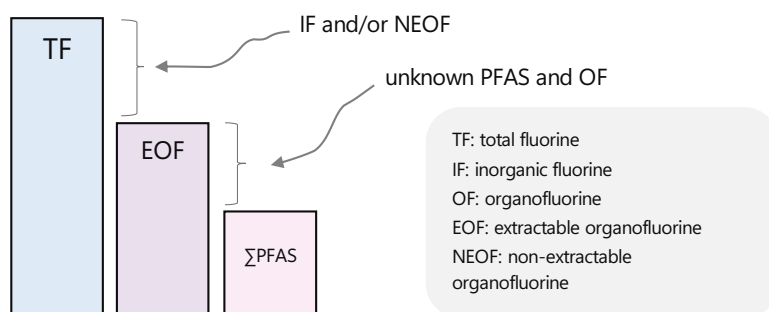


Figure 2 – The fluorine mass balance and what explains the gaps between measurements.

EOF (and by extension, the fraction of non-EOF). Current FMB studies employ procedures designed for capturing (semi-) polar PFAS, but other PFAS classes may be overlooked. For example, fluorinated polymers will contribute to the TF but they are not extractable, while neutral organofluorines might be only partially extractable with current methods (Zweigle et al., 2023a). Large gaps between TF and EOF in the absence of IF can therefore indicate the presence of non-extractable OF, while significant unknown EOF concentrations indicates the presence of novel PFAS, which can potentially be identified using HRMS.

1.3 Discovery of novel PFAS and expanding the investigated chemical space

1.3.1 Novel analytical tools.

Analysis using liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS), typically using triple quadrupole mass spectrometers, is a cornerstone of PFAS research due to its sensitivity and selectivity. However, for discovery of novel PFAS (i.e. via suspect and non-targeted screening (NTS) workflows), acquisition of full scan, accurate mass/high resolution data requires HRMS. LC-based Orbitrap and time-of-flight instruments have been extensively used for this purpose, with a focus on identifying polar and semi-polar species (e.g. PFAAs and PFAA-precursors). Less non-targeted screening work has been done with gas chromatography (GC)-based HRMS. As pointed out by Newton et al. (2025), only a small fraction (i.e. 10% of ~12 000 PFAS) are amenable to LC-ESI-MS analysis, highlighting the need for more GC-based NTS research on PFAS.

GC-MS employing electron ionisation (EI) at 70 eV is used extensively for suspect screening due to its very reproducible fragmentation. However, this approach is limited by both the availability of library mass spectra, and, in the case of non-target screening, often by a lack of molecular ions needed for *de novo* structure elucidation. Alternatively, atmospheric pressure chemical ionisation (APCI) is a soft-ionisation where the (quasi)molecular ion is usually the most abundant, and this technique is gaining increasing popularity for GC-based non-target and suspect screening of environmental contaminants (MacNeil et al., 2022; Shi et al., 2025; Singh et al., 2023). In APCI, reagent gas molecules, typically nitrogen, are ionised by electrons produced by the corona discharge needle in the source (Fang et al., 2020). These ionised reagent molecules then react with analytes, undergoing charge transfer to form radical cations. In the presence of water, charge transfer occurs to form H_2O^+ , followed by a series of reactions that ultimately result in proton transfer to the analytes.

Finally, ion mobility spectrometry (IM) represents a semi-orthogonal approach for suspect- and non-target screening of PFAS which can be coupled to either GC- or LC-instrumental workflows. In IM, ions travel through a gas under an electric field, where larger molecules will have more collisions with the

gas, resulting in a longer drift time through the mobility cell compared to smaller/more compact ones. This enables the determination of collision cross section (CCS) values, which are instrument-independent physicochemical parameters that describe the effective area of an ion. CCS values are particularly useful for non-target prioritisation of halogenated substances (see next section) as they provide additional separation based on analyte size and shape.

1.3.2 Data processing workflows for suspect and non-target screening

Both suspect and non-target screening involve analysing samples in a manner which avoids targeting only specific chemicals. The difference between the two techniques lies primarily in how the data are handled. In suspect screening, the data acquired from a sample are searched against a library, typically consisting of a list of accurate masses for (quasi) molecular ions (e.g. NORMAN Suspect List Exchange) or fragmentation patterns (e.g. NIST electron ionisation database). In contrast, NTS can be used to discover entirely new PFAS (true unknowns) by analysing the full-scan HRMS data for indications of the presence of fluorinated moieties or so-called feature “prioritisation strategies”, which involve using statistical approaches (e.g. case-control, time trend, etc.) to reduce the size of the dataset. The prioritisation strategies used in the present work are explained in the following paragraphs.

