Ministry of Environment and Gender Equality
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Further development of analytical methods for the monitoring of PFAS in environmental, food and human samples

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Preface

This report presents one of seven knowledge building projects, which were launched in 2024 following the report "Begrænsning af menneskers og miljøets eksponering for PFAS i Danmark – Del 1: Identifikation af vidensshuller" (Baun et al., 2024) of the Danish Knowledge Taskforce for PFAS. In the report, the Knowledge Taskforce identified existing knowledge gaps within the PFAS area and proposed twelve knowledge building projects that address some of these knowledge gaps. It was decided to initiate seven of these projects in 2024, as listed below.

Original project number and project title

Project 3: PFAS in residual products for agricultural use

Project 4: Screening of different types of food and feed for PFAS content

Project 5: Plan for biomonitoring for PFAS in the Danish population

Project 6: Contribution of different exposure pathways to the total human exposure to PFAS

Project 8: Further development of analytical methods for PFAS monitoring of environmental, food and human samples

Project 9: Conceptual model for transport and fate of PFAS at contaminated sites

Project 10: Diffuse pollution and pre-existing concentrations of PFAS

The Danish Knowledge Taskforce for PFAS was established in August 2023 with the aim of collecting the existing knowledge on PFAS both nationally and internationally. Based on the available knowledge, the expertise of the Knowledge Taskforce and the results from the described knowledge building projects, the Knowledge Taskforce has in their concluding report suggested a series of actions, which can form the basis for the authorities' future focus and efforts against PFAS pollution.

The Knowledge Taskforce is an independent expert group with the Danish Environmental Protection Agency as secretariat. The Knowledge Taskforce has the following members: Professor Anders Baun, Technical University of Denmark (chairperson); Chief physician Ann Lyngberg, Department of Occupational and Social Medicine, Holbæk Hospital; Professor Anne Marie Vinggaard, Technical University of Denmark; Associate Professor Bjarne W. Strobel, University of Copenhagen; Deputy Head of Department John Jensen, Aarhus University; Professor Katrin Vorkamp, Aarhus University; Professor Poul L. Bjerg, Technical University of Denmark; Professor Tina Kold Jensen, University of Southern Denmark; Associate Professor Xenia Trier, University of Copenhagen.

The present project “Further development of analytical method for PFAS monitoring of environmental, food and human samples” is described as project no. 8 in Baun et al. (2024). The project was carried out in the period 11 April 2024 – 20 December 2024.

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1. Introduction

1.1 Background

Per- and polyfluorinated alkyl substances (PFAS) are a complex group of several thousand individual compounds (Glüge et al., 2020). Buck et al. (2011) originally defined PFAS as all aliphatic compounds with a -C_nF_{2n-1} moiety. This definition was recently revisited by an expert group under the Organization of the Economic Cooperation and Development (OECD), who proposed a definition that included all fluorinated substances with at least one fully fluorinated methyl or methylene carbon atom (without H/Cl/Br/I attached to it), in order to include e.g. aromatic molecules or those with functional groups on both ends of the carbon chain (OECD, 2021). This definition excludes R-CF_2- structures where R = H, Cl, Br or I. It also excludes unsaturated fully fluorinated carbon atoms, as expressed by double bonds, for example in tetrafluoroethylene (CF_2=CF_2).

Given this complexity, the question arises how it can be best addressed in monitoring programmes, in terms of most suitable measurement parameters and analytical methods. Monitoring programmes have the general purpose of generating data to i) establish exposure levels in risk assessments, ii) compare with defined limit values in compliance checks and/or iii) document concentration developments over time, in particular related to effects of regulatory actions. In addition, monitoring data can provide important information in an "early warning" context, i.e. point at potential issues that need further action (Niarchos et al., 2024).

The majority of PFAS are commonly measured with liquid chromatography-tandem mass spectrometry (LC-MS/MS), typically including a number of perfluorocarboxylic acids (PFCAs), perfluorosulfonic acids (PFSAs) and sometimes neutral compounds such as perfluorooctane sulfonamide (PFOSA). Fluorotelomer alcohols (FTOHs) have been recognized as important volatile precursors to PFCAs and require analysis by gas chromatography-mass spectrometry (GC-MS). However, they are not widely included in monitoring programmes, due to their less persistent nature. Which PFAS are commonly analysed has historical reasons, related to the initial focus on perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), but is also related to the matrix in question. In general, little information exists of specific PFAS in use, which could help to prioritize which PFAS might be relevant to measure locally, while still ensuring comparability across locations.

In addition to these target analyses of specific PFAS, more comprehensive methods have been used in recent years and applied in "fluorine mass balance" approaches (Kärrman et al., 2021). The Total Oxidizable Precursor (TOP) assay uses LC-MS/MS techniques to focus on PFAS precursors. PFAS Total measurements aim at capturing e.g. the content of Total Fluorine (TF) and Extractable Organic Fluorine (EOF) in a sample and are usually based on combustion ion chromatography (CIC). The difference between EOF and target analyses often remains unidentified. New analytical techniques in non-target and suspect screening can help to elucidate these unknown (or suspected) compounds in a sample. Non-target and suspect screening are based on high resolution mass spectrometry (HRMS), generating detailed mass spectra and other information for comparison with databases and compound identifications.

Most monitoring programmes focus on target analysis of a set of pre-defined individual PFAS. In Denmark, PFAS are measured in marine and freshwater fish, groundwater, wastewater treatment plants and stormwater under the national monitoring programme for water and nature (NOVANA). PFAS are also monitored in drinking water and included in the monitoring of food and food contact materials. Human biomonitoring of PFAS is only established in few countries, for example in Canada (Government of Canada, 2024), the USA (Sonnenberg et al., 2023), Germany (Duffek et al., 2020) and as part of cohort studies (e.g. Berg et al., 2021; Lind et al., 2021). The European Human Biomonitoring Initiative HBM4EU included PFAS in serum samples from aligned national cohorts (Uhl et al., 2023). This approach is currently developed further in the Horizon Europe Partnership for the Assessment of Risks from Chemicals (PARC)[1].

[1] https://www.eu-parc.eu/

The PARC list includes seven mandatory and additional 15 voluntary PFAS. The proposed revision of the Water Framework Directive of the European Union (EU) includes Environmental Quality Standards (EQS) for a set of 24 PFAS. The EU Drinking Water Directive recently included "PFAS Total" measurements with a corresponding parametric value of 500 ng/L as an alternative to the sum of 20 PFAS, which members have to comply with by January 2026 (EU, 2024). Thus, monitoring programmes differ in the prioritized list of PFAS, recognizing their main occurrence but potentially limiting comparability.

1.2 Terminology

TABLE 1 provides explanations for key terminology used in this report. The explanations reflect the current scientific and regulatory understanding of PFAS and are important for the approaches used in section 6 to quantify and characterise PFAS in environmental, food and human samples.

TABLE 1. Explanation of key terms used in this report

Terminology	Explanation
Sum of PFAS (ΣPFAS)	This term describes the sum of individual PFAS determined with target analysis in this study. The EU Drinking Water Directive defines "Sum of PFAS" as follows: Sum of substances that contain a perfluoroalkyl moiety with three or more carbons (i.e. $–C_nF_{2n}–$, n ≥ 3) or a perfluoroalkylether moiety with two or more carbons (i. e. $–C_nF_{2n}OC_mF_{2m}–$, n and m ≥ 1). This definition excludes ultra-short perfluoroalkyl acids with only two carbon atoms, such as trifluoroacetic acid (TFA). However, the EU Drinking Water Directive restricts "Sum of PFAS" to 20 compounds with 3-13 carbon atoms. In this report, we use "Sum of PFAS" and "ΣPFAS" as the sum of all PFAS determined with target analysis, for example also including longer-chain PFCAs.
PFAS Total	The total amount of all per- and polyfluoroalkyl substances in a sample. It implies the use of an indirect measurement technique that quantifies the fluorine content in a sample as a proxy for PFAS concentration.
Extractable Organic Fluorine (EOF)	A measure of the organic fluorine content that can be extracted from a sample, providing an estimate of PFAS Total.
Sum of PFAS converted to fluorine ($ΣF_{PFAS}$)	For comparisons of concentrations of ΣPFAS and EOF, the concentration of each PFAS in a sample was converted to the content of F according to Equation 1 (Chapter 6.3.3) and then summed for all PFAS.

1.3 Objectives

The primary objective of this project is to further develop analytical methods for PFAS with a view to their application in current or future monitoring programmes. Specifically, the project has the following three aims with related activities:

- Discussion of PFAS lists used in different monitoring programmes across legislations
  The discussion will include questions of standardisation and potential extension of existing lists, also considering their use in products. It will also address potential challenges with currently applied analytical methods, including experiences with methods for PFAS Total.
- Tests of applicability of PFAS Total measurements in combination with target analyses.
  This part of the project has the long-term goal of establishing PFAS Total methods in Denmark. In addition, it is expected to provide data on PFAS Total in a selection of samples.
- Together with the first part of the project, proposals will be developed for PFAS monitoring.

The results of this project have been included in the work of the Danish Knowledge Taskforce for PFAS in 2024.

2. Approaches

The project has been based on a combination of the following methodological approaches:

- A workshop on PFAS monitoring strategies with European experts in the analysis of PFAS was held on 26th June 2024. The workshop included a list of questions to the experts about their experience with target analysis, non-target and suspect screening as well as measurements of PFAS Total, and their recommendations. A workshop report is given in Annex 1.
- Literature searches, including the scientific literature, product registers and information on PFAS in European and other international monitoring programmes.
- Target analysis and analysis of PFAS Total in selected samples, as described in detail in Chapter 6.

An advisory group was established for this project; its members and their affiliations are shown in Annex 2. The objectives and approaches were presented to the advisory group on 10th June 2024.

Preliminary results of the project were presented to the Danish Environmental Protection Agency (EPA) and the Danish Knowledge Taskforce on PFAS on 16th September 2024. Updated results were presented to the advisory group of the Danish Knowledge Taskforce for PFAS on 2nd October 2024.

3. PFAS in EU directives and relevant monitoring programmes

3.1 PFAS lists

Five European countries (Denmark, Germany, the Netherlands, Norway and Sweden) submitted a proposal to the European Chemicals Agency (ECHA) on 13th January 2023 that aims to reduce PFAS emissions to the environment and make products and processes safer for Europeans. According to this proposal, around 10,000 PFAS should be restricted to reduce environmental emissions and enhance safety, aligning with the EU's Chemicals Strategy and Zero Pollution plan. The restriction proposal was published on 7th February 2023, with a potential ban decision expected by 2025 and application by 2026 or 2027. Without action, 4.4 million tons of PFAS could enter the environment over the next 30 years (ECHA, 2023).

Despite regulatory restrictions and voluntary phase-outs, legacy PFAS continue to contaminate water bodies and other environmental compartments from sites with historical use (e.g. firefighting foams), landfills, and wastewater plants (Reinikainen et al., 2024). Additionally, many other PFAS compounds are still manufactured and used globally, with some restricted substances produced elsewhere and imported into the EU (Joerss and Menger, 2023). Consequently, PFAS pollution from both point and diffuse sources, including atmospheric deposition, is likely to persist even if a comprehensive EU ban takes effect. To address ongoing emissions and contamination risks to human health and the environment, the EU has been updating several legal frameworks, setting benchmarks and shifting from regulating individual chemicals to specific PFAS mixtures (Cousins et al. 2020; Reinikainen et al. 2024).

The **Drinking Water Directive (DWD)** is the primary **EU legislation** governing the quality and accessibility of drinking water. The DWD was recently updated, with the recast version EU 2020/2184 adopted in December 2020 (EC, 2020). It lists compounds that must be monitored in water due to their potential impact on public health or scientific concern, including PFAS. The recast of the directive introduces the monitoring of "PFAS Total" and "Sum of PFAS." As explained in TABLE 1, " PFAS Total" refers to the total amount of all PFAS, while "Sum of PFAS" refers to a list of 20 substances which all contain a perfluoroalkyl chain with at least three carbon atoms (CF_{n2n}, where n ≥ 3) or a perfluoroalkyl ether group with at least two carbon atoms ($CF_{n2n}OCF_{m2m}$, where n and m ≥ 1) (EC, 2020). Monitoring of these substances is required when risk assessments indicate their potential presence in water sources. The directive sets parametric values of 500 ng/L for "PFAS Total" and 100 ng/L for "Sum of PFAS" which includes the 20 target substances. Member States were required to transpose the provisions of the DWD into national law and ensure compliance by 12th January 2023. However, they are only obligated to meet the PFAS thresholds ("Sum of PFAS" or "PFAS Total") by 12th January 2026. Then, Member States can decide whether to apply the "Sum of PFAS" or the "PFAS Total" parameter.

The **US EPA** proposed in March 2023 a **National Primary Drinking Water Regulation** (USEPA, 2023) that would establish legally enforceable thresholds for six PFAS: PFOA and PFOS as individual substances and perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), perfluorobutane sulfonate (PFBS), and perfluoro-2-methyl-3-oxahexanoic acid (HFPO-DA) and its ammonium salt (commonly referred to as GenX chemicals) as a group (Table 2). The proposed Maximum Contaminant Levels are 4 ng/L for PFOA and PFOS individually, reflecting the "lowest feasible quantitation level" (USEPA, 2023). The GenX chemicals would have to be monitored individually, and their weighted concentrations (based on toxicity) should not exceed a Hazard Index of 1. The US EPA also proposes a non-enforceable advisory level of zero for both PFOS and PFOA (USEPA, 2023).

While the EU and the USA are tightening regulations on PFAS based on human health concerns, the **World Health Organization** proposed revised guidelines (WHO, 2022) that focused on minimizing treatment costs rather than aligning with health risk studies, raising concern among scientists (Ågerstrand et al., 2022). The WHO

draft document on revised guidelines for drinking water quality (TABLE 2) proposes to limit PFOS and PFOA to 100 ng/L individually (WHO, 2022).

The EU regulates pollutant levels in natural waters through the **Water Framework Directive** 2000/60/EC (EC, 2000), the Environmental Quality Standards Directive 2008/105/EC (EC, 2008), and the **Groundwater Directive** 2006/118/EC (EC, 2006). Since 2013, PFOS has been classified as a priority substance under the Water Framework Directive. In October 2022, the European Commission proposed new priority substances for surface and groundwater pollutants, including a threshold of 4.4 ng/L for a group of 24 PFAS (TABLE 2) in surface and groundwater, along with a limit of 77 ng/kg wet weight in biota for the same group (EC, 2022a). These thresholds are expressed as PFOA equivalents and use a Relative Potency Factor approach to account for the varying potencies of different PFAS when setting the group threshold.

The **European Food Safety Authority (EFSA)** guidelines on PFAS exposure have been significantly revised downward over the past 15 years. In July 2020, EFSA established the new safety threshold for a group of four PFAS—PFOA, PFOS, PFNA, and PFHxS—setting the Tolerable Weekly Intake (TWI) at 4.4 ng per kg of body weight per week. This threshold follows EFSA's guidance for assessing combined exposure to multiple chemicals. The European Commission also states in Recommendation (EU) 2023/915, updated from the 2022/1431, that Member States should test for related compounds with different alkyl chains (TABLE 2), such as perfluorobutanoic acid (PFBA) and PFBS, and for emerging PFAS (EC, 2022b). In the same recommendation, the European Commission advises that Member States, in collaboration with food business operators, monitor the presence of PFOS, PFOA, PFNA, and PFHxS in food during 2022–2025. If the indicative levels are exceeded— for example 10 ng/kg for PFOS and PFOA, 5 ng/kg for PFNA, and 15 ng/kg for PFHxS in fruits, vegetables (except wild fungi), starchy roots, and tubers—further investigation into the causes of contamination should be conducted. The recommendation also provides specific thresholds for wild fungi, milk, and baby food.

While the **Sewage Sludge Directive** (86/278/EEC) (EEC, 1986) regulates the use of sewage sludge in agriculture by setting limits on the concentration of heavy metals, it does not address organic contaminants such as PFAS. However, the EU Joint Research Centre found that significant risks to both humans and soil organisms may originate from a relatively small set of pollutants when present in concentrations levels typically documented for sewage sludge. These priority contaminants — e.g. polychlorinated dibenzodioxins (PCDDs) and -furans (PCDFs), polycyclic aromatic hydrocarbons (PAHs), long-chain PFAS and short and medium-chain polychlorinated paraffins — are persistent in soils and have bioaccumulative and toxic properties (Huygens et al., 2022). Thus, the upcoming evaluation of the Sewage Sludge Directive, along with subsequent studies, could present an opportunity to introduce limits for organic contaminants, including PFAS, during any review of the directive (European Commission: Directorate-General for Environment 2022). This could involve setting limits for "PFAS Total" or for specific PFAS compounds.

Some EU Member States, such as Denmark, have already established PFAS limits in sewage sludge used for agricultural purposes (Huygens et al., 2022). Denmark's regulations, which came into effect in October 2021, set two categories of PFAS limits: a maximum of 10 µg/kg dry matter for the sum of four PFAS (PFOA, PFOS, PFNA, and PFHxS); and a maximum of 400 µg/kg dry matter for the sum of 22 PFAS.

Nonetheless, it has been reported that PFAS losses to the environment via effluents are most likely the main contributor to the total human health risks associated with PFAS. PFAS in sludge may further enhance risks, but a detailed source contribution analysis that also considers wastewater treatment effluents is critical to develop effective mitigation strategies that tackle the root of the contamination issue (EurEau, 2022). In this sense, the European Council gave the final green light for a revised EU directive on urban wastewater treatment on November 5, 2024. Under the new **Urban Wastewater Treatment Directive** known viruses, emerging pathogens, chemical pollutants, including PFAS, microplastics and antimicrobial resistance will be monitored (EU 2022/0345(COD)).

Human biomonitoring programmes have been established in some European countries, but no legislation exists that require EU Member States to systematically monitor the level of chemical pollutants in the European population. The European Human Biomonitoring Initiative HBM4EU studied a variety of compounds, including PFAS, in aligned national biomonitoring initiatives (Gilles et al., 2021). Building on the HBM4EU experience, the Horizon Europe PARC project further pursues the harmonization of human biomonitoring in Europe. The PARC General Survey includes PFAS measurements in the serum of teenagers and adults, with a mandatory set of

PFAS including PFOA, PFNA, perfluorodecanoid acid (PFDA), PFBS, PFHxS, perfluoroheptane sulfonate (PFHpS) and PFOS. In addition, 15 compounds are suggested for voluntary measurements, including various PFCAs, PFSAs, some neutral PFAS and emerging PFAS such as GenX.

3.2 Potential for standardization

Standardization and harmonization in environmental monitoring is essential for generating accurate and comparable data across regions and over time. While standardization defines a method that all users follow, harmonized approaches foresee a flexibility of methods that lead to comparable results. Environmental monitoring typically uses a combination of both, for example standardizing sampling approaches, but allowing different instrumental analyses. Their comparability is then ensured through QA/QC measures, including proficiency testing systems. The question to discuss for PFAS is whether more standardization would enable regulatory bodies and researchers to assess contamination levels more consistently, supporting the development of effective, evidence-based policies.

Without standard protocols, diverse detection limits and methodologies can lead to discrepancies, which challenge trend analyses and potentially restrict the ability to fully comprehend the extent of contamination. However, too rigorous standards might inhibit developments as detection limits improve, and new analytical methods emerge.

In terms of directives for PFAS monitoring, standardizing a core list of PFAS compounds to be monitored across all matrices could enhance consistency and provide a more comprehensive view of PFAS dispersion and dissemination pathways. However, due to the range of physical-chemical properties, it will be beneficial to tailor monitoring approaches based on the compounds' behaviours in specific environmental compartments. For example, while compounds like PFOA and PFOS, which are detected in multiple environmental matrices, could be consistently monitored across all environmental compartments, short-chain PFAS with high mobility, but less bioaccumulative potential will be more relevant to monitor in water samples than in biota.

A practical approach could involve establishing a core list of PFAS compounds for cross-matrix monitoring and assessment, supplemented by matrix-specific lists that consider each compound’s physical-chemical properties and likely dissemination pathways. This balanced method would allow for a standardized yet flexible framework, maximizing the effectiveness of PFAS monitoring while accommodating the unique challenges of diverse matrices.