One common approach for prioritising organochlorine and -bromine substances is to take advantage of their multiple abundant stable isotopes: ^{35}C and ^{37}Cl occurring at 76 and 24% abundance, respectively, and ^{79}Br and ^{81}Br , occurring at 51 and 49% abundance, respectively. Using these distinct isotopic patterns, the total number of Cl or Br atoms in the molecule can be deduced. However, since fluorine only has a single stable naturally occurring isotope, its isotopic pattern cannot be used for prioritising organofluorine compounds. Consequently, other prioritisation approaches have been developed for PFAS.

Homologous-series based prioritisation takes advantage of the tendency of some PFAS to occur in subclasses displaying a common head group (e.g., $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{SO}_2\text{NH}_2$) and differing by a repeating unit (e.g., CF_2 , C_2F_4 , CF_2O). For uncovering these related masses, one can use Kendrick mass defect (KMD) plots using CF_2 and/or other repeating units for normalisation. Molecules belonging to the same subclass will have the same KMD and will appear in a line in a KMD vs m/z plot (Bugsel et al., 2023; Liu et al., 2019; Strynar et al., 2023). Another approach is to use specialised tools such as the R package 'nontarget', which systematically analyse the data to extract peaks that differ in constant m/z units as well as retention time (Loos & Singer, 2017). However, this prioritisation approach is limited by the requirement that two or more homologues are present, with ease of discovery and confidence increasing with the number of homologues present.

In mass spectral fragmentation, PFAS often generate characteristic fragments that are either fluorinated (e.g. $C_2F_5^-$, $C_3F_7^-$) or related to common functional groups. These fragments can be screened for in MS^1 data (in the case of in-source fragments; as in Liu et al., 2015), or in MS^2 data (Strynar et al., 2023). Prioritising the annotation of features that yield these diagnostic fragment ions can help identify PFAS among many other compounds. The same can be done for neutral losses or typical mass differences (e.g. neutral loss of HF or mass difference of CF_2), which allows prioritisation of unknown compounds based on their MS^2 (Bugsel & Zwiener, 2020).

A useful property for prioritisation of PFAS is the mass defect (MD), which refers to the difference between the exact monoisotopic mass of a compound and its nearest integer mass. Fluorine-19 has a monoisotopic mass of 18.9984 Da, resulting in a slightly negative mass defect of -0.0016 Da. As the number of fluorine atoms in a molecule increases, the mass defect shifts further into the negative range. This trend enables rapid filtering of potential PFAS candidates in complex HRMS datasets (Figure 3a), as most naturally occurring compounds exhibit positive mass defects (Strynar et al., 2023). At the same time, many false positives can occur due to the negative MD of other commonly encountered heteroatoms in organic compounds (e.g. oxygen, sulphur and phosphorous).

Another recently developed approach for halogen prioritisation (including PFAS) involves CCS. Organohalogen compounds tend to be quite heavy but relatively compact in size, resulting in lower CCSs compared to hydrocarbons of similar mass (Foster et al., 2022). Substituting a hydrogen with a fluorine atom results in a significant increase in mass but not in size, so the relationship of CCS to m/z can be used to filter large datasets. A threshold at $CCS (\text{\AA}^2) < 0.2 \text{\AA}^2 \times m/z + 100 \text{\AA}^2$ has been shown to greatly minimise false positives when prioritising PFAS candidates (MacNeil et al., 2022; Figure 3b).

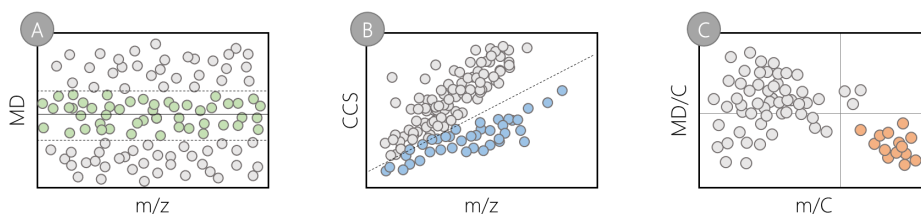


Figure 3 – Visual representation of three prioritisation strategies for PFAS. **A)** PFAS tend to have negative mass defect, usually features within an MD range (dashed lines) that is closely around 0 (horizontal line), are prioritised. **B)** CCS values are plotted as a function of m/z , the $0.2 \text{\AA}^2 \times m/z + 100 \text{\AA}^2$ threshold is represented by the dashed lines, features below this line are prioritised as fluorinated. **C)** The ratio of mass defect to number of carbons (MD/C) is plotted versus the ratio of m/z to number of carbons (m/C), PFAS tend to have high m/C and low MD/C, often below 0, and occupy a specific area of the plot in the bottom right. Features above a certain m/C and below a certain MD/C can be prioritised.

Another related characteristic of PFAS that can be leveraged for prioritisation is their high mass compared to small number of carbons, originating from the presence of several fluorine atoms. This means that their mass-to-carbon ratio (m/C) is quite large, and plotting mass defect-to-carbon ratio (MD/C) vs. m/C can discriminate fluorinated compounds in a large data set (Kaufmann et al., 2022; Zweigle et al., 2023b; Figure 3c). The number of carbon atoms of a detected feature in mass spectrometry data can be estimated from the relative abundance of the $M + 1$ isotope given by the presence of ^{13}C ($C = I_{M+1}/I_M/0.011145$).

Together, these prioritisation strategies enhance the detection and classification of both known and unknown PFAS in environmental samples, improving non-targeted workflow efficiency and contributing to a more comprehensive fluorine mass balance assessment.

1.4 Closing the organofluorine mass balance in the absence of chemical standards

Quantifying PFAS without analytical standards remains a major challenge, limiting our ability to assess exposure and risk. Despite the large number of PFAS detected via liquid chromatography high-resolution mass spectrometry (LC-HRMS), the lack of standards prevents accurate concentration estimation and leaves open the fluorine mass balance. To address this, one strategy has been to use a compound with a similar retention time as a proxy, assuming comparable ionisation behaviour. Another widely applied method relies on homologue-based quantification, where a structurally related PFAS with an available standard serves as a reference. For instance, the concentration of perfluoroheptanesulfonic acid (PFHpS) is often estimated using PFHxS due to their structural similarity (Li et al., 2020). However, these approaches introduce uncertainty since ionisation efficiency (IE) can vary significantly, even among closely related compounds (Kruve, 2020).

Recently, machine learning models have shown promise in predicting ionisation efficiency by incorporating molecular and chromatographic eluent descriptors (P. Liigand et al., 2021). Their accuracies in application, however, can hinge on the diversity of the training data. While these models have been successfully applied to a range of chemical classes, most of the training datasets have included few PFAS (J. Liigand et al., 2020). This could mean that the unique ionisation behaviour of many PFAS has not been fully captured, and further validation is essential. Incorporating more PFAS data into these predictive models would help address this gap, allowing the models to account for the distinctive structural features of PFAS—such as their high fluorine content—which could influence ionisation efficiency differently from more typical organic compounds. Advancing these predictive approaches could significantly improve PFAS mass balance calculations which in turn would enhance exposure assessments.

1.5 Characterising PFAS exposure in marine mammals

Marine mammals accumulate considerable amounts of anthropogenic chemicals over their long lives and are therefore recognised as sentinel species for chemical pollution in the marine environment (Plön et al., 2024). PFAS have been measured in these species globally, with e.g. concentrations reaching up to 19 500 ng/g in dolphin liver (Foord et al., 2024) and 4000 ng/g in polar bear liver (Spaan et al., 2020). Due to the strong affinity of PFAAs for proteins, PFAS accumulation has been mostly investigated in liver and blood, where protein binding is a dominant factor (Ng & Hungerbühler, 2013, 2014). Additionally, PFAAs exhibit increasing interactions with phospholipids as chain length increases and tend to accumulate in phospholipid-rich tissues of highly metabolic organs, such as the liver and brain (Dassuncao et al., 2019).

In contrast, fatty tissues like blubber have been relatively underexplored in PFAS research. While blubber primarily consists of triglycerides (neutral fats; Bories et al., 2021), recent evidence suggests it may also act as a significant reservoir for unknown PFAS. Specifically, Schultes et al. (2020) found the first evidence of high levels of unidentified EOF in blubber of a killer whale from East Greenland. Analyses of eight tissues showed that known PFAS followed expected distribution patterns (highest in the liver and blood), while the highest unknown EOF concentrations were detected in blubber. Given that blubber can constitute up to 50% of the body mass in certain cetacean species at specific life stages (Lockyer, 1976; Perrin et al., 2009), neglecting this tissue could lead to a substantial underestimation of total exposure to organofluorine compounds.

2. Hypotheses and objectives

The overarching hypothesis driving this thesis is that PFAS usually targeted in monitoring studies cover a small fraction of the organofluorine used and released by human activities to the environment. Moreover, the lack of analytical standards hinders us from understanding how much of the extractable organofluorine observed in samples is explained by compounds identified via suspect or non-target screening studies. Therefore, the objectives of this thesis were to:

- 1) Carry out fluorine mass balance studies on a variety of samples to determine the fraction of unknown extractable organofluorine;
- 2) Develop and validate a machine-learning quantification tool for closing the organofluorine mass balance in the absence of standards;
- 3) Investigate the occurrence of neutral extractable organofluorine and
- 4) Identify novel PFAS using suspect and non-target screening approaches with high resolution mass spectrometry.