Considering this, a literature review on PFAS detection via targeted analysis across various matrices could help establish core PFAS groups and identify any additional PFAS that should be included in matrix-specific monitoring lists. However, the detection of PFAS in certain matrices may be affected by a selection bias, typically concentrating on a certain set of PFAS. In order to capture novel or emerging PFAS compounds, suspect and non-targeted screening approaches offer a valuable perspective, allowing the detection of less-known PFAS and emerging contaminants that might otherwise be overlooked.

TABLE 2. Summary of PFAS monitored under the different regulations/directives for natural waters, drinking water, biota, and food. Values indicate the recommended thresholds for PFAS.

PFAS		Natural waters	Drinking water			Biota	Food				
Abbreviation	CAS reg. no.	Amendment EU Directive 2000/60/EC [1]	EU Directive 2020/2184	US EPA (2023)	WHO (2022)	Amendment EU Directive 2000/60/EC [1]	Regulation (EU) 2023/915	Recommendation (EU) 2022/1431 [2] A	Recommendation (EU) 2022/1431 [2] B	Recommendation (EU) 2022/1431 [2] C	Recommendation (EU) 2022/1431 [2] D
PFBA	375-22-4	x	x			x		(x)	(x)	(x)	(x)
PFPeA	2706-90-3	x	x			x		(x)	(x)	(x)	(x)
PFHxA	307-24-4	x	x			x		(x)	(x)	(x)	(x)
PFHpA	375-85-9	x	x			x		(x)	(x)	(x)	(x)
PFOA	335-67-1	x	x	4 ng/L	100 ng/L	x	x*	10 ng/kg	10 ng/kg	10 ng/kg	50 ng/kg
PFNA	375-95-1	x	x	x		x	x*	5 ng/kg	5 ng/kg	50 ng/kg	50 ng/kg
PFDA	335-76-2	x	x			x		(x)	(x)	(x)	(x)
PFUnDA	2058-94-8	x	x			x		(x)	(x)	(x)	(x)
PFDoDA	307-55-1	x	x			x		(x)	(x)	(x)	(x)
PFTrDA	72629-94-8	x	x			x		(x)	(x)	(x)	(x)
PFTeDA	376-06-7	x				x		(x)	(x)	(x)	(x)
PFHxDA	67905-19-5	x				x					
PFODA	16517-11-6	x				x					
PFBS	375-73-5	x	x	x		x		(x)	(x)	(x)	(x)
PFPeS	2706-91-4	x	x			x		(x)	(x)	(x)	(x)
PFHxS	355-46-4	x	x	x		x	x*	15 ng/kg	15 ng/kg	60 ng/kg	50 ng/kg
PFHpS	375-92-8	x	x			x		(x)	(x)	(x)	(x)
PFOS	1763-23-1	x	x	4 ng/L	100 ng/L	x	x*	10 ng/kg	1500 ng/kg	20 ng/kg	50 ng/kg
PFNS	68259-12-1		x					(x)	(x)	(x)	(x)
PFDS	335-77-3	x	x			x		(x)	(x)	(x)	(x)
PFUdS	749786-16-1		x					(x)	(x)	(x)	(x)
PFDoS	79780-39-5		x					(x)	(x)	(x)	(x)

PFAS		Natural waters	Drinking water		Biota	Food				
PFTrS	749786-16-1		x				(x)	(x)	(x)	(x)
PFOSA	754-91-6						(x)	(x)	(x)	(x)
6:2 FTOH	647-42-7	x			x					
8:2 FTOH	678-39-7	x			x					
Fluorotelomer alcohols and sulfonates							(x)	(x)	(x)	(x)
HFPO-DA	13252-13-6			x						
GenX (or acid form)	62037-80-3	x		x	x		(x)	(x)	(x)	(x)
ADONA (or acid form)	958445-44-8	x			x		(x)	(x)	(x)	(x)
C6O4	151772-58-6	x			x					
F53B (acid form)	73606-19-6						(x)	(x)	(x)	(x)
Capstone A	80475-32-7						(x)	(x)	(x)	(x)
Capstone B	34455-29-3						(x)	(x)	(x)	(x)
Sum of PFAS		4.4 ng/L	100 ng/L	Max Hazard Index of 1	77 ng/kg wet weight	1.3 - 50 µg/kg [(3)]				
PFAS Total			500 ng/L							

Legend: *x – included; x* - individually or as a sum; (x) - not mandatory/if possible; (1) - thresholds are expressed as PFOA equivalents and make use of a Relative Potency Factor approach to account for the potencies of the different substances when setting the group threshold ; (2) - indicative values, if exceeded investigation on contamination causes should be carried out; A - fruits, vegetables (except wild fungi), starchy roots and tubers; B - wild fungi; C – milk; D - baby food; (3) – concentration varies depending on type of food, minimum and maximum values presented.*

4. Relevant PFAS for monitoring purposes

4.1 Scientific literature for core PFAS selection

For this project, a review of 32 scientific articles was included, of which ~84% were published within the last five years, and ~52% were published in 2024 (Annex 3). While this analysis provides some insights, it is important to note that it only considers a small subset of the extensive literature on PFAS monitoring and only for PFAS target analysis. The detected PFAS identified in this analysis may not necessarily be the most widespread ones in the environment or in human populations. Instead, their frequent appearance in studies more likely reflects a higher research focus due to their known persistence, regulatory interest, and potential health risks, potentially leading to the selection bias addressed in Chapter 3.2. Expanding this literature survey to include results, for example, from non-target analysis of PFAS which may help to identify new emerging PFAS, may be necessary to achieve a more accurate and comprehensive analysis of current PFAS trends. A more comprehensive summary of human biomonitoring data is given in the parallel project "Plan for biomonitoring of PFAS in the Danish population" (Raun-Petersen et al., 2024). A review of PFAS in food items is included in the parallel project "Contribution of different exposure pathways to the total human exposure to PFAS" (Fauser et al., 2024).

Bearing in mind this likely research bias, the most frequently detected PFAS were PFOS detected in 35 matrices, and PFOA and PFHxS detected in 33 matrices, indicating their widespread environmental and human presence (TABLE 3). PFNA was detected in 28 matrices, while PFDA was found in 26 matrices. Other frequently reported PFAS, such as perfluoroundecanoic acid (PFUnDA), perfluorohexanoic acid (PFHxA), and perfluoroheptanoic acid (PFHpA), were detected in 21–22 matrices.

The eight most frequently detected PFAS are included in most current regulations (TABLE 2). For PFOA, PFOS, PFHxS and PFNA, Recommendation (EU) 2022/1431 even establishes threshold levels in food, which, if exceeded, require an investigation into contamination sources, as Regulation (EU) 2023/915 covers these compounds. However, for the remaining four of the eight core PFAS—PFHxA, PFHpA, PFDA and PFUnDA—monitoring in food is not mandatory and is recommended if possible. Including these PFAS in routine food monitoring could provide more comprehensive data on their presence in food items. This, in turn, may support their eventual inclusion in future revisions of Regulation (EU) 2022/2388 if their profiles align with the criteria for regulatory consideration, such as exposure levels and potential health impacts.Despite the risk of circular reasoning, given the research bias, this overview confirms that the regulations have targeted PFAS which are prevalent in environmental, food and/or human samples and that are known for their persistence, bioaccumulation and potential health risks (

Overview by matrix for the eight core PFAS
PFAS detection in air, both indoor and outdoor, suggests that atmospheric transport significantly contributes to the environmental distribution of PFAS. The high detection frequency (average of 75%; total n = 112) and substantial concentration range (e.g., PFOA in the air up to 9730 pg/m³) reflect PFAS persistence in urban environments. Indoor dust often contains PFAS due to their use in consumer products, while outdoor dust may accumulate PFAS from industrial emissions and urban runoff. The high levels of PFOS and PFHxA in dust also suggest possible contributions from firefighting foams and industrial applications.

TABLE 4). By targeting these high-priority PFAS, regulations aim to reduce human exposure, which could help alleviate the significant health costs associated with PFAS exposure in Europe—estimated at €52-84 billion annually (Goldenman et al., 2019).

This limited literature review highlights that certain PFAS are omnipresent. While short-chain PFAS exhibit lower bioaccumulation potential, they are still widely detected in abiotic media, which further supports the use of matrix-specific PFAS lists for monitoring purposes. These eight most frequently detected PFAS (PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFHxS and PFOS) could be considered as the eight core PFAS to be monitored in all matrices that should be further complemented by matrix-specific compounds.

TABLE 3. Minimum and maximum concentration of the eight most reported PFAS in the reviewed literature and detection frequency (number of samples vary for difference matrices and the PFAS studied; references available in Annex 3).

		Min-Max concentration (Detection frequency in %)							
	Matrix	Short-chain PFCAs		Long chain PFCAs				PFSAs	
		PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFHxS	PFOS
Environ-mental	Air (pg/m^3)	0.50 - 493 (100)	0.001 - 185 (100)	4.4 - 9730 (100)	nd - 26.6 (95)	nd - 9.92 (68)	nd - 4.6 (37)	nd - 4.1 (42)	nd - 46 (79)
	Outdoor dust (ng/g)	0.15 - 108 (100)	nd - 82.3 (88)	4.29 - 8512 (100)	nd - 4.55 (94)	nd - 6.41 (94)	nd - 2.20 (82)	nd - 0.06 (41)	nd - 0.54 (88)
	Indoor dust (ng/g)	nd - 76.9 (62)	nd - 22.6 (54)	nd - 60.0 (77)	nd - 29.8 (64)	nd - 42.0 (54)	nd - 17.5 (51)	nd - 12.2 (26)	nd - 91.5 (72)
	Outdoor/Indoor bulk dust (ng/g)	-	-	nd - 34 (82)	-	-	-	nd - 1100 (91)	nd – 4700 (91)
	Outdoor/Indoor (ng/swab)*	-	-	1.1 - 100 (38)	-	-	-	1 - 930 (73)	1.6 - 8500 (88)
	Snow (pg/L)	-	-	11.9 - 147 (100)	5.0 - 245.5 (100)	1.4 - 21.8 (100)	nd - 25.3 (92)	-	1.4 - 86.0 (100)
	Rain (ng/L)	2.41 - 554 (100)	2.03 - 944 (100)	44.7 - 2752 (100)	nd - 9.79 (95)	nd - 7.39 (85)	nd - 1.58 (50)	nd - 4.46 (55)	nd - 1.35 (60)
	Surface water (ng/L)	nd - 5.89 (29)	nd - 0.84 (25)	nd - 2.28 (28)	nd (0)	nd - 0.54 (20)	nd (0)	nd - 3.15 (35)	nd - 1.17 (99)
	Surface water SPM and sediment (ng/g)	nd - 1.477 (1)	nd - 0.739 (34)	nd - 2.484 (47)	nd - 1.52 (31)	nd - 7.797 (46)	nd - 2.637 (16)	nd - 2.887 (13)	nd - 41.956 (93)
	Surface / Ground-water (ng/L)[g]	2.9 - 20 (100)	nd - 7.3 (71)	nd - 8.0 (71)	-	-	-	nd - 10.5 (71)	-
	Groundwater (ng/L)	nd - 240 (88)	nd - 37 (83)	nd – 4200 (59)	nd - 5 (55)	nd - 4.22 (31)	nd - 0.97 (45)	nd – 86000 (78)	nd – 220000 (75)
	Sea surface sedi-ments (ng/g)	nd - 0.13 (12)	nd - 0.29 (33)	nd - 1.4 (91)	nd - 0.89 (79)	nd - 0.38 (40)	nd - 0.67 (49)	-	nd - 1.7 (80)
	Soil / Sediment (ng/g dw)	0.55 ± 0.51[a] (80)	NA (20)[f]	nd – 14 (71)	0.31 ± 0.23[a] (100)	0.10 ± 0.09[a] (60)	0.20 ± 0.14[a] (100)	nd – 340 (73)	nd – 1400 (99)
Biota	Fish[a] (ng/g dw)	0.11 ± 0.01 (100)	0.78 ± 0.40 (100)	nd (0)	nd (0)	nd (0)	0.03 ± 0.02 (33)	0.49 ± 0.20 (100)	0.71 ± 0.07 (100)

		Min-Max concentration (Detection frequency in %)							
	Fish muscle (ng/g ww)	nd - 2.3 (3)	nd – 4.2 (1)	nd – 3.1(8)	nd – 1.4 (11)	nd – 6.7 (25)	nd – 21.6 (14)	nd – 1.2 (7)	nd – 306 (94)
	Fish liver (ng/g ww)	nd - 3.6 (2)	nd - 16.8 (5)	nd – 118.5 (9)	nd – 8.6 (52)	nd - 96 (90)	nd – 162.8 (98)	nd – 6.4 (3)	0-4 – 728 (100)
	Sea snail (ng/g dw)[a]	nd - 2.32 (46)	nd - 0.76 (35)	nd - 0.08 (21)	nd - 0.10 (40)	nd - 0.08 (26)	nd - 0.07 (15)	nd - 0.25 (65)	nd - 0.47 (85)
	Cape petrel feathers[a] (ng/g dw)	nd (0)	nd (0)	0.06 ± 0.02 (31)	0.06 ± 0.02 (23)	nd (0)	nd (0)	0.19 ± 0.05 (46)	0.77 ± 0.58 (100)
	Penguin feathers[a] (ng/g dw)	nd (0)	nd (0)	nd (0)	nd (0)	0.11 ± 0.14 (20)	nd (0)	0.05 ± 0.02 (60)	0.90 ± 0.65 (100)
	Penguin eggs (ng/g ww)	-	-	0.03 - 0.19 (100)	0.02 - 0.08 (100)	0.04 - 0.16 (100)	0.18 - 0.69 (100)	nd - 0.05 (93)	0.14 - 0.45 (100)
	Freshwater amphipods (ng/g)	-	nd - 0.1 (25)	nd - 3.2 (85)	nd - 1.7 (85)	0.04 - 4.0 (100)	nd - 1.1 (70)	nd - 0.7 (50)	nd - 15.7 (92)
	Algae[a] (ng/g dw)	0.30 ± 0.28 (83)	nd (0)	nd (0)	nd (0)	0.04 ± 0.02 (50)	nd (0)	0.16 ± 0.15 (50)	0.41 ± 0.28
Food & drinking water	**Drinking water (ng/L)**	nd - 1.5 (14)	nd - 0.66 (17)	nd - 0.59 (17)	nd - 0.31 (1)	-	-	nd - 2.1 (32)	nd - 2.7 (94)
	Samples considered to represent drinking water[b] (ng/L)	nd - 1.7 (11)	nd - 0.66 (15)	nd - 0.6 (14)	nd - 0.47 (5)	-	-	nd - 2.1 (23)	nd - 3.9 (99)
	Vegetables / Fruit (ng/g)	-	-	nd - 1 (6)	-	-	-	nd - 180 (38)	nd - 180 (35)
	Fish (e.g. tuna, salmon) from supermarket (ng/g)	nd (0)	nd (0)	nd – 0.105 (4)	nd – 0.106 (16)	nd – 0.151 (32)	nd – 0.888 (40)	nd (0)	nd – 0.195 (26)
	Shellfish (e.g. shrimp, crab)	nd (0)	nd - 234 (5)	nd - 510 (50)	nd - 350 (50)	nd - 105 (35)	nd - 265 (40)	nd - 242 (10)	nd - 388 (25)
	Bivalve mollusks (e.g. clams) (ng/g)	nd – 0.204 (90)	nd – 0.263 (90)	4.307 – 20.133 (100)	0.333 – 0.796 (100)	nd -0.211 (90)	0.85 – 0.260 (100)	0.051 – 0.605 (100)	0.194 – 1.235 (100)
	Seaweed (ng/g dw)	nd (0)	nd (0)	nd - 194 (76)	nd - 87 (39)	nd - 62 (9)	nd (0)	nd - 48 (27)	nd - 772 (48)
	Eggs (ng/g)	nd (0)	nd (0)	nd – 7.6 (41)	nd – 0.057 (45)	nd – 0.083 (43)	nd (0)	nd - 130 (22)	nd - 1700 (70)

		Min-Max concentration (Detection frequency in %)							
	Contacting material (ng/g)	nd - 310 (83)	nd - 41 (55)	nd - 51 (55)	nd – 6.5 (25)	nd – 47.4 (23)	nd – 6.4 (5)	nd – 0.152 (5)	nd – 0.617 (10)
Human	**Pooled blood serum (ng/g)**	.	-	0.2 - 13.1 (100)	0.10 - 1.56 (100)	0.01 - 1.26 (100)	0.01 - 1.31 (100)	0.09 - 3.92 (100)	0.2 - 60.5 (100)
	Maternal plasma / serum (ng/mL)	-	-	nd - 14.54 (99)	0.20 - 5.51 (100)	-	-	nd - 24 (95)	nd - 38.58 (99)
	Cord serum (ng/mL)	-	-	0.60 - 10.56 (100)	0.13 - .24 (100)	-	-	nd - 1.93 (88)	0.53 - 4.71 (100)
	Cord blood (ng/mL)[c]	0.01 - 0.11 (78)	-	0.46 - 1.06 (100)	0.15 - 0.32 (100)	nd - 0.16 (98)	0.11 - 0.26 (100)	nd - 0.14 (92)	1.10 - 1.92 (100)
	Serum / Urinary (ng/mL)[d]	0.04 (10)	0.04 (20)	0.62 (97)	0.26 (97)	0.07 (57)	0.04 (51)	1.16 (98)	1.89 (98)
	Cerebrospinal fluid (pg/mL)	nd - 326 (52)	nd - 67 (76)	nd - 1416 (98)	nd - 416 (78)	nd - 76.0 (48)	nd - 112 (55)	nd - 299 (90)	nd - 2291 (99)
	Tissue samples (ng/g ww)[e]	nd - 569 (63)	nd - 638 (36)	nd - 234 (47)	nd - 150 (13)	nd - 204 (22)	nd - 55.4 (4)	nd - 47.6 (11)	nd - 405 (49)

SPM - suspended particulate matter; NA - not available; nd – not detected; dw - dry weight; ww – wet weight

** Surface swab dust samples were collected from the air conditioning system, fire truck, firefighting boat, door panel, kitchen, and dining areas (Tefera et al., 2022).*

[a] Only average values available; for the sea snails the minimum and maximum are average values for different snail species (Gao et al., 2020).

[b] Source water samples from waterworks not employing activated carbon in their treatment (Grung et al., 2024).

[c] Minimum and maximum considered to be the values presented for Median Q1 and Q3 (Guo et al., 2024).

[d] Only the geometric mean is presented (Eick et al., 2024).

[e] 5 tissue samples (liver, bone, brain, lung, kidney) from 20 individuals, total n=100 (Pérez et al., 2013)

[f] Detection frequency available; but for samples with detection frequencies below 20%, mean concentrations were not calculated (Gao et al., 2020).

[g] PFAS concentrations in groundwater-surface water interface, samples from fishponds included (McFarlan et al., 2024).

4.2 Overview by matrix for the eight core PFAS

PFAS detection in air, both indoor and outdoor, suggests that atmospheric transport significantly contributes to the environmental distribution of PFAS. The high detection frequency (average of 75%; total n = 112) and substantial concentration range (e.g., PFOA in the air up to 9730 pg/m³) reflect PFAS persistence in urban environments. Indoor dust often contains PFAS due to their use in consumer products, while outdoor dust may accumulate PFAS from industrial emissions and urban runoff. The high levels of PFOS and PFHxA in dust also suggest possible contributions from firefighting foams and industrial applications.

TABLE 4. Uses and health effects of the eight most detected PFAS based on the literature review.