The results will contribute to identify emerging PFAS of concern that might need stricter control.

3. Experimental section

3.1 Overview of samples

For **Paper I**, three different components of artificial turf (backing, blades and infill) were collected from 17 football fields in Stockholm following a stratified sampling design which included different years of installation and different materials (plastic, rubber and organic materials).

For **Paper II**, liver of 8 white-beaked dolphins (3 from Sweden and 5 from Greenland) and 5 pilot whales from Greenland were studied. For **Paper III** we obtained blubber from 4 killer whales (3 from Greenland, 1 from Sweden). One of these Greenland killer whales had been already characterised in a previous study for EOF and target PFAS, revealing high concentrations of unknown EOF (Schultes et al., 2020); blubber was reanalysed here, as well as liver. All marine mammal samples were provided by the Swedish Museum of Natural History or the Greenland Institute of Natural Resources. Marine mammals are sentinel species for the monitoring of persistent organic pollutants in the marine environment and therefore can provide insight into novel persistent and mobile contaminants reaching remote areas of the globe.

For **Paper IV**, sludge from the Henriksdal municipal wastewater treatment plant (Sweden), and NIST reference material (dust and sludge) were studied. Sludge and dust offer an integrated snapshot of chemicals used for commercial and industrial purposes, but also in consumer products, for example cosmetics, textiles, electronics, as well as pesticides and pharmaceuticals.

3.2 Sample extraction

Measurements of TF did not require any sample preparation; therefore, portions of sample materials were weighed directly onto ceramic sample carriers (“boats”) for CIC analysis. The same extract was used for EOF determination by CIC, target PFAS and suspect/non-target analysis. Extractions were carried out with either acetonitrile and ultra-sonic bath (**Paper I**) or bead blending (**Paper II and III**). Samples were extracted twice, the aliquots combined, concentrated and cleaned with ENVI-Carb (**Papers I, II**) or subjected to lipid and ionic compounds removal (**Paper III**). The extracts were then split and used for EOF analysis via CIC (**Papers I to III**) and LC-MS/MS target analysis (**Paper I**), LC-HRMS target and suspect screening (**Paper II**), or GC-APCI-IM-HRMS (**Paper III**). Sludge and dust studied in **Paper IV** were extracted for PFAS (methanol and ultrasonication) and cleaned with ENVI-Carb, then analysed by LC-ESI-IM-HRMS. They had been previously characterised for EOF (Spaan et al., 2023).

3.3 Sample analysis

Methods for determination of TF and EOF with CIC were based on previous publications and were the same for all papers (Schultes et al., 2018). Briefly, a combustion oven HF-210 and absorption unit GA-210, both Mitsubishi, were coupled to a Dionex Integriion ion chromatograph (IC) from Thermo Fisher Scientific. Liquid (100-200 μ l) or solid (5-100 mg) samples were placed in pre-baked ceramic boats (containing quartz glass wool in the case of liquid samples) which were introduced in a quartz glass combustion tube, heated to 1100 °C and, with a flow of argon, water vapor and oxygen, the combustion gasses were bubbled through water in a gas absorption tube. An aliquot of this solution (50-200 μ l) was injected onto the IC equipped with an anion exchange column (2 \times 50 mm guard column Dionex IonPac AS19-4 μ m and 2 \times 250 mm analytical column Dionex IonPac AS19-4 μ m) operated at 35 °C with a gradient of aqueous hydroxide mobile phase ramped from 8 to 100 mM at flow rate of 0.25 ml/min.

For **Paper I**, target analysis was carried out by injecting 5 μ l onto an Acquity ultraperformance liquid chromatograph (UPLC) using an ethylene bridge hybrid (BEH) C₁₈ column (2.1 mm \times 50 mm, 1.7 μ m) coupled to a Xevo TQS tandem mass spectrometer (MS/MS) from Waters, operated in negative electrospray ionisation (ESI), multiple reaction monitoring mode. The mobile phase consisted of a mixture of water and acetonitrile at 90% and 10% (A) and at 99% and 1% (B), both with ammonium acetate at 2 mM. The following gradient elution program was used at a flow rate of 0.4 mL/min: 0-0.5 minutes, 10% B; 0.5-8 minutes, 80% B; 8-8.1 minutes, 100% B; 8.1-11 minutes, 100% B; 11-11.1 minutes, 10% B; 11.1-13 minutes, 10% B. The column temperature was 30°C.

Target analysis and suspect screening for **Paper II** were carried out simultaneously using a Dionex Ultimate 3000 RS UHPLC system (Thermo Scientific) coupled via ESI source to a Q-Exactive™ Orbitrap™ mass spectrometer. LC conditions were the same as in paper 1. The MS was operated in negative ionisation, Full Scan (200-1800 m/z) Data-Dependent MS² mode, based on an inclusion list of molecular ions for 324 known PFASs compiled from literature on marine mammals and marine birds.

The non-target analysis using GC-APCI-IM-HRMS in **Paper III** was carried out using the SELECT SERIES™ Cyclic™ IMS (Waters). The ion mobility cell was operated with nitrogen, a traveling wave (TW) of 15 and 5 pushes per bin (ppb). Wet source conditions were used. Extracts (1 μ l) were injected in pulse splitless mode using a programmed inlet temperature for vaporisation. Analytes were separated on a 30 metre column with helium carrier gas at a constant flow of 1.5 mL min⁻¹. The GC oven temperature programme was 70 °C for 1 min; increased at 10 °C min⁻¹ to 310 °C, and then held for 15 min (for a total run time of 40 minutes). The MS was operated in the MS^E mode (100-1200 amu) with a collision energy

of 6 eV and 15-50 eV at the low and high energy mode, respectively. The scan time was 0.3 s for each mode.

For **Paper IV**, NTS using negative LC-ESI-IM-HRMS was carried out on the same IM instrument as for paper III (with the same IM settings), while LC specifications were the same as for paper I and II.

3.4 Suspect and non-target screening

In **Paper II**, suspect screening was implemented using a data processing method in TraceFinder™. Compounds with peak heights exceeding 10 000 cps were considered significant. The MS² spectra of each suspect were scrutinised for characteristic fragments, which were then compared with literature data to increase confidence in the annotation. Additionally, for compounds belonging to a homologous series, an increasing retention time with chain length served as an additional criterion for annotation confidence.

In **Paper III**, features detected in blubber were selected for further investigation if their CCSs were lower than one-fifth of their $m/z + 100 \text{ \AA}^2$, with $150 \text{ \AA}^2 < \text{CCS} < 250 \text{ \AA}^2$. The resulting features were then further prioritised if $m/z > 400 \text{ Da}$, a threshold proposed for prioritising bioaccumulative PFAS, and mass defect between -0.1 and +0.05 Da. Finally, the most intense features were manually inspected and annotated and if a structure was assigned, the presence of its homologues was manually checked. For confirmation and quantification, custom-synthesised standards of confidently annotated compounds were purchased.

In **Paper IV**, features detected in sludge and SRMs extracts were prioritised for further investigation when three criteria were fulfilled: $\text{CCS} (\text{\AA}^2) < 0.2 \text{ \AA}^2 \times m/z + 100 \text{ \AA}^2$ (with $100 \text{ \AA}^2 < \text{CCS} < 250 \text{ \AA}^2$), when $-0.1 < \text{MD} < 0.05$ and finally if $30 < m/C < 80$ and $\text{MD}/C < 0.003$. The approach was first evaluated using a mixture of PFAS standards, and thereafter applied to samples. The features prioritised by all three approaches were further investigated for homologous series of the following repeating units: C_nF_{2n} ($n=1,2,3$), CF_2O , $\text{C}_2\text{F}_2\text{O}$, $\text{C}_2\text{F}_3\text{H}$, $\text{C}_2\text{F}_2\text{H}_2$ and C_2FH_3 , using the R package 'nontarget' (Loos & Singer, 2017). Series with ≥ 3 homologues were further inspected. Prioritised features also underwent suspect screening.

Confidence levels (CLs) were assigned based on the five-level identification framework proposed by Schymanski et al., (2014), incorporating CCS values as an additional identification parameter. In this system, Level 1 represents confirmed structure identification with reference standard matching across multiple parameters (e.g., retention time, MS/MS, and CCS). Level 2 indicates probable structure identification, often supported by spectral library/literature information (2a) or diagnostic evidence

(2b). Level 3 refers to tentative candidates where some structural information is available, but ambiguity remains. Level 4 is used when only molecular formula information can be assigned, while Level 5 represents features of interest with no further structural information.

3.5 Machine-learning based quantification

In **Paper II**, the scope of a previously published quantification model (J. Liigand et al., 2020) was expanded by incorporating measurements of 33 PFAS standards into the dataset used for training and validation of the model. The original dataset was based on 100 unique chemicals measured under different experimental conditions, resulting in 1286 datapoints. The chemical structures were translated into numerical molecular descriptors for training the model. A gradient-boosted tree model was then developed to predict log ionisation efficiency ($\log IE$) in ESI negative mode by using 80% of data for training the model and 20% for evaluation. Thereafter, the model was applied to quantify PFAS lacking an analytical standard detected via suspect screening in marine mammal livers.