PFAS	Uses	Health effects[1]
PFOS	Firefighting foams and stain-resistant fabrics (PFOS is now mostly phased out)	Thyroid hormone disruption, immune system effects, liver toxicity, and developmental issues; a possible carcinogen.
PFOA	Manufacturing non-stick cookware and water-repellent textiles.	Linked to various health issues, including kidney and testicular cancer, liver damage, thyroid disease, high cholesterol, and immune system effects. Recognized carcinogen according to the International Agency for Research on Cancer (IARC).
PFHxS	Firefighting foams; metal plating, textiles, leather and upholstery, polishing agents and cleaning/washing agents, coatings, impregnation/proofing, manufacturing of electronics and semiconductors.	Associated with thyroid hormone disruption, liver damage, and neurodevelopmental effects.
PFNA	Production of non-stick, stain repellent and chemically inert coatings.	Linked to liver damage, developmental toxicity, and changes in immune function.
PFDA	Production of fluoropolymers, stain and greaseproof coating for furniture, packaging and carpet.	Disruption of lipid metabolism, potentially leading to liver damage and increased cholesterol levels; may interfere with hormone regulation, affecting thyroid function and reproductive health; some evidence linking PFDA exposure to developmental effects, including reduced birth weights in offspring.
PFUnDA	Production of fluoropolymers, textiles waterproofing, firefighting foams	Liver function, immune suppression, and alterations in thyroid hormone levels; concerns about its potential to impair reproductive health and fetal development. Animal studies suggest that it may affect liver enzymes and cause developmental toxicity.
PFHxA	Water and stain-resistant proofing, and food packaging	Althouh considered to have lower toxicity than some other PFAS, it has still been linked to potential adverse effects on kidney function and liver enzymes. Chronic exposure may lead to mild liver damage, though the evidence is not as strong as for longer-chain PFAS.
PFHpA	Production of other PFAS, particularly for short-chain fluorinated compounds, water proofing.	Limited evidence on the specific health risks of PFHpA, but it may share similar toxicological effects with other PFAS, including potential liver toxicity and effects on cholesterol levels. It could also pose risks to developmental health and immune function.

[1] *Schrenk et al. (2020); Tefera et al. (2022); Gao et al. (2020); Grung et al. (2024); Guo et al. (2024); Eick et al. (2024)*

High PFAS levels in snow and rain (e.g., PFOS up to 86.0 pg/L) confirm atmospheric deposition as a key route. Detection frequencies over 50% (n=58) suggest global distribution, due to PFAS volatility and long-range transport. Volatile precursors play an important role in the atmospheric long-range transport of PFAS, whereas ionic PFAS are primarily transported over long distances with ocean currents (Lohmann et al., 2024).

High concentrations reported in fish tissue (e.g., PFOS in fish liver with up to 728 ng/g wet weight and with 100% detection frequency) reflect bioaccumulation in aquatic food chains. Short-chain PFAS have generally lower concentrations in fish, supporting findings at longer-chain PFAS tend to accumulate more in biological tissues due to their higher hydrophobicity. The consistently high detection frequencies (up to 100%) for several PFAS in bivalves highlight their potential as bioindicators of PFAS contamination in aquatic ecosystems.

In certain food matrices, such as eggs and shellfish, PFAS concentrations vary widely (e.g., PFOS in eggs from a fire station presented values up to 1,700 ng/g; Tefera et al., 2022). This variability results from different exposure routes, including bioaccumulation in aquatic environments and surface contamination from PFAS-containing materials. The review of PFAS in food items that was included in Fauser et al. (2024) also highlighted a large variation of PFAS concentrations in food, spanning several orders of magnitude.

The detection of PFAS in human serum samples, particularly for PFOA and PFOS, highlights human exposure resulting from e.g. contaminated drinking water, food and consumer products. High detection frequencies across human samples, with PFOS up to 60.5 ng/g and 100% detection frequency, underscore the persistence and bioaccumulative nature of PFOS and related compounds. This raises significant concerns for human health, as certain PFAS are linked to adverse outcomes, including immunotoxicity, cancer, and endocrine disruption (

Overview by matrix for the eight core PFAS
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TABLE 4). Maternal plasma and cord blood detections also indicate PFAS transfer to the fetus, which may affect its development. PFOS, PFOA and PFHxS, (but also N-methylperfluorooctanesulfonamidoacetic acid (N-MeFOSAA)) were the most frequently detected PFAS in cerebrospinal fluid, with a detection frequency exceeding 90% (Hu et al., 2023). As mentioned above, a more detailed analysis of human exposure data has been provided by Raun-Petersen et al. (2024).

4.3 New compounds suggested to be included in monitoring efforts

4.3.1 Ultrashort PFAS

Ultrashort PFAS, typically characterized as having two or three carbon atoms, did not receive much attention in the past regarding regulatory and analytical actions. However, a growing number of publications demonstrate their presence in the environment, especially in water. One ultrashort PFAS that has received increasing attention is trifluoroacetic acid (TFA) (Van Hees, 2024). TFA is a very mobile and very persistent fluorinated chemical which is ubiquitous in our environment and often present in higher concentrations than other PFAS. The substance is used in the chemical industry, but it is also the final metabolite of many PFAS. Several studies have highlighted the widespread presence of TFA in water and rain samples (e.g. see review by Ateia et al., 2019). For example, TFA has been detected in 34 out of 36 European tap water samples from eleven EU countries, with values ranging from below detection limits to 4,100 ng/L (PAN Europe and Global 2000, 2024). The samples were tested for 24 PFAS, and TFA accounted for more than 98% of the total PFAS load across all tested samples (PAN Europe and Global 2000, 2024). TFA was also among the most frequently detected PFAS (of eleven targeted PFAS) with concentrations between 4.6–220 ng/L in surface waters from Canada (Wang et al. 2024). Furthermore, in precipitation samples from Ohio, USA, the ΣPFAS concentrations ranged between 50-850 ng/L, with TFA being the dominant compound (~90%) (Pike et al. 2021), results that align with previous studies as reported by the author (e.g. Scott et al. 2006; Wang et al. 2014).

Thus, and considering also the results from the PFAS workshop on monitoring strategies (see report in Annex 1), ultrashort PFAS such as TFA will be relevant to include in the monitoring of aqueous matrices. In Denmark, TFA is included in the monitoring of water quality (surface water and groundwater), with a national limit value of 9 µg/L (Retsinformation, 2023).

In the study conducted in European tap waters, half of the samples analysed exceeded the parametric value of 500 ng/L for " PFAS Total" in drinking water (PAN Europe and Global 2000, 2024). Thus, it was concluded that if TFA was to be included in PFAS regulations for drinking water, it would involve investments in the multi-digit billion range to technologically upgrade the European drinking water supply to ensure that the limit value of 500 ng/L was not exceeded (PAN Europe and Global 2000, 2024). Nonetheless, the analytical method(s) for monitoring the parameter “PFAS Total” remain unclear in this point at present, stating that it should be clarified whether or not TFA is included in the methodological approach.

4.3.2 Precursors

Human exposure to PFAS has been linked to numerous adverse health effects, with seafood recognized as a significant dietary source of these chemicals (Miranda et al., 2021). Some fish consumption guidelines already exist to help mitigate exposure risks, particularly for highly bioaccumulative legacy PFAS like PFOS (Massachusetts Department of Public Health, 2021). However, PFAS precursors, which constitute the majority of PFAS in consumer products and many contaminated aquatic environments, remain a concern (Pickard et al., 2022 and references therein).

The short-chain perfluorobutane sulfonamide (FBSA), an electrochemical fluorination precursor, is among the compounds reported to be present in all fish samples analysed by Pickard et al. (2022). FBSA was detected in fish at an average concentration of 1.1 ± 1.8 ng/g, exceeding the levels of all other targeted precursors. As a degradation product and key metabolite of certain aqueous film-forming foam formulations and surface treatment products, FBSA has recently been identified in environmental samples (Chu et al., 2016; Kaboré et al., 2022a;b). Thus, it will also be a relevant candidate to be included in the monitoring of biota samples to assess the extent of its distribution.

Volatile PFAS, particularly FTOHs, are among the most significant PFAS types emitted from waste treatment facilities. FTOHs are also released from consumer products, such as clothing, which contribute substantially to estimated air emissions (Lassen et al., 2024). FTOHs are the commonly reported PFAS precursors in air samples (Bossi et al., 2016) that have also been detected in snow (see references in Annex 3). Beyond the studies summarized in Annex 3, numerous reports have documented FTOHs in indoor and outdoor air and dust (Haug et al., 2011; Goosey and Harrad, 2012; Fromme et al., 2015; Padilla-Sánchez et al., 2017). In air samples collected from landfill sites, volatile FTOHs were the dominant PFAS class, with 8:2 FTOH being the most prevalent (measured at concentrations of 1,290–17,380 pg/m³). In contrast, 6:2 FTOH was the dominant PFAS detected over sewage treatment plants. This suggests that the sources of FTOHs at landfills are older, as 8:2 FTOH has been increasingly replaced by the shorter-chain 6:2 FTOH in more recent years (Lassen et al., 2024). FTOHs are neutral precursors to PFCAs.

Given the environmental relevance and transformation of FTOHs, including their degradation to TFA (namely from 6:2 FTOH and 4:2 FTOH (Sun et al. 2020)), special attention should be directed toward monitoring their emissions and environmental concentrations, namely 8:2 FTOH, 6:2 FTOH and 4:2 FTOH, with the first two already included in the monitoring of natural waters and biota (Amendment EU Directive 2000/60/EC).

The polyfluoroalkyl phosphate diesters (diPAP) have also been gaining attention in recent years. Polyfluoroalkyl phosphate esters (PAPs) have been used in the pulp and paper industry e.g. in food contact materials but also in personal care products, cosmetics, cleaning products, coatings and paints (Eriksson and Kärrman, 2015). DiPAPs are precursors of the group of PAP and are known to biotransform into several intermediates. They have been detected in indoor dust, consumer products, sewage sludge, surface water and biota (De Silva et al., 2012; Sun et al., 2020; Göckener et al., 2023) as well as in human serum (D’eon et al., 2009; Lee and Mabury, 2011; Yeung et al., 2013). One of the studies showed that there was no clear decline of diPAPs in human samples over decades (1982–2009), indicating ongoing exposure and their potential contribution to PFCA levels in humans (Yeung et al., 2013). In another study, conducted in Canada, the levels of diPAPs in indoor dust were about 20 times higher than those of other PFAS (De Silva et al., 2012). Considering that humans spend around 90% of their time indoors (Klepeis et al., 2001), the contribution from the indoor environment to human exposure to PFAS needs to be accounted for (Axmon et al., 2014; Eriksson and Kärrman, 2015). The parallel project “Contribution of different exposure pathways to the

total human exposure to PFAS" also identified diPAPs and FTOHs as relevant precursors for human exposure (Fauser et al., 2024).

It should also be noted that the degradation of PAPs to PFCAs has been demonstrated in both rats and microbial systems (D'eon and Mabury, 2011). During this process, intermediates such as saturated and unsaturated fluorotelomer aldehydes (FTAL and FTUAL), fluorotelomer carboxylic acids (FTCAs) and unsaturated carboxylic acids (FTUCAs) were formed, which were several orders of magnitude more toxic than PFCAs. Given the widespread presence of diPAPs in various environmental and consumer matrices, their persistence in human samples, and their transformation into highly toxic intermediates and PFCAs, it is relevant to consider the inclusion of diPAPs in comprehensive monitoring efforts to better understand their environmental fate, human exposure pathways, and potential health impacts.

4.4 PFAS in products

PFAS are not produced in Denmark, but used in the manufacture of products. This can lead to human exposure at the workplace, along with emissions to the environment via industrial and municipal wastewater, emissions to air, waste from production processes and the use and disposal of PFAS-containing goods.

Danish manufacturers are obliged to report hazardous chemical ingredients in products and mixtures to a product register. This applies to chemicals above 2% (m:m) in the product, with a limit of 0.1% for carcinogenic compounds. Chemicals that are not classified as hazardous do not have to be reported, which can be the case for several PFAS, including fluoropolymers such as teflon. Imported products not processed further in Denmark will not have to be reported either. Consequently, PFAS applications may exist that are not included in the product register. The Danish product register is part of the Nordic SPIN (Substances in Preparations In Nordic Countries) database[2], which is considered one of the most detailed databases for chemical substances in Europe.

4.5 Discussion

PFAS have been commonly divided according to their chain length: ultrashort PFAS C1-C4, short-chain PFAS C4-C6, and long-chain PFAS >C6 (with small variations for PFCAs and PFSAs, since carboxylic acids have a C atom in the functional group). Regulatory frameworks often focus on long-chain PFAS due to their higher potential for bioaccumulation. Despite the lower bioaccumulation, the frequent detection of short-chain PFAS (C4-C6) in environmental samples supports the need for monitoring programmes to include these substances, especially in areas where groundwater or drinking water sources are at risk. Although some short-chain PFAS, e.g. PFBA and PFBS, are already included in monitoring, and Denmark has also included TFA in the monitoring of surface water and groundwater, their systematic analysis is not as common as it is for the long-chain PFAS. TFA monitoring still poses analytical challenges (see report on PFAS workshop; Annex 1), for example in terms of background contamination. However, its inclusion in monitoring programmes would potentiate the efforts on developing new analytical methods, thus allowing to better assess its presence in aquatic matrices.

Considering the brief literature survey conducted here, PFAS monitoring should also consider the specific requirements at different locations, especially concerning the inclusion of short-chain PFAS and emerging compounds. It is worth noting that monitoring programmes might follow different strategies, for example reflecting diffuse background pollution and avoiding polluted locations. Depending on the purpose of the monitoring programme, it might be useful to also consider site-specific contamination situations, such as production sites where unique PFAS profiles may be present

This review supports that the list of monitored PFAS should be flexible, reflecting the physical-chemical properties of the compounds as well as the likelihood of their presence. However, it will be meaningful to define a core set of PFAS that all monitoring initiatives have in common, in order to enable comparisons across environmental compartments as well as between environment, food and humans. The alignment of monitoring strategies with evolving

[2] http://spin2000.net/

PFAS profiles, including legacy and emerging compounds, is critical for effective regulation and public health protection.

Given the large number of PFAS in use, including a “PFAS Total” approach in monitoring campaigns might provide a more comprehensive assessment of exposure than an extension of the list of target PFAS, which will almost inevitably be insufficient. This would help address the issue of unknown or less studied PFAS that may still contribute to overall exposure and health impacts. For example, in one of the studies that aimed to evaluate potential sources of firefighter exposure to PFAS inside fire stations, target PFAS and total fluorine (TF) were analysed in dust samples from 15 Massachusetts fire stations, many of which no longer (or rarely) used aqueous film-forming foams that contained PFAS (Young et al., 2021). In this study, the authors reported values between 6170 and 952000 ng/g for TF and the sum of 24 PFAS was between 16.8 and 2170 ng/g – with the maximum TF being 438x higher than the maximum for the sum of the 24 PFAS studied. In cases where TF and sum of PFAS (from target analysis) differ considerably, additional non-target and suspect screening analyses will be particularly relevant.

5. Use of PFAS Total in monitoring and research

5.1 Total Oxidizable Precursors (TOP)

This method consists of a first step in which precursors are oxidised, followed by a second step in which the formed PFAAs are measured by LC-MS/MS. Thus, it includes precursor compounds that cannot be analysed directly but can be converted by oxidation to PFAAs (such as PFCAs and PFSAs), after which these PFAAs can be analysed by standard LC-MS/MS. This process requires two analyses; before and after oxidation. TOP analyses can be used to address the potential for precursor transformation in a given medium. It should be noted that only the substances are identified that are included in the analytical method, and therefore the method is not a PFAS Total method. In addition, perfluorinated ether compounds, and possibly other classes, do not oxidize to PFAAs. While mainly applied to water, applications to other matrices, such as technical products, e.g. AFFF, soil, sediment, sewage sludge, dust, and biota have been reported in recent years. Some of the studies on other matrices than water used modified TOP assay methods, which are mainly characterised by harsher reaction conditions (higher concentration of oxidation reagent).

5.2 PFAS Total

The range of substances included in the parameter “PFAS Total” is defined as “the totality of per- and polyfluoroalkyl substances” in the EU DWD. At present, no single analytical method is fully capable of covering or quantifying all possible substances in this large class of compounds with a wide range of molecular weights and various chemical and structural properties. A summary of recent studies using “PFAS Total” approaches is given in TABLE 5.

Currently, the methods for measuring the parameter “PFAS Total” are neither standardized nor harmonized. Furthermore, the assessment of the methods does not usually cover the sample preparation methods. In the few interlaboratory calibrations that have been conducted, variation in sample preparation was identified as a key cause of variation between laboratory performances (Kärrman et al., 2021). Another key issue is the varying background concentration, which is visible in blank samples and typically decreases over time as residuals gradually leach out of the instruments. CIC instruments for determination of PFAS Total might contain fluorine in their components. This is a similar situation to that of PFAS target analysis on LC-MS/MS instruments in the past, which was solved as instrument producers changed polymer and elastomer materials.

Total fluorine (TF) can be measured with adsorbable or extractable organic fluorine approaches. **Adsorbable organic fluorine (AOF)** can be used for water samples, where the sample is passed through a column with an adsorbent such as activated carbon. The column is rinsed to remove inorganic fluorine, and organic fluorine compounds on the adsorbent are analysed directly using CIC. This method can be used to determine all organic fluorine compounds that can be adsorbed on the column.

For the determination of **extractable organic fluorine (EOF)**, the sample is extracted with a solvent that is purified of inorganic fluorine, then evaporated and analysed for organic fluorine using CIC. EOF can be used on solid samples and water samples that are concentrated on solid phase extraction (SPE) columns, and then eluted. The eluate is evaporated and analysed using CIC. The method can only be used to measure organic fluorine compounds that can be extracted under the given conditions, which is why standardized conditions are required to obtain comparable results.

Total organic fluorine (TOF) is used to determine the total content of organically bound fluorine. This method can only be used on solid matrices, which are analysed directly by CIC after inorganic fluorine has been removed from the sample. In CIC, the sample is heated to between 900-1100°C in a moist oxygen-rich atmosphere. Here, organic fluorine compounds are oxidized and decomposed, and the organic fluoride content is converted to hydrogen fluoride. The combustion gases, including the formed hydrogen fluoride, are driven with argon through an absorption solution, which is subsequently analysed for fluoride content using ion chromatography.

The methods are inclusive and quantitative for “PFAS Total” according to EU Directive C/2024/4910, and there is a low risk of underestimating the PFAS load at the concern level. Quantitative results are fluorine concentrations (ng F/L or ng F/g), which must be converted to a proxy-PFAS mass concentration to be comparable with e.g. limit values for PFAS. As discussed in Chapter 3.1, the parametric level for “PFAS Total” has been set at 500 ng/L in the EU DWD. Typically, PFOA equivalents (PFOAeq) are used to convert the fluoride concentration into a PFAS mass. The conversion factor to obtain PFOAeq from fluorine mass concentrations is 1.45, i.e. a mass concentration of 345 ng F/L corresponds to a mass concentration of 500 ng/L PFOAeq. Which PFAS is chosen as the conversion compound, is a choice – and should ideally be the PFAS occurring in the highest level in a given matrix. This choice will directly influence the converted value, showing that the conversion would also benefit from more standardization. The background of other possible F-compounds is not well studied and may result in concentrations that exceed the parametric value of 500 ng/L.

TABLE 5. Summary of studies applying “PFAS Total” methods. LOD: Limit of detection. EOF: Extractable organic fluorine. CIC: Combusion ion chromatography. UPLC: Ultra-performance liquid chromatography. MS: Mass spectrometry. AOF: Adsorbable organic fluorine

Reference	PFAS target	Matrix	LOD	Analysis	Comments
Aro et al. (2021)	45 PFAS	Blood and water	-	EOF-CIC	Less than 100% combustion efficiency or differences in calibrating with Inorganic F or Organic F could lead to underestimating EOF
Aro et al. (2021)	37 PFAS	Surface water, sediment, fish liver, sewage		Target: UPLC-MS/MS, PFAS Total: EOF-CIC	
Aro et al. (2022)	63 PFAS	Blood	107 ng F/mL[a)]	EOF-CIC	Not possible to detect PFAS at ng/mL level. Suitable screening method to detect elevated levels
Kärrman et al. (2019)		Fish		EOF-CIC	
Forster et al. (2023)		Wastewater, river water, air	AOF-CIC: 300-500 ng F/L EOF-CIC: 100-200 ng F/L	AOF-CIC, EOF-CIC	

[a)] Limit of quantification (LOQ)

5.3 Screening methods based on High Resolution Mass Spectrometry (HRMS)

In the last few years, significant developments have taken place in the field of suspect and non-target screening methods based on high resolution mass spectrometry (HRMS), encompassing quadrupole time-of-flight (QTOF)-MS, Orbitrap-MS, and Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS). Orbitrap-MS has a sensitivity between 3-20 ng/L for fluorine and an accuracy of ≤5 ppm (Liu et al. 2015).