4. Results and discussion

4.1 Unextractable PFAS in plastic and rubber of artificial turf (Paper I)

TF was detected in all 51 artificial turf (AT) samples, with concentrations ranging from 16 to 313 $\mu\text{g F/g}$ in the backing, 12 to 310 $\mu\text{g F/g}$ in the filling, and 24 to 661 $\mu\text{g F/g}$ in the blades. In contrast, EOF and target PFAS were present in less than 42% of the samples, with measured levels below 200 ng F/g and 1 ng F/g, respectively. Water-based extractions confirmed the absence of fluoride in the samples and the total oxidisable precursor assay (TOPA) yielded minimal PFAAs formation across all sample types, suggesting that the fluorinated compounds in AT are not PFAA precursors or side-chain fluorinated polymers. These findings point to the presence of polymeric organofluorine species, such as fluoroelastomers, PTFE, or polyvinylidene fluoride (PVDF), in line with information from patent literature.

The observed recalcitrance to extraction and oxidation suggests that the fluorine content in AT is unlikely to pose an immediate exposure risk to users. However, concerns regarding the environmental impact associated with AT manufacturing, disposal, and the potential release of microplastic particles from the filling and blades should be further explored. Moreover, AT represents a case study of the pervasive presence of PFAS in plastic materials and illustrates a broader challenge of contamination of the plastic waste stream, making PFAS problematic in the context of the circular economy envisioned by the EU.

4.2 Closing the organofluorine mass balance using machine learning (Paper II)

HRMS-based suspect and non-target screening methods have recently revealed an ever-growing number of novel PFAS in the environment. While these advanced screening techniques have greatly expanded current knowledge of PFAS diversity, the lack of corresponding analytical standards for many compounds renders it challenging to accurately determine their contribution to overall PFAS exposure. This gap can hinder effective risk assessment and regulatory decision-making.

To address this limitation, recent advances in machine learning for predicting *IE* have provided a possible solution for quantifying suspect compounds without authentic standards. In the present study, a gradient-boosted tree-based model was developed and validated to predict the logarithm of *IE* ($\log IE$) in negative ESI mode. The model was trained and evaluated using a previously used dataset of measurement on 100 different compounds combined with additional data from analysis of 33 PFAS standards. Its performance was evaluated using root-mean-square errors (RMSE), it achieved RMSEs of 0.43 and 0.79 $\log IE$ units for training and test sets, respectively—corresponding to average

deviations within a factor of 2.7–6.2. Importantly, performance on the PFAS subsets was significantly better, with an RMSE of 0.26 for the training set and 0.29 log IE units for the test set (2× error), suggesting the model is well-suited for PFAS applications.

Subsequently, the model was applied to quantify suspect PFAS compounds in liver samples collected from marine mammals—specifically, pilot whales from East Greenland (n = 5) and white-beaked dolphins from West Greenland (n = 5) and Sweden (n = 3). While the Greenland dolphins had a closed FMB, other samples contained a substantial fraction (13-70%, median: 32%) of unidentified extractable organofluorine, along with 35 suspect PFAS compounds annotated with varying CLs (2–4). By employing *IE*-based quantification, the fraction of unidentified organofluorine was reduced to between 0%-27% (median: 17%), demonstrating the potential of this approach to improve fluorine mass balance estimates even in the absence of analytical standards.

Overall, these results illustrate that the integration of machine-learning models with HRMS data not only enhances the quantification of PFAS in complex matrices but also improves exposure assessments. This advancement holds considerable promise for future environmental monitoring and risk management strategies for these persistent contaminants.

4.3 Novel lipophilic PFAS (Paper III)

The recent detection of high levels of unidentified EOF in the blubber of a Greenland killer whale suggests that, despite current paradigms, certain fluorinated compounds preferentially accumulate in storage lipids and not in protein or phospholipids rich tissues (Schultes et al., 2020). Building on this initial observation, the same killer whale plus three additional killer whales (two from Greenland and one from Sweden) were further investigated in **Paper III**. Blubber from all four specimens was extracted with acetonitrile, extracts underwent lipid-removal and were analysed using CIC for EOF determination and by GC-APCI-IM-HRMS for NTS. By applying a CCS filter, an MD filter and a *m/z* filter, molecular features indicative of high fluorination were prioritised and five were selected for manual annotation.

Custom-synthesised standards confirmed the presence of 5 fluorotelomer sulfones (Figure 4) in all 3 of the Greenland blubber samples: 10:2 and 12:2 fluorotelomer methylsulfones ($C_{13}H_7F_{21}SO_2$ and $C_{15}H_7F_{25}SO_2$; 15.1-72.5 ng/g), 10:2 and 12:2 fluorotelomer chloromethylsulfones ($C_{13}H_6ClF_{21}SO_2$ and $C_{15}H_6ClF_{25}SO_2$; <0.3 to 1.1 ng/g), and 6:2 bisfluorotelomer sulfone ($C_{16}H_8F_{26}SO_2$; 3.6-6.2 ng/g). Together they account for 34–75% of the total EOF in blubber. No fluorotelomer sulfones and EOF were observed in the fourth killer whale found in Sweden. None of these substances were detected in liver, reinforcing the hypothesis of preferential partitioning into storage lipids. This represents the first

identification of fluorotelomer sulfones in wildlife and the first documented occurrence of highly fluorinated, lipophilic PFAS. Moreover, given the larger percentage of body mass of a marine mammals that the blubber constitutes, the body burden of these substances could exceed that of more well-studied PFAS such as PFAAs.

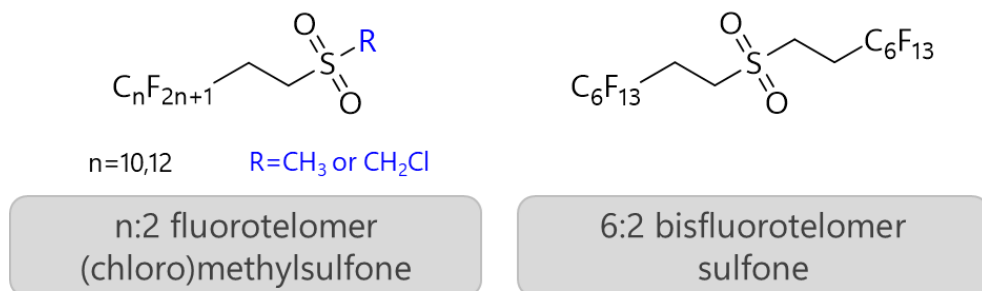


Figure 4 – Structures of novel fluorotelomer compounds found in the blubber of killer whales by GC-APCI-IM-HRMS.

4.4 Nontarget PFAS screening using ion mobility (Paper IV)

In **Paper IV**, the unknown EOF fraction in sewage sludge (73%) from Henriksdal wastewater treatment plant and certified reference materials of dust and sludge were investigated using non-target screening by LC-ESI-IM-HRMS. A workflow based on three criterion was applied to prioritise features likely to be fluorinated. The first filter was based on a mass defect range characteristic of many PFAS ($-0.1 < MD < 0.05$). The second was based on ion mobility, and only features where $CCS (\text{\AA}^2) < 0.2 \text{\AA}^2 \times m/z + 100 \text{\AA}^2$, with $100 \text{\AA}^2 < CCS < 250 \text{\AA}^2$, were kept. The third prioritisation uses the first isotopic (containing one ^{13}C) to monoisotopic (containing only ^{12}C) peak intensity ratio to estimate the number of carbons in the molecule, then the ratio of mass to carbon is calculated (m/C), since PFAS have few carbons but high masses due to the presence of several fluorine atoms, features were prioritised if $30 < m/C < 80$ and $MD/C < 0.003$.

A mixture of PFAS standards was used for evaluating the workflow and of the 36 detected PFAS, 32 matched all three of the prioritisations. Two were excluded by the CCS filter: one because it was above 250\AA^2 and another because it was slightly above the threshold. Four were excluded by the MD/C - m/C filter, even if theoretically they should not have been: the first monoisotopic peak was either falsely too high or too low, leading to over- or underestimating the number of carbon atoms.

After blank subtraction, LC analysis revealed 41,206 features, a total of 7,440 of which matched one or more prioritisations, and 565 of which matched all three and were further investigated for the presence of homologous series and by suspect screening. The presence of 36 PFAS at CL 1-2b, including perfluoroalkyl acids, their precursors, and their branched isomers, was validated in Henriksdal sludge and SRMs of dust/sludge. Additionally, 39 features were assigned CL 3-5, and many were present as homologous series, among them are perfluoro sulfinic acids, unsaturated and H-substitute PFAS.

Overall, this study demonstrated the advantage of combining several prioritisation strategies in order to remove >98% of features from the initial dataset. Such a reduction makes it possible to minimise data processing time for suspect screening and homologous series extraction as well as minimise workload for the analyst manually evaluating matches, series and fragmentation information, which is greatly beneficial for application in complex matrixes.