HRMS approaches are used for suspect and non-target screening analyses, where various MS fragments can be identified and assigned as PFAS compounds due to carbon-fluorine bonds. These approaches can include:

- Identification of substances as known PFAS and confirmation with an analytical standard. These substances can be quantified with standard analysis methods (target analysis) in relation to certified reference standards.
- Tentative identification of substances as suspected PFAS using suspect screening lists based on mass, fragmentation patterns, and, ideally, retention time, but whose identity cannot be finally confirmed without an analytical standard (suspect screening). Semi-quantification can be possible (Cao et al., 2023).

- Tentative identification of substances that may be PFAS compounds due to carbon-fluorine bonds but are not on a pre-defined suspect list (non-target screening). The identity cannot be finally confirmed without an analytical standard. Semi-quantification may be possible in the same way as for suspect screening.

It is usually part of the workflow of compound identification to compare the obtained data with large international databases or computer-simulated mass spectra.

For the analysis of precursors, the analytical methods are usually limited by the availability of standards for the substances in question, but include the following techniques (Pancras et al., 2016; Backe et al., 2013):

- LC-MS/MS, as mentioned above. However, this method is not applicable to non-polar PFAS and FTOHs.
- GC-MS using non-polar or mixed solvents, e.g. for non-polar PFAS and FTOHs (Bossi et al., 2016).
- Headspace GC-MS for volatile PFAS.
- GC-PCI-MS/MS (gas chromatography with positive chemical ionization and tandem mass spectrometry) for volatile PFAS.

Non-target screening approaches are powerful in addressing unknown contamination, but so far, the available methods are strongly based on expert judgment and only provide tentative identification, possibly in combination with semi-quantitative results. The selectivity of suspect and non-targeted methods depends on the HRMS data processing workflow, which provides a lower specificity level than target analysis. A risk of false negatives and false positives exists, which depends on the significance level of assigning detected signals to a specific PFAS.

5.4 Other methods

Proton induced gamma emission (PIGE): The PIGE spectroscopy method is a non-commercially available method that measures gamma rays emitted from a surface by proton bombardment. The method measures the total content of organic fluorine compounds in the material's surface down to a depth of 250 µm. For water samples, PFAS is first absorbed onto activated carbon filters in the laboratory, making it possible to measure the PFAS content in the material's surface. The method can detect fluorine down to around 50 ppt F in a 2 L water sample (Tighe et al., 2021) and 10 ppb F in a 50 mL water sample (Peaslee, 2020).

^{19}F-Nuclear Magnetic Resonance (NMR) is a powerful technique, which is mostly used in compound discovery to determine chemical structures. NMR exploits that atoms that have an unpaired (single) electron in their outer shell, have an electronic spin which can resonate (absorb energy) when exposed to a strong magnetic field. This is the case for fluorine (^{19}F) and other atoms. How much energy is absorbed is proportional to the number of atoms with single electrons in a molecule. Since fluorine has no isotopes (all are in form of ^{19}F) and because there typically are many fluorine atoms in a PFAS, the ^{19}F-NMR signal is strong. Splits in the NMR signal can indicate structures where electrons on atoms close to each other interact. Not only is it possible to see interactions between similar atoms, but also between different atoms (e.g. H-F, C-F). The method was used in the early 2000, but only few environmental laboratories had these instruments. With the need to differentiate between PFAS and fluorinated non-PFAS, NMR has regained interest, particularly as a method for screening or confirmation (Gauthier and Mabury, 2022). It has hence been proposed as one of the tools for the universal PFAS restriction (Vestergren et. al., 2024).

6. Analysis of PFAS and PFAS Total in selected samples

6.1 Selection of samples

The project plan included 40 samples for the determination of PFAS Total of which 20 samples should be analysed with target analysis. This number was lower because some of the samples selected for the analysis of PFAS Total had previously been analysed for PFAS, for example as part of NOVANA.

The selection of samples for the chemical analyses followed two main lines:

- The samples should include environmental, food and human samples.
- Selected samples of parallel PFAS projects on fertilizers (Jensen et al., 2024) and diffuse PFAS pollution in soil (Strobel et al., 2024) should be included because data for PFAS Total were required for these two parallel projects.

TABLE 6. Summary of samples analysed in this project. AU: Aarhus University

Sample type	Number	Availability of target analysis	Comments
Fertilizer	5	From commercial laboratory (Jensen et al., 2024)	5 mineral fertilizers analysed; additional organic fertilizers might be analysed later, Results used by Jensen et al. (2024)
Soil	5	From commercial laboratory (Strobel et al., 2024)	Results used by Strobel et al. (2024)
Fish (liver)	3	Available from NOVANA	Samples were available at AU, i.e. perch *(Perca fluviatilis)* from Danish lakes collected under NOVANA
Shellfish (blue mussels; crabs)	6	Available from other projects at AU	Samples were available at AU
Terrestrial animals (muscle of wild birds)	5	Available from another project at AU	Samples were available at AU
Surface water	4	Included in this project	Collected from small streams in Jutland by the Danish EPA
Groundwater	1	Included in this project	One sample collected by NIRAS. More groundwater samples may be analysed later.
Sediment	3	Available from another project at AU	Samples were available at AU from previous sampling in Nivå Bugt.
Human samples (serum; milk)	1 pool of each matrix	Included in this project	Milk sample not yet analysed, needs method tests. Serum samples were analysed in 3 replicates
Fish (muscle)	5	Available from another project at AU	Samples available at AU

Proposals for sample lists were presented at the following meetings:

- Initial meeting with the advisory group on 10th June 2024.
  This initial list included samples of fertilizers, soil, biota (fish, shellfish, terrestrial biota), surface water, groundwater, sediment, food and human samples.
- Meeting with the Danish Knowledge Taskforce for PFAS and the Danish EPA on 16th September 2024.
  Due to time constraints the feedback at this meeting was i) to use existing samples and ii) to reduce the variation of samples. Specifically, it was agreed to use fish samples as food items. Thus, the project would include fish liver (representing environmental samples) and fish muscle (representing food samples). DTU Food provided human samples (pooled samples of serum and human milk).

Meeting with the advisory group of the Danish Knowledge Taskforce for PFAS on 2nd October 2024.
Following the advice of the previous meeting, it was suggested to use surface water samples only. This suggestion was rejected by the advisory group who considered groundwater samples highly important and offered to assist with the collection of groundwater samples.The final list of samples is given in TABLE 6. Since the surface and groundwater samples were collected close to the research visit to Stockholm University (SU), they could not be prepared for the CIC analyses. These analyses have subsequently been analysed on the CIC instrumentation at Aarhus University (AU), using preliminarily developed methods which were not fully validated by the time of the project (TABLE 7). Some additional samples might still be analysed after the end of this project, such as the samples of human milk that needed more method development. If relevant, they will be reported separately.

6.2 PFAS Total analysis – Extractable organic fluorine

6.2.1 Methods

Instrumentation for CIC analysis was purchased at AU in the spring of 2024 and intended to be used in this project. Following installation in August 2024, however, technical issues occurred which jeopardized the time schedule for this project. This risk had been communicated in the project description and was also discussed in all meetings with the advisory group, the Danish Knowledge Taskforce for PFAS and the Danish EPA. A mitigation action was to carry out the analyses of PFAS Total in laboratories with experience with these analyses, such as Örebro University (Anna Kärrman) and Stockholm University (SU) (Jon Benskin; Merle Plassmann) in Sweden. At the meeting of 16th September 2024, it was suggested by the project leader and approved by the Danish Knowledge Taskforce for PFAS and the Danish EPA that the experts in Sweden would be contacted with regard to hosting and training AU staff in the PFAS Total analysis.

Both universities kindly agreed to this plan. AU staff visited Stockholm University (with CIC instrumentation similar to that at AU) from 21st-25th October 2024 to be trained in the PFAS Total analysis and to analyse the samples in TABLE 6.

The samples had previously been extracted in the laboratories of AU, together with appropriate samples for quality assurance/control (QA/QC), following guidance by the experts from Stockholm University and using in-house validated methods for target PFAS. Unlike preparation methods for target analysis (see Chapter 6.3), the samples for EOF analysis were not spiked with internal standards. It should be noted that the CIC method cannot differentiate between different forms of fluorine. Thus, the sample extraction methods need to account for the removal of inorganic fluorine before CIC analysis. All extracts were analysed by CIC according to the conditions presented in TABLE 7. The water samples were analysed at AU using the sample pretreatment described for target analysis in Chapter 6.3.1.

TABLE 7. Combustion Ion Chromatography (CIC) analysis conditions.

Parameter	Conditions Stockholm University	Conditions Aarhus University*
Sampling preparation	Extracts were manually placed in a ceramic sample boat containing quartz wool	VECTRA autosampler. Extracts were automatically placed in a ceramic sample boat containing quartz wool
Combustion System	HF-210 furnace (Mitsubishi) + ceramic inner combustion tube.	XPREP C-IC Combustion (Trace Elemental Instruments)

Combustion temperature	1100°C	1000°C
Combustion gases and flow rates	Oxygen (400 mL/min), argon (200 mL/min), and argon mixed with water vapor (100 mL/min) for 5 min.	Oxygen (400 mL/min), argon (200 mL/min), and argon mixed with water vapor (100 mL/min) for 5 min.
Absorption	GA-210 gas absorber unit (Mitsubishi). Absorption in ultrapure water.	XPREP C-IC Fraction collector (Trace Elemental Instruments). Absorption in H_2O_2 solution in ultrapure water (100 mg/L)
Volume of absorption solution injected onto IC	200 µL	200 µL
Ion Chromatograph	Dionex Integrion (Thermo Fisher Scientific)	Dionex Inuvion (Thermo Fisher Scientific)
Ion Chromatography columns & column temperature	Aqueous hydroxide ramped from 8 mM to 100 mM at a flow rate of 0.25 mL/min	35 mM aqueous hydroxide at a flow rate of 0.25 mL/min; Conductivity Suppressor at 22 mA; Oven temperature of 30 ºC.
Detection	Conductivity	Conductivity
Quantification	Eight-point calibration curve prepared from NaF at concentrations ranging from 50 to 10000 µg/L fluoride	Calibration curve prepared from fluorobenzene at concentrations ranging from 50 to 10000 µg/L fluorine.
Sample loads	Liquid extract onto quartz wool - 100 µL	Liquid extract onto quartz wool - 50 µL

** The AU methodology is currently under development and optimization and not yet fully validated.*

6.2.2 Quality assurance and control (QA/QC)

For the measurements at SU, all boats loaded with samples for CIC analysis were combusted prior to the analysis of real samples to minimize background contamination. Before analysis ~5 instrumental blanks (empty boats) were run to ensure the background was low and reproducible before combusting real samples. After every matrix sequence run, another blank was run followed by an instrumental standard every ~10 runs (1 µg/mL NaF).

Each sequence started and ended with a calibration curve. The accuracy of the CIC analysis was assessed through quadruplicate direct combustions (i.e. no extraction) of a QC standard of a mixture of PFOS and PFOA, which revealed good agreement between measured versus expected concentration, 79.40 *vs* 73.98 ng of F, respectively (107 ± 4%).

The CIC limit of detection (LOD) was calculated through the residual standard deviation of the calibration curve (Sx) multiplied by 3. The limit of quantification (LOQ) is the value of the LOD multiplied by 3. These values were then used to calculate methods LOD and LOQ for each sample category, i.e. biota; soil, sediment and fertilizer; serum.

As explained in Chapter 6.1, the water samples were not available in time to be included in the EOF analyses at SU. Therefore, they were subsequently analysed at AU according to the parameters in TABLE 7. The analytical method is currently undergoing development and optimization of its performance. Therefore, LOD and LOQ have not been firmly established yet. At the time of the analyses, the background level was still higher than it had been for the analyses at SU.

6.3 PFAS target analysis

The target analysis of PFAS followed validated methods that are commonly used in the NOVANA programme. Soil and fertilizer samples were analysed for target PFAS by Eurofins as part of the parallel PFAS projects (Jensen et al., 2024; Strobel et al., 2024).

6.3.1 Methods

Biota tissues: The PFAS analysis of biota samples was based on the method described in Ahrens et al. (2009), with minor modifications. Around 1 g of homogenised sample was weighed in polypropylene tubes and spiked with a mix

of ^{13}C-PFAS as internal standards. The extraction was carried out by adding two times 5 mL of acetonitrile. The extracts were evaporated under nitrogen to 2 mL, then cleaned up using Supelclean™ ENVI-Carb™ cartridges (100 mg, 1 mL, 100–400 mesh, Supelco, USA) conditioned with 2 mL of acetonitrile and 1 mL of glacial acetic acid 20% in acetonitrile. After adding 50 µL of glacial acetic acid to the extracts, analytes were eluted with 3 mL of methanol. The purified extracts were evaporated to dryness under nitrogen and reconstituted with 500 µL of a methanol/2 mM ammonium acetate buffer (50:50, v:v). Samples were analysed for target PFAS by LC-MS/MS.

Sediments: 0.5 g of homogenized sample was weighed into 10 mL polypropylene tubes and spiked with a mix of ^{13}C-PFAS as internal standards. The extraction was carried out with 5 mL of methanol. The samples were shaken for 5 min, placed in an ultrasonic bath for 15 minutes, and then centrifuged at 1000 rpm for 5 minutes. The supernatant was transferred to a new polypropylene tube. The extraction step was repeated and the extracts combined. The combined extract was evaporated under nitrogen to 2 mL, then cleaned up using Supelclean™ ENVI-Carb™ cartridges (100 mg, 1 mL, 100–400 mesh, Supelco) conditioned with 2 mL of acetonitrile and 1 mL of glacial acetic acid 20 % in acetonitrile, i.e. in the same way as the biota samples. After adding 50 µL of glacial acetic acid to the extracts, they were passed through the ENVI-Carb™ column. The polypropylene tubes from the preparation were rinsed 3 times with 1 mL of methanol, which was also passed through the SPE column and collected with the acetonitrile extract. The target extract was evaporated to dryness with nitrogen and resuspended in 500 µL methanol/5 mM ammonium acetate buffer. A vortex mixer was used for a few seconds to better redissolve the samples. The extract was filtered through a white syringe filter (nylon) directly into a 1.5 mL polypropylene vial with a screw cap and stored. Samples were analysed for target PFAS by LC-MS/MS.

Human serum: A 100 µL aliquot of homogenised pooled serum samples was transferred into a 2 mL Eppendorf tube and spiked with a mix of ^{13}C-PFAS as internal standards. Then, 1.5 mL of 0.1 M formic acid was added, and the solution was shaken for 3 minutes and placed in an ultrasonic bath for 10 minutes. Oasis HLB SPE columns (60 mg, 3 cc Vac Cartridge, Waters) were conditioned by adding 2 mL of methanol, followed by 2 mL of 0.1 M formic acid. The serum samples were then added to the conditioned SPE columns, and the sample vial was washed with 1 mL of Milli-Q water. The column was then washed with 2 mL of Milli-Q water and dried under vacuum for 30 minutes. The SPE column was eluted with 2 mL of methanol, and the eluate was collected in a 2 mL Eppendorf vial, which was left open overnight in a fuming cupboard to evaporate. If any solvent remained, it was evaporated to dryness under a nitrogen stream at 30°C. The dried sample was reconstituted in 100 µL of methanol/5 mM ammonium acetate (50:50, v:v) and transferred to a Nanosep vial with a 0.2 µm Bio-Inert filter (Pall Life Science). The sample was then centrifuged at 14,000 rpm for 10 minutes. After centrifugation, the sample was transferred to HPLC vials for final analysis by LC-MS/MS.

Water: Surface water samples (1 L) were processed using Oasis WAX (6 mL, 150 mg) columns. The columns were conditioned with 4 mL of 0.1% NH_4OH in methanol, followed by 4 mL of Milli-Q water. Samples were loaded onto the columns at a flow rate of 1–1.5 mL/min. Afterwards, the sample flasks were rinsed with 50 mL of Milli-Q water, and the rinse was added to the columns. The columns were then dried for approximately 30 minutes under maximum vacuum. To elute the analytes, 5 mL of methanol was used to rinse the flasks and subsequently passed through the columns, followed by 4 mL of 0.1% NH_4OH. The eluates were collected in 15 mL polypropylene tubes. The extracts were evaporated to dryness under a nitrogen stream and reconstituted in 500 µL of methanol/5 mM ammonium acetate (50:50, v:v) for PFAS target analysis (200 µL of methanol for PFAS Total analysis, see Chapter 6.2.1). Finally, the extracts were filtered and transferred to plastic vials. The PFAS target analysis was performed by LC-MS/MS.

6.3.2 Quality assurance and control (QA/QC)

Each batch underwent rigorous QA/QC measures to ensure data reliability and accuracy. These measures included laboratory blanks to monitor potential contamination and control samples to verify analytical performance. Control samples may consist of certified reference materials or intercalibration samples with assigned values for target compounds. For these batches, the latter was used. Additionally, random test samples were analysed in duplicate across different batches to assess reproducibility. These practices ensured the validity of results and adherence to high analytical standards.

6.3.3 Calculations

Results from the target analysis are typically given in ng/g for solid samples and ng/L (or ng/mL) for liquid samples. For the comparison with PFAS Total, a conversion to fluorine content is necessary, as EOF provides ng F/g or ng F/L (TABLE 1). Therefore, the concentration for each detected PFAS was converted to its equivalent fluorine content according to Eq. 1. Then, these values were summed for all individual PFAS to obtain the total F equivalent content ($\sum F_{PFAS}$):

$$F_{PFAS} = \frac{n_F \times M_F}{M_{PFAS}} \times C_{PFAS} \qquad \textbf{Equation 1}$$

n_F is the number of fluorine atoms in the PFAS molecule
M_F is the molar mass of fluorine (19 g/mol)
M_{PFAS} is the molar mass of the specific PFAS (g/mol)
C_{PFAS} is the concentration of the specific PFAS (ng/g or ng/L)
F_{PFAS} is the fluorine content equivalent of a specific PFAS (ng F/g or ng F/L)

This approach allows for a direct comparison between the sum of target PFAS and EOF results, both expressed in terms of fluorine content.

6.4 Non-target screening of neutral PFAS

A GC-HRMS method was developed at the University of Copenhagen (KU) to analyse neutral PFAS in environmental and food samples. Several biota samples (including seafood; fish and shellfish), manure and soil have been obtained for the identification of neutral PFAS in non-target screening analysis on GC-QTOF-HRMS. The preliminary list of samples, the extraction method and instrumental parameters are given in Annex 4. The method development included spike of shrimp and soil samples with six neutral PFAS, including FTOHs and perfluoroalkylsulfonamido ethanols (Annex 4). Recovery rates were > 50%, which was considered sufficient for the purpose of non-target screening. The results of this initiative are not yet available and will be provided in a separate report.

6.5 Results of EOF and PFAS target analyses

The results for EOF analyses, the sum of PFAS (ΣPFAS) and the PFAS concentrations converted to fluorine (ΣF_{PFAS}) are summarized in TABLE 8. Results for individual PFAS derived from traditional target analyses are shown in Annex 5, i.e. ΣPFAS is broken down to individual compounds in Annex 5. In FIGURE 1 to FIGURE 5, EOF results are compared with ΣF_{PFAS}, i.e. the cumulative fluorine content in the individual PFAS in a sample. It should be noted that these figures do not show PFAS concentrations, but the fluorine content according to Equation 1, to be directly comparable with the EOF values (TABLE 1).

6.5.1 EOF results

In the PFAS Total analyses, elevated fluoride contents were observed in extraction blanks, which is a common problem in CIC analysis (Kärrman et al. 2021). These had an impact on the analytical sensitivity, raising the LODs and LOQs, which has implications in the analysis of samples with low EOF concentrations. For example, biota extraction blanks presented ~38 ng F, with the analysis of a methanol blank (no extraction) presenting ~6.4 ng F. These background levels are attributed to fluorine cross-contamination from materials used in the extraction process, such as solvents and filters. To address this, a comprehensive evaluation of the materials and solvents used in the methodology is necessary to identify and mitigate sources of F contamination, thereby lowering LODs and LOQs and enhancing overall analytical sensitivity.