5. Conclusions and future perspectives

The overarching hypothesis that targeted PFAS in monitoring studies cover a small fraction of the organofluorine used and released by human activities to the environment was confirmed throughout all chapters of this thesis. Fluorine mass balance experiments revealed a significant fraction of unknown EOF in artificial turf, marine mammals (blubber and liver), sludge and dust samples or SRMs. HRMS-based suspect and NTS methods revealed novel PFAS, including neutral substances, and an ionisation efficiency prediction tool for quantification of PFAS in the absence of standards was trained and validated.

Paper I revealed the presence of high levels of non-extractable fluorine in AT components, and all analytical results and patent literature point to the presence of fluoropolymers. This study highlights the importance of total fluorine methods for screening of consumer products containing non-extractable PFAS, which are otherwise hard to analyse. Moreover, it sheds light on the widespread use of PFAS in plastic production and the need for stricter controls on PFAS use in plastics, in order to ensure safer and more sustainable waste management strategies aligned with the European Union circular economy goals.

In **Paper II**, marine mammal liver samples were characterised for EOF, target and suspect PFAS. Results showed significant concentrations of unknown EOF in certain samples, and a number of features were confidently annotated or assigned a possible structure. Usually, the contribution to the FMB of other PFAS annotated during suspect screening remains unknown. To bridge this gap, a machine-learning model was trained to predict ionisation efficiency based on eluent and molecular descriptors. Data derived from analysis of PFAS standards was collated to a previously gathered dataset of 100 substances and used to train and validate a model for ionisation efficiency prediction ($\log IE$) that allows quantification without the use of standards. When applied to the samples, IE -based quantification reduced the unknown EOF to 0-27%, revealing that the suspects possibly covered a significant portion of the unknown EOF but a gap still exists.

In **Paper III**, the unknown EOF in blubber of killer whales was investigated using GC-APCI-IM-HRMS and non-target screening. CCS, MD and m/z filters were applied to prioritise features and several novel fluorotelomer sulfones were confidently annotated and then ultimately confirmed and quantified using custom-synthesised standards. The newly discovered PFAS accounted for 34–75% of the total EOF in blubber. The results underscore how the investigated chemical space for PFAS needs to be expanded by using different tools in order to fully capture PFAS exposure. Moreover, this is the first report of highly fluorinated lipophilic PFAS and it challenges the paradigm that most PFAS bioaccumulate through protein and phospholipids interaction.

Non-target screening was carried out in **Paper IV** using LC-ESI-IM-HRMS. A prioritisation workflow for PFAS based on MD, CCS and MD/C-m/C was proposed and evaluated on a standard mixture resulting in very good performance with few exceptions. When applied to samples of sewage sludge and certified reference material (sludge and dust) with significant unknown EOF, the workflow prioritised 565 (1.34%) of the 41206 initial features. Of these 30 were identified at CL 1 and 45 were annotated at CL 2b-5.

Overall, this thesis leveraged CIC and HRMS to comprehensively characterise different sample types. Screening samples using CIC analysis is highly valuable as an initial step, providing insight into the overall fluorine content and identifying where significant portions of unknowns require further investigation. Although identifying all fluorine measured by CIC remains challenging, suspect and non-target screening are powerful approaches, and machine learning can serve as a crucial link between putative identification and quantification in the absence of analytical standards. In some cases, analytical results may only be able to exclude certain PFAS classes or inorganic fluorine, as it was the case in Paper I, and qualitative knowledge and a holistic approach (e.g. patent literature, knowledge of industry practices) can be essential in assigning the most likely PFAS candidates.

Another key consideration is the need to expand the investigated chemical space, as recent findings suggest that a significant portion of PFAS is more amenable to GC-based analysis rather than the more commonly used LC-based methods (Newton et al., 2025). This shift in analytical focus could be especially relevant for future exposure studies, as the detection of non-polar PFAS in killer whale blubber in this thesis demonstrated that exposure may have been underestimated. Future studies should also investigate different extractions to enhance the recovery of these underrepresented PFAS classes. Complementary approaches, such as fractionation of extracts prior to analysis or employing CIC at different stages of sample clean-up, could also aid in tracking fluorine when concentrations are high enough for reliable detection.

One major step toward improving the utility of NTS data is the development and validation of machine learning models for quantification. The *IE* prediction model trained in this thesis represents a key advancement in this direction, allowing for semi-quantitative estimates of PFAS concentrations even in the absence of analytical standards. Expanding such models with additional training data, case studies, as well as across diverse ionisation methods and conditions, could enhance their applicability, making NTS more reliable for exposure assessments and regulatory decision-making. If robustly validated, these models could help regulatory bodies act on NTS findings with greater confidence and speed.

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