Despite the methodological constraints, the EOF in liver of freshwater fish samples ranged from 331 to 561 ng F/g ww, with no values below the LOQ. This consistent detection suggests significant PFAS contamination and bioaccumulation of these substances in freshwater biota. EOF levels in blue mussel samples were above the LOQ in one sample (180 ng F/g ww), with two other samples below the LOQ of this study (56.9 ng F/g ww). EOF was 69 ng F/g ww in one crab sample at, while two other samples of crabs were below the LOD.

Results for muscle of marine fish samples were mixed, with two samples showing detectable EOF levels (78 and 110 ng F/g ww) and three others below the LOQ or LOD. The samples above LODs were muscle samples of European plaice *(Pleuronectes platessa)* and European flounder *(Platichthys flesus)*, while the samples below the LOD included eelpout *(Zoarces viviparus)* and another sample of flounder. The differences observed between the same species samples likely reflect the variability of PFAS contaminations in sampling locations in combination with natural variability. The EOF variation among different species may reflect differences in PFAS exposure across species or locations, but could also be influenced by the elevated blank values.

For the birds, the EOF values were below the LOQ or LOD in most samples, except one mallard *(Anas platyrhynchos)* muscle sample with an EOF level of 95 ng F/g ww.

In the case of soil, sediments and fertiliser, the method blank presented ~62 ng F. For the soil extraction, the recoveries were low (~26%), thus further method optimisation is required. Still, one of the soil samples showed a detectable EOF concentration of 142 ng F/g ww, while the others were below the LOD. The detectable EOF level in the soil sample could indicate localised PFAS contamination, perhaps from nearby anthropogenic sources, but needs verification. EOF was only detected in one fertiliser sample at a high concentration of 767 ng F/g ww, while others were below the LOD. All marine sediment samples had EOF values below the LOD.

EOF levels in serum samples were predominantly below the LOQ or LOD (113.8 and 37.9 ng F/mL, respectively), with one detectable concentration at 121 ng/mL. This is unexpected since all three samples were taken from the same pool of serum samples and indicates analytical variability, especially for values close to the LOQ, which should be decreased for routine analysis. An LOQ of 107 ng F/ml was reported for serum by Aro et al. (2022), confirming the LOQs of this study. For serum, good recovery was observed (76 $\pm$ 6%, after subtracting sample signal without spiking). The methodology blank presented ~34 ng F.

The water samples showed undetectable EOF levels. It should be mentioned that the method is being optimized, e.g. the amount of sample injected for combustion (currently 50 μL is sampled for combustion, Table 7) and single combustion *vs* multiple combustion cycles prior to injection, among other parameters. However, the groundwater sample from Flyvestation Skrydstrup showed a relatively high level of ΣPFAS. Converting this to the ΣF_{PFAS} parameter, the groundwater sample (from Flyvestation Skrydstrup) has a ΣF_{PFAS} of 468 ng F/L (TABLE 8). The undetectable EOF concentration might thus be a matter of non-optimized CIC methods at AU.

TABLE 8. Preliminary results of the PFAS Total (EOF) analysis of biota, soil, sediments, fertilizer, water and serum, compared with sum of PFAS (ΣPFAS) from target analysis and their conversion to the fluorine content in the sample (ΣF_{PFAS}). Graphical illustrations are given in FIGURE 1-FIGURE 5.

AU-Code	Sample	Type of sample	EOF	ΣF_{PFAS}	ΣPFAS
		Unit	ng F/g ww	ng F/g ww	ng/g ww
2023-22135	Perch 1	Liver	560.61	26.59	40.38
2023-22139	Perch 2	Liver	331.13	34.39	50.21
2023-22145	Perch 3	Liver	382.58	300.46	463.98
2024-22849	Blue mussel	Whole	180.48	0.47	0.71
2024-23082	Blue mussel	Whole	<56.88	0.07	0.11
2024-23083	Blue mussel	Whole	<56.88	0.01	0.02
2022-21717	Crab 1	Whole	69.09	0.16	0.23
2022-21537	Crab 2	Whole	<18.96	1.65	2.37
2022-21536	Crab 3	Whole	<18.96	0.05	0.07
2023-22384	Eelpout 1	Muscle	<56.88	0.28	0.42
2023-22386	European plaice	Muscle	78.22	0.48	0.74
2023-22388	Eelpout 2	Muscle	<18.96	0.61	0.93
2023-22409	European flounder 1	Muscle	<56.88	0.18	0.28
2024-22507	European flounder 2	Muscle	109.83	0.27	0.42

AU-Code	Sample	Type of sample	EOF	ΣF_{PFAS}	ΣPFAS
2023-22438	Greylag goose	Breast meat	<56.88	9.95	15.00
2023-22452	Eurasian teal 1	Breast meat	<18.96	20.71	31.81
2024-22558	Eurasian teal 2	Breast meat	<18.96	146.26	223.64
2024-22571	Mallard 1	Breast meat	<56.88	29.91	46.15
2024-22574	Mallard 2	Breast meat	94.86	41.60	63.20
		Unit	**ng F/g dw**	**ng F/g dw**	**ng/g dw**
2024-23579	Marine sediment 1	-	<18.96	0.69	1.03
2024-23581	Marine sediment 2	-	<18.96	0.12	0.17
2024-23582	Marine sediment 3	-	<18.96	8.35	12.33
2024-23593	Soil 1	-	141.56	(a)	(a)
2024-23595	Soil 2	-	<37.92	(a)	(a)
2024-23596	Soil 3	-	<37.93	(a)	(a)
2024-23600	Soil 4	-	<37.94	(a)	(a)
2024-23602	Soil 5	-	<37.95	(a)	(a)
2024-23678	Fertilizer 1	-	<37.96	(a)	(a)
2024-23679	Fertilizer 2	-	<37.97	(a)	(a)
2024-23680	Fertilizer 3	-	<37.98	(a)	(a)
2024-23681	Fertilizer 4	-	<37.99	(a)	(a)
2024-23682	Fertilizer 5	-	766.73	(a)	(a)
		Unit	**ng F/L**	**ng F/L**	**ng/L**
2024-23970	Surface water 1	Brande Å	(b)	4.05	6.17
2024-23972	Surface water 2	Søby Å	(b)	4.90	7.34
2024-23973	Surface water 3	Elkjær Bæk	(b)	17.28	26.14
2024-23974	Surface water 4	Isen Bæk	(b)	0.65	0.99
2024-24001	Groundwater	Flyvestation Skrydstrup	(b)	467.89	708.53
		Unit	**ng F/mL**	**ng F/mL**	**ng/mL**
2024-23631-1	Serum 1		<113.77	3.62	5.48
2024-23631-2	Serum 2		<37.98	3.38	5.14
2024-23631-3	Serum 3		120.75	3.26	4.97

(a) Analysed by EUROFINS for target PFAS, not detected. Details in Jensen et al. (2024) and Strobel et al. (2024). (b) Preliminary analysis carried out at AU, not detected; method currently under development and not yet fully validated. ww- wet weight; dw – dry weight. Species names are given in the text except for greylag goose (Anser anser) and Eurasian teal (Anas crecca). ΣPFAS varies in terms of number of PFAS included. Details are given in Annex 5.

The elevated fluoride levels in methodology blanks have increased the LOD and LOQ, impacting the detectability of PFAS in the samples. This limitation hinders a comprehensive analysis of PFAS distribution in these samples, as several readings fall below these thresholds. Despite these challenges, detectable levels in certain matrices, particularly liver of freshwater fish, soil, and fertiliser, suggest that these samples may contain notable PFAS concentrations above the sum of PFAS from target analysis. Additional method refinement to lower blank-related fluoride content could enhance analytical sensitivity, improving detection in matrices with lower PFAS concentrations.

Based on these results, several specific improvements can be made to enhance the accuracy of the CIC methodology for PFAS analysis:

Sample preparation:

- Evaluate the materials and solvents used in the methodology to identify and mitigate, whenever possible, the sources of F contamination.
- Increase sample volume or mass to concentrate analytes.
- Improve extraction efficiency.

CIC performance:

- Optimize combustion conditions, specifically temperature and oxygen flow, for complete PFAS conversion.
- Assess and optimize the use of multiple combustions prior to injection to concentrate the extract.
- Use matrix-matched calibration standards if needed.

6.5.2 Target PFAS analysis

Detailed results of individual PFAS from target analysis of PFAS are shown in Annex 5. It should be noted that the individual PFAS included in the target analyses were not identical for all the different samples. This is due to the fact that existing data were used from previous analyses, prior to recent extensions of the PFAS list for target analysis.

The results in Annex 5 show diverse contamination patterns across the various environmental matrices, but are generally similar to the patterns summarized in TABLE 3. PFOS was still the predominant compound in all biota samples. In freshwater fish liver samples, PFOS accounted for 80-97% of ΣPFAS in two samples, with one sample reaching 450 ng/g ww (sample AU id. 2023-22145). A third sample showed a more diverse profile, with PFOS and PFTrA accounting for 43% and 35% of ΣPFAS, respectively (sample AU id. 2023-22139). Marine fish muscle samples has a similar profile, but were much lower in ΣPFAS concentrations. Interestingly, despite a general low level of ΣPFAS, both the marine fish and the blue mussels contained the cyclic PFAS perfluoroethylcyclohexane (PFECHS) at detectable levels.

Blue mussels and crabs exhibited low ΣPFAS levels (0.02-2.37 ng/g ww). The highest PFAS concentration in one of the blue mussel samples was for perfluorooctanesulfonamide (PFOSA), i.e. 0.31 ng/g ww. This sample also contained the fluorotelomercarboxylic acid (FTCA) 7:3 FTCA. The three crab samples varied strongly in their concentration, with PFOA detected in one of the samples at a concentration of 1.21 ng/g ww. The crab samples originated from a previous project and had only been analysed for a reduced set of PFAS (PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA and PFOSA).

The muscle samples of wild birds were also dominated by PFOS (57-85% of ΣPFAS), with long-chain PFCAs, such as PFOA, PFNA, and PFDA, also present in high concentrations. One Eurasian teal sample showed a notable PFNA concentration of 25.6 ng/g ww (accounting for 11% of ΣPFAS; sample AU id. 2024-22558). The cyclic PFECHS was also detected in these samples, as well as 6:2 FTCA. 7:3 FTCA, which was detected in blue mussels, had not been included in the analyses of the wild bird samples.

Marine sediments consistently contained PFOA, PFOS, PFNA, PFDS, and PFUnDA. In addition, 7:3 FTCA was also detected in all sediment samples. The highest concentration was of PFOA (6.32 ng/g dw in sample AU id. 2024-23581) followed by PFOS (4.37 ng/g dw, also in sample AU id. 2024-23581). This was the sample with the highest ΣPFAS content of 12.3 ng/g dw. This sample also contained detectable amounts of ADONA and some long-chain PFCAs such as PFDoDA and PFTrDA. However, one of the other marine sediment samples (AU id. 2024-23579) contained the short-chain PFBA and PFPeA, which were not present in the other sediment samples.

As expected, the PFAS pattern was different in the water samples. The groundwater sample from Flyvestation Skrydstrup had high PFAS levels, with PFPeA dominating (268.6 ng/L, 38% of ΣPFAS), followed by PFHpA and PFHxA (138.6 and 117.4 ng/L, respectively). The sample still contained PFOS, PFOA and PFNA, but none of the longer chain molecules at detectable levels. However, the groundwater sample also contained 6:2 FTSA at a level of 30.9 ng/L, as well as many short-chain PFSAs. Since this sample originated from an airport, it seems likely that it represents a local source of PFAS.

The surface water samples had lower PFAS levels, with Elkjær Bæk sample showing the highest concentrations, primarily PFHxA (8.88 ng/L), followed by PFPeA, PFBA, PFTrA, PFOA and PFHpA. While the surface water samples also had a pattern that was dominated by short-chain PFCAs and, to a lesser extent, short-chain PFSAs, they also

contained detectable amounts of long-chain PFCAs, such as PFTrDA. 6:2 FTSA was also present in these samples. It should be noted that the frequently detected 7:3 FTCA was not included in the water analyses.

Human serum samples, analysed in triplicate, showed varying results due to low internal standard recovery in one sample. The two samples with good recoveries contained 10 PFAS compounds each, with PFOS consistently detected at the highest concentrations (3.06-3.80 ng/mL), followed by PFOA, PFNA and PFHxS. Long-chain PFCAs were also present, but not above a chain length of C11. Interestingly, ADONA, PFECHS and the chlorinated PFAS 9-Cl-PF3ONS were detected as well. The short-chain PFAS, such as PFBA, PFBS and PFPeA, were below LODs.

6.5.3 Comparative Analysis of EOF and ΣFPFAS

Figure 1 to Figure 5 compare the EOF results with the summed F content equivalent to the concentrations of individual PFAS from target analysis (ΣF_{PFAS}). In freshwater fish livers, EOF values are significantly higher than ΣF_{PFAS}, with the difference ranging from 82.1 to 534.0 ng F/g ww. Despite the strong predominance of PFOS in these samples, a part of EOF remains unexplained by the sum of PFAS.

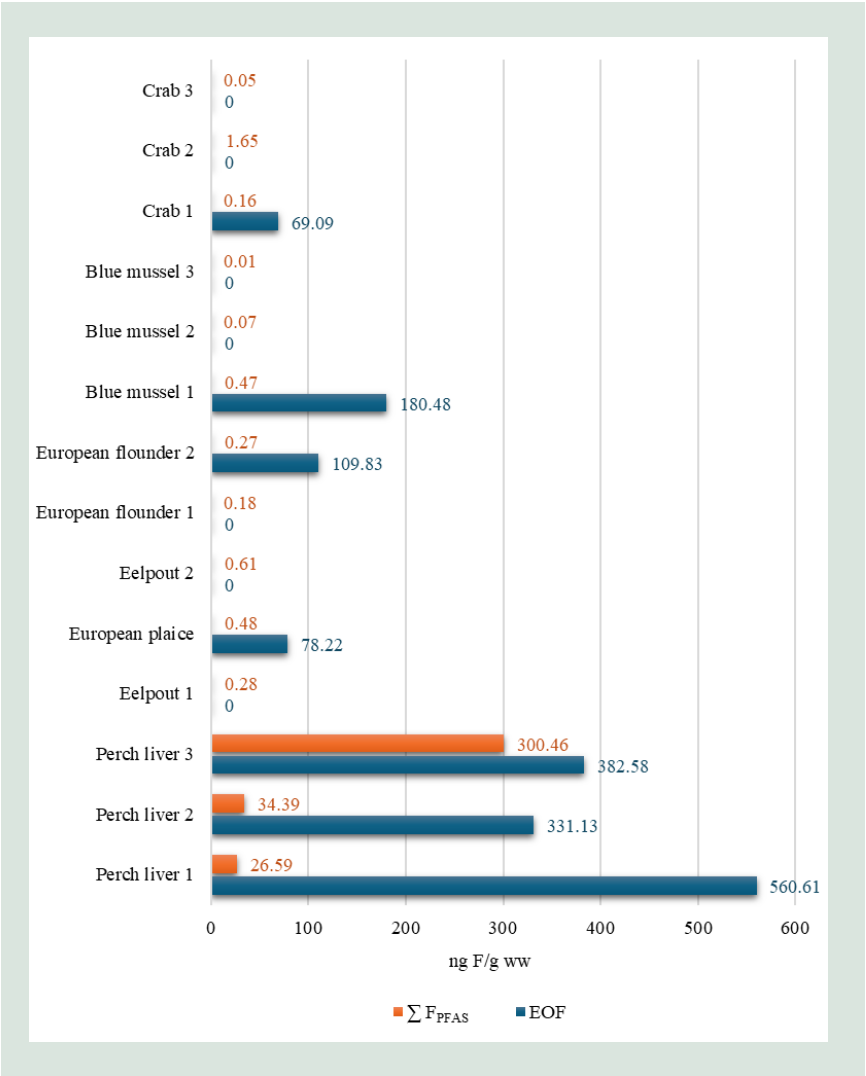

FIGURE 1. Aquatic biota EOF and ΣFPFAS in ng F/g wet weight (values below LOD/LOQ were considered 0). The data are also given in Table 8.

The results are less clear for the marine fish muscle samples, which generally have much lower levels than the fish liver samples. Consequently, some samples have EOF values below detection limits with ΣF_{PFAS} between 0.18 and 0.61 ng F/g ww, while others show much higher EOF than ΣF_{PFAS} (78 and 109 ng F/g ww). These results indicate

that i) the relatively high LODs of the EOF analyses limit comparisons with ΣF_{PFAS}, i.e. the results from target analyses and ii) for some fish samples there is a large difference between EOF and ΣF_{PFAS}, reflective of significant contributions from PFAS that are not included in the target analysis. This might suggest a larger variability in PFAS Total than indicated by the ΣPFAS results.

Blue mussels and crabs generally show higher EOF values than ΣF_{PFAS} when detectable. For blue mussels, the sample with the highest concentration of ΣPFAS also had the highest EOF concentration, however, with a factor of nearly 400 between EOF and ΣF_{PFAS}. For crabs, the sample with the highest concentration of ΣPFAS was below detection limits for EOF, while another sample with lower ΣPFAS had a relatively high concentration of EOF. Again, this indicates cases of significant unknown contributions to EOF.

The bird samples raise questions because of inconsistent results. Some show EOF values below detection limits despite measurable ΣF_{PFAS}. In fact, for one of the samples the calculated ΣF_{PFAS} concentration is higher than the EOF value, i.e. the sampled should have been above detection limits based on its ΣF_{PFAS} concentration. The reasons for this disagreement are not clear and need further investigation of potential challenges and limitations in the EOF determination. While the risk of false positives has been addressed, given the presence of background F in the samples, the risk of potential false negatives needs further study.

Marine sediment samples show EOF below detection limits, with low but detectable ΣF_{PFAS}. The large variation of ΣPFAS in the sediment samples is not captured in the EOF measurements. However, the results from PFAS target analysis are all consistent with being below LODs for EOF. For soil samples, soil 5 shows a detectable EOF concentration (141.6 ng F/g dw), but no target PFAS were detected, giving a ΣF_{PFAS} of zero (below LODs). The same trend was observed for the fertilizer samples, with one sample showing an EOF of 767 ng F/g dw, but no target PFAS were detected. The CIC analysis is currently under optimization for the water samples, so no comparison can be carried out at this stage.

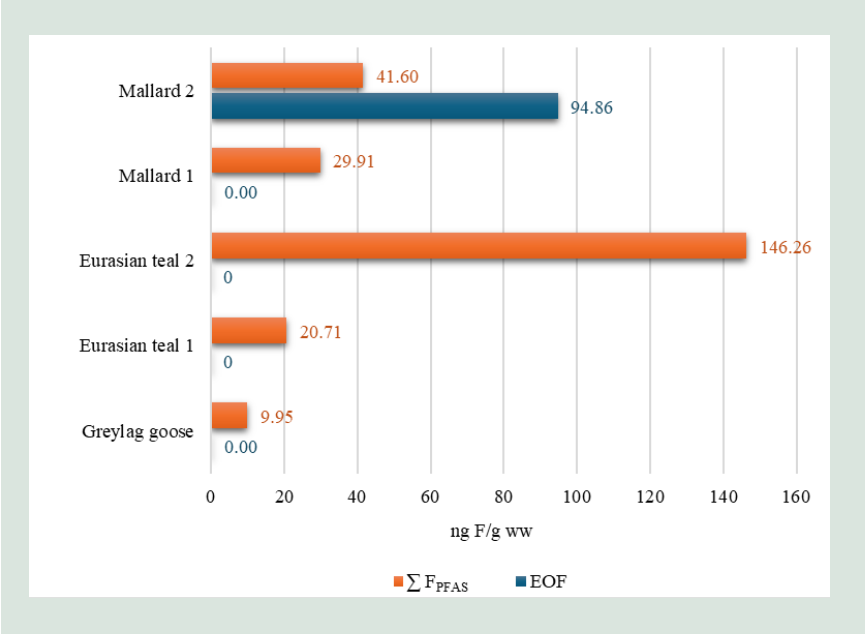

FIGURE 2. Wild birds EOF and ΣFPFAS in ng F/g wet weight (values below LOD/LOQ were considered 0). The data are also given in Table 8.

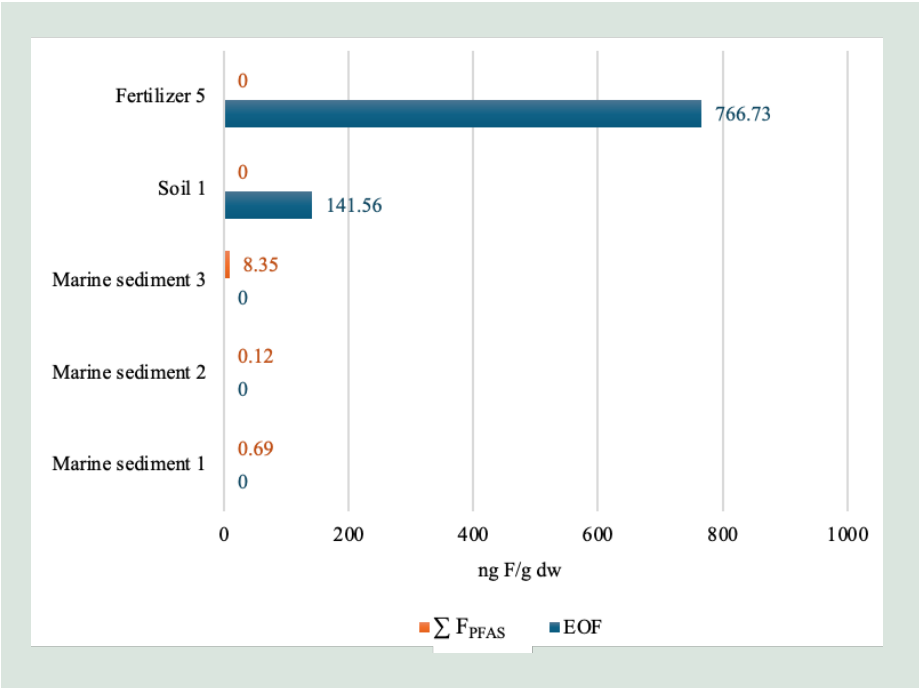

FIGURE 3. Sediment, soil and fertilizer EOF and ΣFPFAS in ng F/g dry weight (values below LOD/LOQ were considered 0; soil sam-ples 2-5, fertilizer samples 1-4 not included as both ΣFPFAS were below respective LODs/LOQs). The data are given in Table 8.

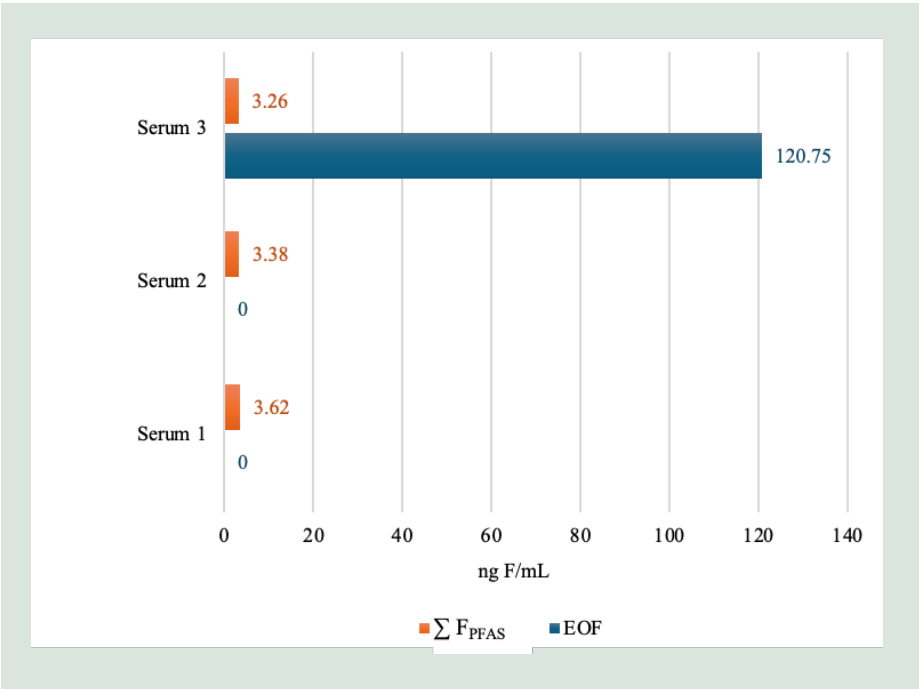

FIGURE 4. Adult serum samples EOF and ΣFPFAS in ng F/mL (values below LOD/LOQ were considered 0). The data are also given in Table 8.

The comparison between EOF results and the sum of F equivalent content from target PFAS (ΣF_{PFAS}) provides valuable insights into the presence of both identified and unidentified organofluorine compounds in various environmental matrices. The consistently higher EOF values in freshwater fish livers indicate a significant presence of unidentified organofluorine compounds, which may include unknown PFAS or their precursors. However, the EOF might also suggest a larger variation in contributions from unknown PFAS than indicated in cases where target analyses result

in similar concentrations of ΣPFAS. The discrepancies between EOF and ΣF_{PFAS} emphasize the importance of non-target screening techniques to identify unknown PFAS and other organofluorine compounds in environmental samples.

The lack of detectable EOF in some samples with measurable ΣF_{PFAS} (e.g., bird samples, serum) may indicate matrix-specific challenges in EOF analysis or differences in method sensitivity. Thus, further method development and optimization for EOF analysis will be necessary to improve sensitivity and reliability across different sample matrices. In general, a better understanding is needed of the possibilities and limitations of the PFAS Total approach.

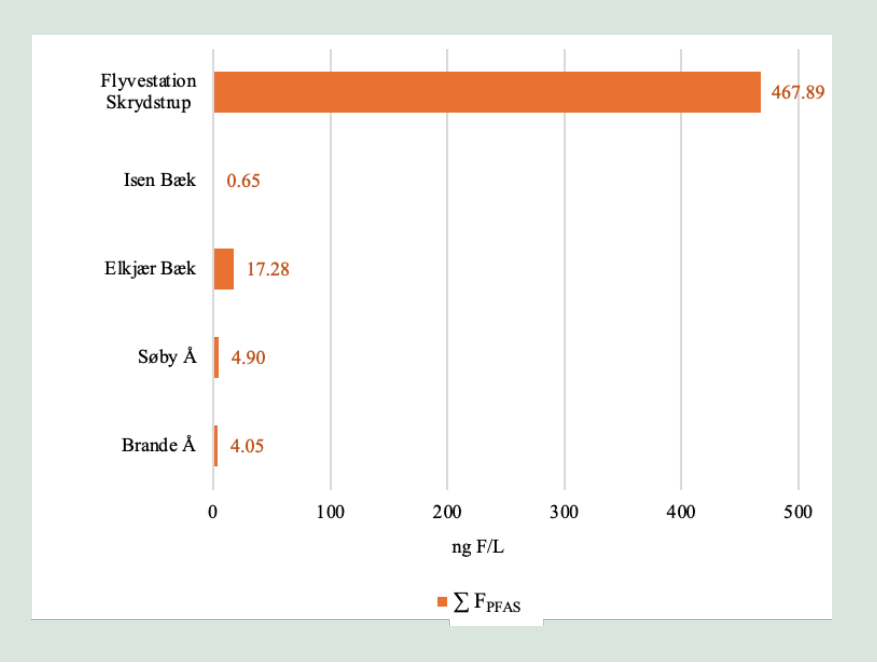

FIGURE 5. Surface and groundwater samples ΣFPFAS in ng F/L. For preliminary results of EOF in these samples, see Table 8 and comments in the text.

7. Concepts for future monitoring

The combined results of this project, obtained in the workshop, the literature survey and the PFAS analyses point at new concepts for future monitoring of PFAS, which, however, still need to gain maturity for high quality routine applications. One aspect is the extension of existing list for PFAS target analyses and their optimization for specific matrices. As discussed in this report, they could consist of a set of (eight) core PFAS and matrix-specific additions. Including PFAS Total, a tiered approach in environmental monitoring programs could enable a balance between comprehensive coverage and resource efficiency, ultimately leading to more effective and informed decision-making in environmental management. For PFAS monitoring, different approaches are possible (Figure 6):

- Option 1: as suggested in the workshop, a tiered approach could be designed, with PFAS Total for an initial screening and prioritization of subsequent target and/or non-target screening analysis.
- Option 2: the tiered approach could also include both PFAS Total and PFAS target analyses and define a certain threshold for its difference. Above this threshold, non-target and suspect screening could be applied to identify the unknown PFAS part.

Considering the operational application of these tiered approaches or similar concepts for PFAS monitoring, there is a need to include quantitative thresholds for transitioning between tiers that need to account regulatory limits, risk analysis and analytical method capabilities. Also, the development of tailored tiered approaches for different environmental matrices (water, soil, biota) needs to be considered to account for variations in analytical parameters and differences in PFAS patterns. To guide analysts through the tiered process, there is also a need to create detailed decision trees to ensure consistent applications across laboratories.

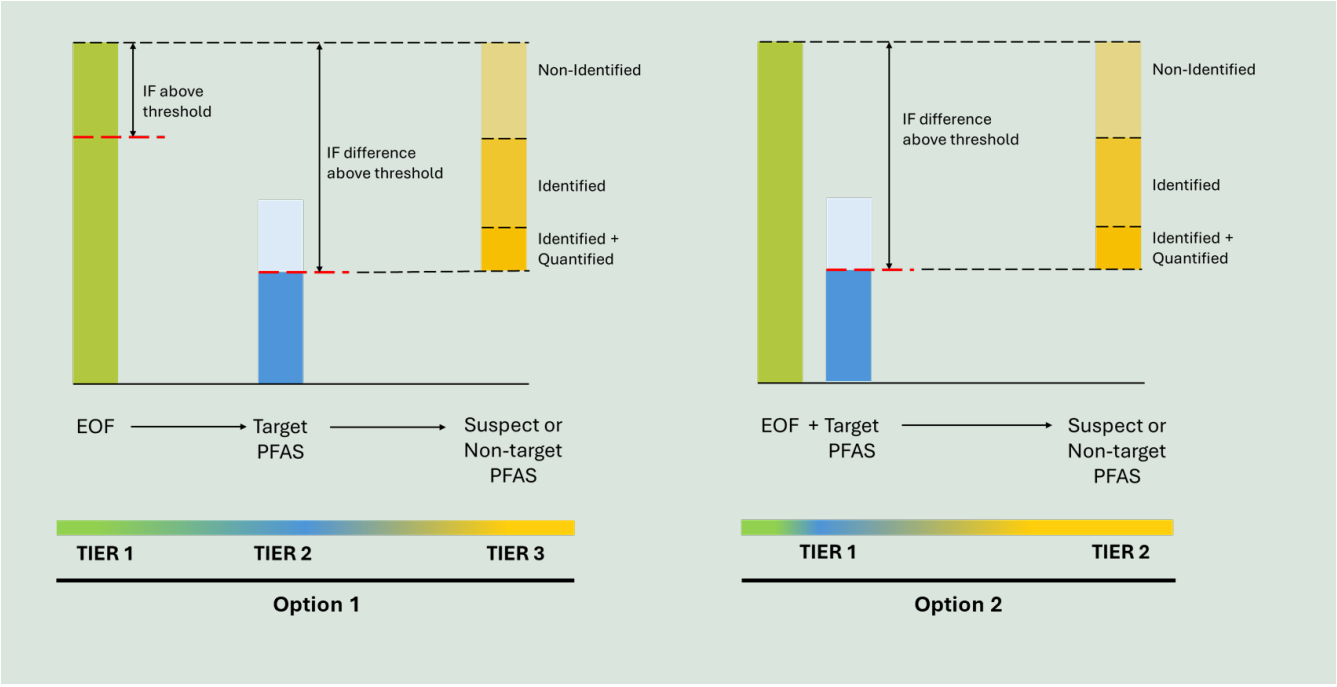

FIGURE 6. Conceptual framework of the tiered approaches for PFAS analysis

PFAS Total analyses are still in the early stages of routine applications and need further method development and consolidation by international expert laboratories for use in monitoring programmes. However, PFAS Total offers a great potential to approach the complexity of PFAS in a feasible way. To include PFAS Total analyses in routine monitoring, internal QA/QC measures will be necessary to ensure robust, accurate and precise data. These include the development of comprehensive protocols specific to PFAS Total analyses, implementing measures to avoid and monitor blanks, and improving the precision of PFAS Total measurements. The establishment of criteria for method

blanks, matrix spikes and precision and recovery is also required. However, they should also include external QC for an independent control of PFAS Total measurements, i.e. in round robin and proficiency testing exercises where samples of unknown PFAS Total concentrations are analysed by interested laboratories to check accuracy and precision of their analyses. Harmonization of PFAS Total analyses will be crucial, as well as the development of matrix-matched certified reference materials to support method validation and quality control. By implementing robust QA/QC measures, PFAS Total analyses can be effectively integrated into routine monitoring programs, ensuring the generation of accurate, precise, and comparable data across different laboratories and studies. This will significantly enhance the reliability of PFAS monitoring.

The proposed concepts also include suspect screening and non-target analyses, which is an obvious technique to identify unknown or overlooked PFAS. One of the crucial aspects of non-target analysis is to balance sensitivity and selectivity. Achieving a balance between matrix removal and preserving as many substances as possible in the extracts is one of the main challenges in non-target analysis. More “aggressive” extraction and clean-up methods can remove interfering matrix components and thus lower detection limits, but they may also eliminate some PFAS compounds of interest. Less selective methods preserve more substances but can lead to reduced sensitivity due to matrix effects.

Another key point is the importance of standardizing data reduction and prioritization techniques, such as chemical mass defect (MD) analysis and Kendrick mass defect (KMD) analysis (Zweigle et al. 2023). This standardization can enhance consistency in identifying potential PFAS compounds. Although many efforts have been made towards more comparable results in this field of research, e.g. the NORMAN guidelines (Hollender et al., 2023), recent intercomparisons still indicate a large variability of results (Dürig et al., 2023). Suspect screening studies of PFAS might be easier to harmonize than those of chemically more diverse compound groups (e.g. pesticides or plastic additives) since sample preparation methods are relatively simple (i.e. avoiding losses and thus false negatives) and comprehensive databases are available. However, besides the analytical quality, the high use of resources makes these approaches less likely to be implemented in large-scale monitoring, but will limit them to few selected samples.

The analytical challenges posed by PFAS monitoring necessitate ongoing advancements in methodologies and broader integration of quality assurance measures. While suspect and non-target screening analyses are time consuming and still subject to research and method development, they might provide relevant information on overlooked and unknown PFAS. As outlined in Figure 6, it could be useful to include them in cases of large unknown fractions of PFAS, provided that resources are available. To improve monitoring outcomes, it is also essential to optimize methods tailored to specific matrices, such as water, soil, and biota, recognizing the unique behaviours and challenges associated with each.

Even though PFAS Total analysis with CIC provides a promising method for detecting PFAS, its economic feasibility needs to be evaluated. Smaller laboratories may be unable to acquire CIC equipment due to the high initial cost. However, with the introduction of PFAS Total into the EU DWD, the measurements might become more common. Furthermore, because CIC may help to select samples with high amounts of unknown PFAS compounds, non-target analysis can be more focused, resulting in a more cost-effective streamlined analysis.

8. Conclusions

Matrix-specific PFAS lists are a meaningful approach in monitoring. However, given the widespread occurrence of PFAS and the resulting human exposure, environmental, food and human biomonitoring should be regarded as a continuum rather than separate disciplines. While methods and compound lists do not need to be standardised, more links should be established. It might be useful to define a set of core PFAS, such as the eight PFAS suggested in this report (PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFOS), that all monitoring efforts have in common to allow cross-disciplinary comparisons. Matrix-specific PFAS could be added to specific programmes, for example the ultrashort-chain PFAS to water monitoring. Some short-chain PFAS, such as TFA, should be included in the monitoring of relevant matrices, in particular water matrices. While methods are established in research contexts, challenges still exist for routine monitoring of short-chain PFAS.

Environmental monitoring can be used as early warnings of e.g. bioaccumulative PFAS which could also indicate a risk of human exposure that needs more detailed study. Lower trophic levels, e.g. benthic organisms and molluscs can be useful indicators close to emission points (to track sources), in particular for PFAS precursors (because of lower transformation potential).

Rather than a standardization of PFAS monitoring methods, their harmonization should be ensured, for example via proficiency testing schemes covering a variety of compounds and matrices. It is crucial for managing and regulating this class of contaminants to be able to rely on high quality and comparable data. Potential changes in methods should still ensure comparability of results, especially for time trends.

Measurements of PFAS Total are an obvious choice to account for the complexity of PFAS. However, the results of this project show that the method is not sufficiently robust yet for routine applications in environmental, food and human (bio-)monitoring. While the literature indicates that EOF can be applied to water samples, challenges remain for more complex matrices and those with relatively low PFAS Total levels. Background contamination has to be reduced (to achieve lower LODs/LOQs) and precision needs to be improved. It should be studied further where imprecisions are introduced, i.e. at the stage of sample preparation or instrumental analysis. External QA/QC such as proficiency testing is needed to improve the harmonization of these emerging techniques towards routine applications.

Likewise, TOP assays can provide useful results on the amount of precursors in a sample. Unlike the PFAS Total measurements, TOP assays do not need specific instrumentation, but can be based on established PFAS analyses by LC-MS/MS. However, harmonization and standardization are necessary to achieve comparable results, along with general method development and understanding of its possibilities and limitations.

The number of commercially available PFAS standards has increased significantly in recent years, but remains limited compared to the large number of PFAS in commerce. This means that screening approaches will be relevant to identify potentially overlooked PFAS. As discussed in Chapter 7, non-target and suspect screening studies can be part of a tiered monitoring strategy. These screening methods currently still have a lower method performance than target analyses, which i) should be improved through constant method development and ii) might be acceptable in some cases as fit for the purpose of indicating compound presence, which then can trigger more detailed studies.

The preliminary results of this project confirm that target analyses only explain a part of PFAS Total. This means that potentially, some exposure occurs that remains unobserved, despite extensions of PFAS compound lists. The explicable part of PFAS Total (that is covered by target analyses) varies depending on the matrix and the location of sampling. The literature indicates that the percentage of PFAS typically included in target analysis is high in top predators, but low close to sources. However, despite a clear predominance of PFOS in the biota samples of this study, they also included an unknown part of PFAS as expressed by EOF. Non-target and suspect screening can close the gap between PFAS Total and target analysis, at least in a qualitative way. While it is currently not sufficiently harmonised and extremely time-consuming, promising developments might allow more routine approaches in the future.

9. References

Ågerstrand et al. (2023) Letter from 116 scientists (November 2022) The World Health Organization Should Significantly Revise or Withdraw. Green Science Policy Institute.

Ahrens, L.; Siebert, U.; Ebinghaus, R. (2009) Total body burden and tissue distribution of polyfluorinated compounds in harbor seals *(Phoca vitulina)* from the German Bight. Mar. Pollut. Bull. 58, 520–525. DOI

Aro, R.; Eriksson, U.; Kärrman, A.; Yeung, L.W.Y. (2021) Organofluorine mass balance analysis of whole blood samples in relation to gender and age. Environ. Sci. Technol. 55, 13142-131515.

Aro, R.; Eriksson, U.; Kärrman, A.; Jakobsson, K.; Yeung, L.W.Y. (2022) Extractable organofluorine analysis: A way to screen for elevated per- and polyfluoroalkyl substance contamination in humans? Environ. Int. 159, 107035.

Ateia, M.; Maroli, A.; Tharayil, N.; Karanfil T (2019) The overlooked short- and ultrashort-chain poly- and perfluorinated substances: A review. Chemosphere 220, 866–882

Axmon, A.; Axelsson, J.; Jakobsson, K. et al (2014) Time trends between 1987 and 2007 for perfluoroalkyl acids in plasma from Swedish women. Chemosphere 102, 61–67. DOI

Backe, W.J.; Day, T.C.; Field, J.A. (2013) Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS. Environ. Sci. Technol. 47, 5226–5234.

Baun, A.; Bjerg, P.L.; Jensen, J.; Jensen, T.K.; Lyngberg, A.; Strobel, B.W.; Vinggaard, A.M.; Vorkamp, K.; Trier, X (2024). Begrænsning af menneskers og miljøets eksponering for PFAS i Danmark – Del 1: Identifkation af videnshuller. Rapport fra Videnstaskforce for PFAS forurening. Link til rapport.

Berg, V.; Sandanger, T.M.; Hanssen, L.; Rylander, C.; Nøst, T.H. (2021) Time trends of perfluoroalkyl substances in blood in 30-year old Norwegian men and women in the period 1986-2007. Environ. Sci. Pollut. Res. 28, 43897-43907.

Bossi, R.; Vorkamp, K.; Skov, H. (2016) Concentrations of organochlorine pesticides, polybrominated diphenyl ethers and perfluorinated compounds in the atmosphere of North Greenland. Environ. Pollut. 217, 4-10.

Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; de Voogt, P.; Jensen, A. A.; Kannan, K.; Mabury, S. A.; van Leeuwen, S. P. (2011) Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr. Environ. Assess. Manag. 7 (4), 513-541.

Chu, S.; Letcher, R.J.; McGoldrick, D.J.; Backus, S.M. (2016) A New Fluorinated Surfactant Contaminant in Biota: Perfluorobutane Sulfonamide in Several Fish Species. Environ. Sci. Technol. 50, 669–675. DOI

Cousins, I.T.; Dewitt, J.C.; Glüge, J. et al (2020) Strategies for grouping per-and polyfluoroalkyl substances (PFAS) to protect human and environmental health. Environ. Sci. Process. Impacts 22, 1444–1460.

De Silva, A.O.; Allard, C.N.; Spencer, C. et al (2012) Phosphorus-containing fluorinated organics: Polyfluoroalkyl phosphoric acid diesters (diPAPs), perfluorophosphonates (PFPAs), and perfluorophosphinates (PFPIAs) in residential indoor dust. Environ. Sci. Technol. 46, 12575–12582. DOI

D'eon J.C.; Crozier, P.W.; Furdui, V.I. et al (2009) Observation of a commercial fluorinated material, the polyfluoroalkyl phosphoric acid diesters, in human sera, wastewater treatment plant sludge, and paper fibers. Environ. Sci. Technol. 43, 4589–4594. DOI

D'eon, J.C.; Mabury, S.A. (2011) Exploring indirect sources of human exposure to perfluoroalkyl carboxylates (PFCAs): Evaluating uptake, elimination, and biotransformation of polyfluoroalkyl phosphate esters (PAPs) in the rat. Environ. Health Perspect. 119, 344–350. DOI. Duffek, A.; Conrad, A.; Kolossa-Gehring, M.; Lange, R., Rucic, E.; Schulte, C.; Wellmitz, J. (2020) Per- and polyfluoroalkyl substances in blood plasma – results of the German Environmental Survey for children and adolescents 2014-2017 (GerES V). Int. J. Hyg. Environ. Health 228, 113549.

Dürig, W., Lindblad, S.; Golovko, O.; Gkotsis, G.; Aalizadeh, R.; Nika, M.-C.; Thomaidis, N.; Alzgizakis, N.A.; Plassmann, M.; Haglund, P.; Fu, Q.; Hollender, J.; Chaker, J.; David, A.; Kunkel, U.; Macherius, A.; Belova, L.; Poma, G.; Preud'Homme, H.; Munschy, C.; Aminot, Y.; Jaeger, C.; Lisec, J.; Hansen, M.; Vorkamp K.; Zhu, L.; Cappelli, F.; Roscioli, C.; Valsecchi, S.; Bagnati, R.; González, B.; Prieto, A.; Zuloaga, O.; Gil-Solsona, G.; Gago-Ferrero, P.; Ridriguez-Mozaz, S.; Budzinski, H.; Devier, M.-H.; Dierkes, G.; Boulard, L.; Jacobs, G.; Voorspoels, S.; Rüdel,

H.; Ahrens, L. (2023) What is in the fish? Collaborative trial in suspect and non-target screening of organic micropollutants using GC- and GC-HRMS. Environ. Int. 181, 108288
East, A.; R.H. Anderson, C.J. Salice (2021) Per- and Polyfluoroalkyl Substances (PFAS) in Surface Water Near US Air Force Bases: Prioritizing Individual Chemicals and Mixtures for Toxicity Testing and Risk Assessment, Environ. Toxicol. Chem. 40, 859–870. DOI
EC (2000) European Commission, Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy
EC (2006) European Commission, Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration
EC (2008) European Commission, Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC.
EC (2020) European Commission, Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast)
EC (2022a) European Commission, Directive of the European Parliament and of the Council amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy, Directive 2006/118/EC on the protection of groundwater against pollution and deterioration.
EC (2022b) European Commission. Commission Recommendation (EU) 2022/1431 of 24 August 2022 on the monitoring of perfluoroalkyl substances in food.
EC (2023) European Commission. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006.
ECHA (2023) ECHA publishes PFAS restriction proposal ECHA/NR/23/04 (available at https://echa.europa.eu/-/echa-publishes-pfas-restriction-proposal, accessed on 04 November 2024)
Eick, S.M.; Y. Tan, K.R. Taibl, P. Barry Ryan, D.B. Barr, A. Hüls, J.A. Eatman, P. Panuwet, P.E. D'Souza, V. Yakimavets, G.E. Lee, P.A. Brennan, E.J. Corwin, A.L. Dunlop, D. Liang (2024) Prenatal exposure to persistent and non-persistent chemical mixtures and associations with adverse birth outcomes in the Atlanta African American Maternal-Child Cohort, J. Expo. Sci. Environ. Epidemiol. 34, 570–580. DOI
Eriksson, U.; Kärrman, A. (2015) World-wide indoor exposure to polyfluoroalkyl phosphate esters (PAPs) and other PFASs in household dust. Environ Sci Technol 49, 14503–14511. DOI
EU (2024) European Union. 2022/0345(COD), Directive of the European Parliament and of the Council concerning urban wastewater treatment (recast)
Fauser, P.; Zhu, L.; Bossi, R.; Vorkamp, K. (2024) Contribution of different exposure pathways to the total human exposure to PFAS. Projektrapport for Videnstaskforcen for PFAS-forurening. Miljøstyrelsen.
Forster, A.L.B.; Zhang, Y.; Westerman, D.C.; Richardson, S.D. (2023) Improved total organic fluorine methods for more comprehensive measurement of PFAS in industrial wastewater, river water, and air. Water Research 235, 119859
Fromme, H.; Dreyer, A.; Dietrich, S. et al (2015) Neutral polyfluorinated compounds in indoor air in Germany - The LUPE 4 study. Chemosphere 139, 572–578. DOI
Gao, K.; X. Miao, J. Fu, Y. Chen, H. Li, W. Pan, J. Fu, Q. Zhang, A. Zhang, G. Jiang (2020) Occurrence and trophic transfer of per- and polyfluoroalkyl substances in an Antarctic ecosystem, Environ. Pollut. 257, 113383. DOI.
Gauthier, J.R.; Mabury, S.A. (2022) Noise-reduced quantitative fluorine NMR spectroscopy reveals the presence of additional per- and polyfluorinated alkyl substances in environmental and biological samples when compared with routine mass spectrometry methods. Anal. Chem. 94, 3278-3286.
Gilles, L.; Govarts, E.; Rambaud, L.; Vogel, N.; Castano, A.; Esteban López, M.; Rodriguez Martin, L.; Koppen, G.; Remy, S.; Vrijheid, M.; Montazeri, P.; Birks, L.; Sepai, O.; Stewart, L.; Fiddicke, U.; Loots, I.; Knudsen, L.E.; Kolossa-Gehring, M.; Schoeters, G. (2021) HBM4EU combines and harmonises human biomonitoring data across the EU, building on existing capacity – The HBM4EU survey. Int. J. Hyg. Environ. Health 237, 113809.
Glüge, J.; Scheringer, M.; Cousins, I.T.; DeWitt, J.C.; Goldenman, G.; Herzke, D.; Lohmann, R.; Ng, C.A.; Trier, X.; Wang, Z. (2020) An overview of the uses of per- and polyfluoroalkyl substances (PFAS). Environ. Sci. Process Impacts 22, 2345.
Göckener, B.; Fliedner, A.; Weinfurtner, K. et al (2023) Tracking down unknown PFAS pollution – The direct TOP assay in spatial monitoring of surface waters in Germany. Sci. Total Environ. 898, 165425. https://doi.org/10.1016/j.scitotenv.2023.165425

Goldenman, G.; Fernandes, M.; Holland, M.; Nordin, A.; Schoumacher, C.; McNeill, A. (2019) The cost of inaction: A socioeconomic analysis of environmental and health impacts linked to exposure to PFAS, Nordic Council of Ministers (available at http://norden.diva-portal.org/smash/get/diva2:1295959/FULLTEXT01.pdf, accessed on 05 November 2024).

Goosey, E.; Harrad, S. (2012) Perfluoroalkyl substances in UK indoor and outdoor air: Spatial and seasonal variation, and implications for human exposure. Environ. Int. 45, 86–90. DOI

Government of Canada (2024) Updated draft state of per- and polyfluoroalkyl substances (PFAS) report. Environment and Climate Change Canada; Health Canada; July 2024.

Grung, M.; D. Hjermann, T. Rundberget, K. Bæk, C. Thomsen, H.K. Knutsen, L.S. Haug (2024) Low levels of per- and polyfluoroalkyl substances (PFAS) detected in drinking water in Norway, but elevated concentrations found near known sources, Sci. Total Environ. 947, 174550. DOI

Guo, Y.T.; J. bo Huang, J. chuan Xue, S.J. Qin, D. Cai, Q.Z. Wu, C. Chu, C. lan Tang, Y. qi Xie, L.Z. Lin, G.H. Dong, X.W. Zeng (2024) Intrauterine exposure to long-chain perfluorocarboxylic acids (PFCAs) were associated with reduced primary bile acids in three-year-old children: Findings from a prospective birth cohort study, Environ. Pollut. 360, 124583. DOI

Haug, L.S.; Huber, S.; Schlabach, M. et al (2011) Investigation on per- and polyfluorinated compounds in paired samples of house dust and indoor air from Norwegian homes. Environ. Sci. Technol. 45, 7991–7998. DOI

Hollender, J.; Schymanski, E.L.; Ahrens, L.; Alygizakis, N.; Béen, F.; Bijlsma, L.; Brunner, A.M.; Celma, A.; Fildier, A.; Fu, Q.; Gago-Ferrero, P.; Gil-Solsona, R.; Haglund, P.; Hansen, M.; Kaserzon, S.; Kruve, A.; Lamoree, M.; Margoum, C.; Meijer, J.; Merel, S.; Rauert, C.; Rostkowski, P.; Samanipour, S.; Schulze, B.; Schulze, T.; Sigh, R.S.; Slobodnik, J.; Steininger-Mairinger, T.; Thomaidis, N.S.; Togola, A.; Vorkamp, K.; Vuilliet, E.; Zhu, L.; Krauss, M. (2023) NORMAN guidance on suspect and non-target screening in environmental monitoring. Environ. Sci. Europe 35:75. DOI

Hu, W.; M.Y. Zhang, L.Y. Liu, Z.F. Zhang, Y. Guo (2023) Perfluoroalkyl and polyfluoroalkyl substances (PFASs) crossing the blood-cerebrospinal fluid barrier: Their occurrence in human cerebrospinal fluid, J. Hazard. Mater. 442, 130003. DOI

Jensen, J.; Strobel, B.; Trier, X. (2024) PFAS i restprodukter til landbrugsmæssig anvendelse. Projektrapport for Videnstaskforcen for PFAS-forurening. Miljøstyrelsen.

Joerss, H.; Menger, F. (2023) The complex 'PFAS world' - How recent discoveries and novel screening tools reinforce existing concerns. Curr. Opin. Green Sustain. Chem. 40, 100775.

Kaboré, H.A.; Goeury, K.; Desrosiers, M. et al (2022a) Novel and legacy per- and polyfluoroalkyl substances (PFAS) in freshwater sporting fish from background and firefighting foam impacted ecosystems in Eastern Canada. Science of the Total Environment 816, 151563. DOI

Kaboré HA, Goeury K, Desrosiers M, et al (2022b) Fish Exhibit Distinct Fluorochemical and δ15N Isotopic Signatures in the St. Lawrence River Impacted by Municipal Wastewater Effluents. Front. Environ. Sci. 10, 833164. DOI.
Kärrman, A.; Wang, T.; Kallenborn, R.; Langseter, A.M.; Grønhovd, S.M.; Ræder, E.M.; Lyche, J.L.; Yeung, L.; Chen, F.; Eriksson, U.; Aro, R.; Fredriksson, F. (2019) PFASs in the Nordic environment – Screening of poly- and perfluoroalkyl substances (PFASs) and extractable organic fluorine (EOF) in the Nordic Environment. TemaNord 2019: 515. DOI

Kärrman, A.; Yeung, L.W.Y.; Spaan, K.M. et al (2021) Can determination of extractable organofluorine (EOF) be standardized? First interlaboratory comparisons of EOF and fluorine mass balance in sludge and water matrices. Environ. Sci. Process. Impacts 23,1458–1465. DOI

Klepeis, N.E.; Nelson, W.C.; Ott, W.R. et al (2001) The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants. Journal of Exposure Analysis and Environmental Epidemiology 11, 231–252

Lassen, C.; Maag, J.; Krag, A.; Dau, M.S. (2024) Substance flow analysis of PFASs in Denmark. Ministry of Environment of Denmark.

Lee, H.; Mabury, S.A. (2011) A pilot survey of legacy and current commercial fluorinated chemicals in human sera from United States donors in 2009. Environ. Sci. Technol. 45, 8067–8074. DOI

Lind, P.M.; Lind, L.; Salihovic, S.; Ahlström, H.; Michaelsson, K.; Kullberg, J.; Strand, R. (2022) Serum levels of perfluoroalkyl substances (PFAS) and body composition – A cross-sectional study in a middle-aged population. Environ. Res. 209, 112677.

Liu, Y.; dos Santos Pereira, A.; Martin, J.W. (2015) Discovery of C_5–C_{17} poly- and perfluoroalkyl substances in water by in-line SPE-HPLC-Orbitrap with in-source fragmentation flagging. Anal. Chem. 87, 4260-4268.

Lohmann, R.; Abass, K.; Bonefeld-Jørgensen, E.C.; Bossi, R.; Dietz, R.; Ferguson, S.; Fernie, K.J.; Grandjean, P.; Herzke, D.; Houde, M.; Lemire, M.; Letcher, R.J.; Muir, D.; De Silva, A.O.; Ostertag, S.K.; Rand, A.A.; Søndergaard, J.; Sonne, C.; Sunderland, E.M.; Vorkamp, K.; Wilson, S.; Weihe, P. (2024) Cross-cutting studies of per- and polyfluorinated alkyl substances (PFAS) in Arctic wildlife and humans. Sci. Total Environ. 954, 176274.

Massachusetts Department of Public Health (2021) Freshwater Fish Consumption Advisory List.

McFarlan, E.L.; L.D. Lemke (2024) Per- and polyfluoroalkyl substances (PFAS) fate and transport across a groundwater-surface water interface, Sci. Total Environ. 951, 175672. DOI

Miranda, D.A.; Benskin, J.P.; Awad, R. et al (2021) Bioaccumulation of Per- and polyfluoroalkyl substances (PFASs) in a tropical estuarine food web. Sci. Total Environ. 754, 142146. DOI

Niarchos, G.; Alygizakis, N.; Carere, M.; Dulio, V.; Engwall, M.; Hyötyläinen, T.; Kallenborn, R.; Karakitsios, S.; Karakoltzidis, A.; Kärrman, A.; Lamoree, M.; Larsson, M.; Lundqvist, J.; Mancini, L.; Mottaghipisheh, J.; Rostkowski, P.; Sarigiannis, D.; Vorkamp, K.; Ahrens, L. (2024) Pioneering an effect-based early warning system for hazardous chemicals in the environment. Trends Anal. Chem. 180, 117901.

Nicolajsen, E.S.; Tsitonaki, K. (2016) Kortlægning af brancher der anvender PFAS. Miljø- og Fødevareministeriet. Miljøprojekt nr. 1905, November 2016.

OECD (2021). Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances: Recommendations and Practical Guidance. OECD Series on Risk Management, No. 61, OECD Publishing, Paris.

Padilla-Sánchez, J.A.; Papadopoulou, E.; Poothong, S.; Haug, L.S. (2017) Investigation of the best approach for assessing human exposure to poly- and perfluoroalkyl substances through indoor air. Environ. Sci. Technol. 51,12836–12843. DOI

PAN Europe and Global 2000 (2024) TFA The Forever Chemical in the Water We Drink.

Pancras, T.; Schrauwen, G.; Held, T.; Baker, K.; Ross, I.; Slenders, H. (2016) Environmental fate and effects of poly- and perfluoroalkyl substances (PFAS). *Concawe Soil and Groundwater Taskforce (STF/33)*, (8), p.121. Available at: DOI.

Peaslee, G. (2020) Developing PIGE into a Rapid Field-Screening Test for PFAS. SERDP Project ER19-1142, October 2020.

Pickard, H.M.; Ruyle, B.J.; Thackray, C.P. et al (2022) PFAS and precursor bioaccumulation in freshwater recreational fish: Implications for fish advisories. Environ. Sci. Technol. 56, 15573–15583. DOI

Pike, K.A.; Edmiston, P.L.; Morrison, J.J.; Faust, J.A. (2021) Correlation analysis of perfluoroalkyl substances in regional U.S. precipitation events. Water Res 190, 166685. DOI

Raun-Petersen, C.; Rausgaard, N.L.K; Vorkamp, K.; Lyngberg, A.C.; Jensen, T.K. (2024) Plan for biomonitorering for PFAS i den danske befolkning - Forslag til national handleplan for fortløbende systematisk biomonitorering af danskernes blodniveauer af PFAS. Projektrapport for Videnstaskforcen for PFAS-forurening. Miljøstyrelsen.

Reinikainen, J.; Bouhoulle, E.; Sorvari, J. (2024) Inconsistencies in the EU regulatory risk assessment of PFAS call for readjustment. Environ. Int. 186, 108614. DOI

Retsinformation (2023) Bekendtgørelse om vandkvalitet og tilsyn med vandforsyningsanlæg. Journalnummer Miljøministeriet 2023-5529.

Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; del Mazo, J.; Grasl-Kraupp, B.; Hogstrand, C.; Hoogenboom, L.; Leblanc, J.C.; Nebbia, C.S.; Nielsen, E.; Ntzani, E.; Petersen, A.; Sand, S.; Vleminckx, C.; Wallace, H.; Barregård, L.; Ceccatelli, S.; Cravedi, J.P.; Halldorsson, T.I.; Haug, L.S.; Johansson, N.; Knutsen, H.K.; Rose, M.; Roudot, A.C.; Van Loveren, H.; Vollmer, G.; Mackay, K.; Riolo, F.; Schwerdtle, T. (2020) Risk to human health related to the presence of perfluoroalkyl substances in food, EFSA Journal 18. DOI

Scott, B.F.; Spencer, C.; Mabury, S.A.; Muir, D.C.G. (2006) Poly and perfluorinated carboxylates in north American precipitation. Environ. Sci. Technol. 40, 7167–7174. DOI

Sonnenberg, N.K.; Ojewole, A.E.; Ojewole, C.O.; Lucky, O.P.; Kusi, J. (2023) Trends in serum per- and polyfluoroalkyl substances (PFAS) concentrations in teenagers and adjults, 1999-2018 NHANES. Int. J. Environ. Res. Public Health 20, 6984.

Strobel, B. W.; Jakobsen, C.; Trier, X.; Abrahamsen, P.; Holbak, M.; Jensen, J. (2024) Diffus forurening og i forvejen forekommende niveauer af PFAS i danske jorde. Projektrapport for Videnstaskforcen for PFAS-forurening. Miljøstyrelsen

Sun, M.; Cui, J.; Guo, J. et al (2020) Fluorochemicals biodegradation as a potential source of trifluoroacetic acid (TFA) to the environment. Chemosphere 254, 126894. https://doi.org/10.1016/j.chemosphere.2020.126894

Tefera, Y.M.; Gaskin, S.; Mitchell, K.; Springer, D.; Mills, S.; Pisaniello, D. (2022) Food grown on fire stations as a potential pathway for firefighters' exposure to per- and poly-fluoroalkyl substances (PFAS), Environ. Int. 168, 107455. DOI

Tighe, M.; Jin, Y.; Whitehead, H. D.; Hayes, K.; Lieberman, M.; Pannu, M.; Plumlee, M. H.; Peaslee, G. F. (2021) Screening for per- and polyfluoroalkyl Substances in water with particle induced gamma-ray emission spectroscopy. ACS ES&T Water 1 (12), 2477-2484.

Uhl, M.; Schoeters, G.; Govarts, E.; Bil, W.; Fletcher, T.; Haug, L.S.; Hoogenboom, R.; Gundacker, C.; Trier, X.; Fernandez, M.F.; Castaño Calvo, A.; Esteban López, M.; Coertjens, D.; Santonen, T.; Murínová, L.P.; Richterová, D.; De Brouwere, K.; Hauzenberger, I.; Kolossa-Gehring, M.; Halldórsson, Þ.I. (2023) PFASs: What can we learn from the European Human Biomonitoring Initiative HBM4EU. Int. J. Hyg. Environ. Health 250, 114168.

USEPA (2023) PFAS National Primary Drinking Water Regulation Rulemaking.

Van Hees P (2024) "Novel" and Ultrashort PFAS: Two Groups of Growing Concern. White paper, Eurofins.Vestergren, R.; Appelblom, A.; Bălan, S.A.; Brandsma, S.H.; Bruton, T.A.; Cousins, I.T.; Gauthier, J.R.; Heggelund, A.; Ivarsson, J.; Kärrman, A.; Melymuk, L.; Olisah, C.; Rosen, A.; Savvidou, E.; Schellenberger, S.; Skedung, L.; Talasniemi, P.; Wickman, T.; Zweigle, J.; Zwiener, C.; Benskin, J.P. (2024) A systematic workflow for compliance testing of emerging international classwide restrictions on PFAS. Environ. Sci. Technol. 58, 14968-14972.

Wang, Q.; Wang, X.; Ding, X. (2014) Rainwater trifluoroacetic acid (TFA) in Guangzhou, South China: Levels, wet deposition fluxes and source implication. Science of the Total Environment 468–469, 272–279. DOI

Wang, Y.; Chang, W.; Wang, L. et al (2019) A review of sources, multimedia distribution and health risks of novel fluorinated alternatives. Ecotoxicol. Environ. Saf. 182, 109402.

Wang, Y.; Liu, M.; Vo Duy, S. et al (2024) Fast analysis of short-chain and ultra-short-chain fluorinated organics in water by on-line extraction coupled to HPLC-HRMS. Sci. Total Environ. 943, 173682. https://doi.org/10.1016/j.scitotenv.2024.173682

WHO (2022) PFOS and PFOA in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality

Yeung, L.W.Y.; Robinson, S.J.; Koschorreck, J.; Mabury, S.A. (2013) Part I. A temporal study of PFCAs and their precursors in human plasma from two German cities 1982-2009. Environ. Sci. Technol. 47, 3865–3874. DOI

Young, A.S.; E.H. Sparer-Fine, H.M. Pickard, E.M. Sunderland, G.F. Peaslee, J.G. Allen (2021) Per- and polyfluoroalkyl substances (PFAS) and total fluorine in fire station dust, J. Expo. Sci. Environ. Epidemiol. 31, 930–942. DOI.

Zweigle, J.; Bugsel, B.; Zwiener, C. (2023) Efficient PFAS prioritization in non-target HRMS data: systematic evaluation of the novel MD/C-m/C approach. Anal. Bioanal. Chem. 415, 1791–1801. DOI

Appendix 1. PFAS workshop on monitoring strategies

On 26th June 2024, a workshop focusing on the analysis of per- and polyfluoroalkyl substances (PFAS) was held online, bringing together 34 experts from across Europe. US experts were informed, but could not attend because of the morning time slot in Europe. The project advisory group had been invited to the workshop as well. The workshop was structured to facilitate active participation and information sharing among attendees. To guide the discussions, 16 questions were provided in a shared Google document, allowing participants to contribute their experience and insights before, during, and after the event (Table A1).

The workshop resulted in 24 responses to the posed questions, representing the perspectives of different European participants. The outcomes include valuable information on PFAS analytical methods and monitoring strategies with a broad European scope, as well as strengthened professional networks and the development of this workshop report.

TABLE A1. Questions shared in the Google document for the PFAS Workshop

Q1	Are you involved in a monitoring programme for PFAS? Does it include environmental monitoring, food monitoring or human biomonitoring?
Q2	What is the main purpose of the monitoring activity (e.g. time trends, compliance checks, screening etc.?)
Q3	Which matrices are included in your monitoring activities?
Q4	How frequently are the matrices collected and analysed?
Q5	How are the matrices and locations selected?
Q6	Which individual PFAS are included in the analysis? If possible, list all individual PFAS (or a reference to the legislation)
Q7	What quality assurance/control (QA/QC) measures are included in the PFAS analysis for monitoring?
Q8	Do you use any external QC (e.g. proficiency testing, certified reference materials)? If so, which one?
Q9	What do you experience as challenging regarding the PFAS analysis?
Q10	Where do you see the main obstacles and gaps in an accurate and precise PFAS analysis (e.g. QA/QC, availability of analytical standards)?
Q11	Which other PFAS would you suggest for your monitoring activity (and why)?
Q12	Do you have experience with PFAS Total measurements? If so, please specify, including instrumentation.
Q13	Do you have experience with TOP Assay?
Q14	Do you have experience with suspect/non-target screening of PFAS?
Q15	What is your view on the possibilities of combining PFAS Total and target analysis (and, potentially, suspect screening) for monitoring purposes?
Q16	Do you also monitor PFAS in products or intend to develop methods in this field?

Appendix 1.1 Participants overview

Of the 34 participants attending the workshop, 24 participants responded to the questions in the Google document. The respondents represented 11 European countries (Figure A1) and diverse backgrounds, including environmental agencies, academia, and research institutions. They were actively involved in both environmental and human monitoring programmes at local, national, and international levels, providing a broad and representative perspective on PFAS monitoring from a European standpoint.

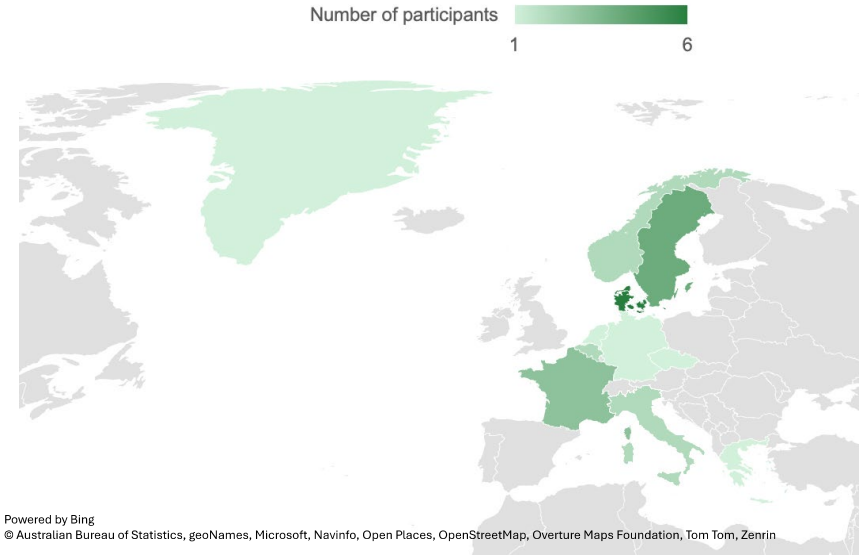

FIGURE A1. Number of questionnaire responses per country.

Monitoring frequency in national monitoring programmes and research projects varied, with sampling conducted on a weekly, monthly, or annual basis, while some projects followed ad hoc or project-specific timelines. The purpose of monitoring programmes varied among participants, encompassing the assessment of time trends, compliance with regulatory standards, source tracking, contaminant screening, and advancing research on the environmental and health impacts of PFAS. The types of matrices monitored included a wide range of environmental and biological samples, such as surface and groundwater, soil, air, wastewater, sediments, sludge, biota (e.g. fish and marine species), and human samples (blood and serum).

The responses regarding the specific monitoring of PFAS in products (answers to Q16, Table 1) indicate a diverse range of experiences. Some participants have actively monitored PFAS in various products, particularly textiles and food packaging materials, and paper and cardboard, although some do not categorize their work as monitoring. Some participants indicated plans to expand their monitoring efforts to include consumer products and other relevant matrices, suggesting a growing recognition of the importance of monitoring PFAS across various product categories. However, although they expressed interest, they also referred to challenges such as a lack of funding or limited interest from clients.

Considering the methodologies employed, almost all participants had hands-on experience with PFAS target analysis. The number of PFAS analysed differed by institution and sample type, with target analyses ranging from 16 to 90 individual PFAS. A few participants also employed broader approaches, such as PFAS Total analysis using extractable organic fluorine (EOF) and total oxidizable precursor assay (TOPA). Some participants mentioned that they were starting to explore PFAS Total measurements and would soon acquire instruments for total fluorine determination.

Regarding expertise in suspect and non-target screening (NTS), a significant number of participants had experience in both methodologies, often using advanced instrumentation like GC- and LC-Orbitrap. Some participants provide suspect screening as a service, while others focus on non-target screening for research purposes.

Overall, the participants reflect a range of experiences and capabilities in PFAS analysis in a wide range of matrices, with wide expertise in established and emerging methodologies.

Appendix 1.2 Insights from participant responses to the questionnaire

Appendix 1.2.1 PFAS lists and differences

The number of PFAS analysed (answers to Q6, Table 1) varied across institutions and sample types, with target analyses typically including between 22-40, being expanded up to 90 individual compounds in specific projects/samples (Table 2). Among these, 18 PFAS were analysed by more than four institutions, establishing them as the "Core PFAS" used in target analyses (Table A2).

The target list can be expanded to incorporate additional compounds of interest based on specific monitoring programmes, sample matrices, or project requirements. For instance, more compounds were typically included in water analyses or research projects that aimed for a broader scope, especially newer PFAS. Common extensions to the PFAS target lists included emerging contaminants such as GenX and ADONA, short-chain PFAS (e.g. TFA), fluorotelomer alcohols and sulfonates (e.g. 4:2 FTOH, 4:2 FTSA), and chlorinated acids (e.g. 6:2 Cl-PFESA and 8:2 Cl-PFESA). The choice of compounds depended on institutional priorities, the type of sample matrix, and the specific analysis requirements.

The number of compounds considered in suspect screening varied widely. In some cases, lists included over 5,000 potential PFAS, as reported by one participant, demonstrating the diverse approaches used in PFAS monitoring.

TABLE A2. Main common PFAS compounds and differences reported.

Category	Commonly analysed PFAS
Number of PFAS analysed	Between 22-40 PFAS, expanding up to 90 compounds
Core PFAS in target analysis	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS
Differences in PFAS lists	Some lists include GenX and ADONA, short-chain PFAS (e.g. TFA), Fluorotelomer alcohols and sulfonates (e.g. 4:2 FTOH, 4:2 FTSA), Cl-substituted acids (e.g. 6:2 Cl-PFESA and 8:2 Cl-PFESA)
Differences by matrix	The number of PFAS analysed varies significantly across matrices such as sediment, fish, soil, and human samples.
Non-target and suspect screening	Advanced methods use wide-scope LC-HRMS with suspect lists that include up to 5,000 compounds.

Appendix 1.2.2 Suggested PFAS for inclusion in the monitoring programs

Participants provided various suggestions for expanding PFAS monitoring lists (Table A3). Overall, participants emphasized the need to expand monitoring lists to assess a more comprehensive range of PFAS, considering the specific requirements of different matrices, the inclusion of short-chain PFAS and emerging compounds.

TABLE A3. Recommended PFAS for inclusion in monitoring programs.

Category	Comment
Matrix-specific PFAS	Several participants noted the importance of tailoring the PFAS target list to the specific matrix (e.g. water, biota, air). Certain PFAS may not be relevant for all matrices, suggesting that a more refined approach based on sample type could improve monitoring outcomes.
Short-chain PFAS	There was a recommendation to include short-chain PFAS like TFA, especially to evaluate human exposure due to their increasing detection in the environment. The inclusion of ultrashort-chain PFAS in air (e.g., measuring neutral monomers), was advised to capture a more comprehensive picture of atmospheric PFAS pollution.
New and emerging PFAS	Emerging PFAS like GenX and other analytes from the PARC project were suggested for inclusion, reflecting their relevance in current contamination trends. Incorporating precursors and semi-quantitative determination of PFAS identified through non-target screening was recommended to improve the detection of compounds that may transform into more persistent PFAS in the environment.

Besides specific PFAS categories, it was referred that the list of monitored PFAS should also be flexible, with an emphasis on site-specific contamination sources, such as production sites where unique PFAS profiles may be present.

The need for more comprehensive approaches was also emphasized. The use of more holistic approaches to monitoring that include as many PFAS as possible, along with suspect screening for novel compounds, was referred as being important to provide a wider overview of PFAS contamination. It was also suggested to use fluorine sum parameters or TOPA together with target and non-target analyses to better capture the full spectrum of PFAS present, especially in complex matrices. Also, performing TOP-type oxidation prior to analysis was recommended to identify residual perfluorinated compounds through non-target analysis, especially for environmental samples where unknown PFAS may be present.

Appendix 1.2.3 QA/QC procedures and challenges of analysing PFAS

The questionnaire included questions addressing various challenges associated with PFAS analysis (Q7-Q10, Table A1), that aimed to gather insights into PFAS analysis, current practices on QA/QC and difficulties encountered by participants aiming to identify common issues and best practices.

Participants reported a range of quality assurance/quality control (QA/QC) practices in PFAS analysis, including procedural measures, use of standards, and participation in external quality control programs. The main practices include the use of:

- **Blanks**: Procedural blanks, field blanks, extraction blanks, and injection blanks were widely used to detect and control contamination during the analysis process.
- **Spiked samples**: Spiked samples, including different levels of spiked serum or other matrices, were commonly employed to check the accuracy and recovery of the analytical methods. Some participants used spiked samples from established programs (e.g., HBM4EU, G-EQUAS QA rounds).
- **Internal standards**: Most participants used labelled internal standards (IS), commercial or in some cases synthetized in house, for quality control.
- **Reference materials**: Certified reference materials (CRMs), such as Standard Reference Materials (SRMs) from NIST, and in-house reference samples were used for QA/QC purposes.
- **Matrix effects and pool samples**: Matrix effect calculations, recovery evaluations, and the use of pooled samples were part of QA/QC procedures to address variability in sample composition.
- **Instrument-specific QA/QC**: Participants conducted regular instrumental calibration, monitored instrument drift, and applied procedures to ensure reproducibility and sensitivity. Some participants also verified results using different instruments or techniques (e.g., LC vs. supercritical fluid chromatography (SFC) and MS/MS vs. HRMS).
- **External QC**: Many participants took part in several external QC/proficiency testing programs, such as QUASIMEME, G-EQUAS, HBM4EU-ICI/EQUAS, AMAP, EURL programmes for food and feed and EURL POPs trials. A few participants indicated that they did not participate in proficiency testing.
- **NTS Specific Practices**: Non-target screening approaches included additional QA/QC measures such as monitoring extraction blanks, instrumental blanks, and instrument drift over time.

The identified analytical challenges on PFAS analysis can be categorized into 5 key areas: recovery, sensitivity, stability and reproducibility, interferences and background contamination, challenges in non-target screening.

- **Recovery**: Low recoveries were commonly reported as a challenge, particularly for long-chain PFAS (C>14) due to adsorption during extraction, or due to matrix complexity (e.g. biota). Low recoveries were also reported for certain polar compounds such as ultrashort-chain PFAS (e.g. TFA) in various matrices.
- **Sensitivity**: Achieving low detection limits for specific PFAS, such as PFBA and chlorinated PFAS (e.g., 6:2 Cl-PFESA), was a challenge due to low sensitivity (high LOD especially in complex matrices like food) or cross-contamination.
- **Stability and reproducibility**: Ensuring the stability and reproducibility of measurements, especially for short-chain PFAS and in methods such as the TOPA, was a recurring concern.
- **Interferences and background contamination**: High laboratory backgrounds, interferences in human samples, and contamination during analysis (e.g. from blank issues) were noted, especially for short-chain PFAS like TFA and for PFAS Total measurements (combustion ion chromatography (CIC)).

- **Challenges in NTS**: Non-target screening methods were described as time-consuming and lacking standardization. The availability of comprehensive mass spectra databases for confirming unknown compounds was considered critical.

In terms of method development and optimization, it was reported that different PFAS groups required specific extraction and analytical techniques, and that methods needed to be optimized for various matrices (e.g. biota, food, water) to ensure reliable recovery and detection. For PFAS Total difficulties in achieving complete mass balances and identifying all PFAS in total fluorine assays were noted as significant obstacles. For example, TOPA was considered challenging, as achieving comprehensive conversion of all PFAS compounds is difficult, which affects the ability to accurately quantify total PFAS precursors.

Regarding QA/QC, a major gap identified was the limited availability of high-quality and mass-labelled standards, including ^{13}C-labelled compounds, especially for less common PFAS, neutral PFAS, and precursors. The availability of CRMs for different matrices is still limited, hindering the accurate assessment of method performance. And, although proficiency programmes have improved, it was referred that they still cover only a limited number of compounds, particularly for human biomonitoring. Overall, the need for standardized methods was emphasized as a means to improve consistency across laboratories, especially for semi-quantification of suspect and non-target screening.

Appendix 1.2.4 Integrating PFAS Total and target analysis: opportunities and considerations

Participants expressed a generally positive outlook on the possibilities of integrating PFAS Total and target analysis, along with potential suspect screening for enhanced monitoring purposes (Q15, Table A1).

A few participants referred to a tiered strategy as an effective tool for PFAS monitoring, with the use of methodologies such as TOPA or EOF to serve as effective pre-screening tools, followed by comprehensive suspect and/or non-target screening. Nonetheless, the methodologies employed are dependent on the matrix being analysed. The tired strategy could allow a more comprehensive and focused analysis of matrices that exhibit higher levels of contamination, which would optimize resource allocation and enhance the efficiency of monitoring efforts. It was also highlighted that combining these analytical approaches is particularly valuable for identifying emerging PFAS and ensuring more comprehensive monitoring.

However, although integrating PFAS Total and target analysis can be a good strategy, participants acknowledged several operational challenges. Conducting comprehensive combined measurements, particularly in large-scale studies like human cohort research, can be resource intensive. The limited capacity for performing simultaneous analyses also presents as a significant barrier to implementation. Optimizing workflows and establishing clear guidelines for when to utilize each method could alleviate some of these challenges, ensuring that the most relevant analyses are prioritized without overwhelming laboratory resources. Also, participants noted that without a consistent framework for measuring PFAS Total across various matrices - such as water, soil, and biota - the reliability of results could be compromised.

Appendix 1.3 Main conclusions

The workshop successfully fostered discussions on PFAS monitoring strategies and research efforts. It highlighted the diverse approaches and challenges faced in PFAS monitoring, from selecting appropriate matrices to sampling frequencies. There was a strong emphasis on the need for continued collaboration to develop standardized methodologies and improve data comparability across monitoring programs. Participants agreed that PFAS monitoring should be adaptative and comprehensive, incorporating a wide array of compounds based on matrix, site specificity, and emerging contamination concerns.

PFAS analysis remains one of the main challenges. Despite continuous technological improvements, there were still significant issues with recovery rates, contamination, sensitivity, and the complexity of analysing a broad spectrum of PFAS. Tackling these challenges requires the development of more robust methods, new standards, and the expansion of databases for non-target screening.

Combining PFAS Total, target analysis, and suspect screening shows significant potential for monitoring purposes, though challenges remain. A tiered approach in which PFAS Total methods (e.g. TOPA or EOF) could be used for initial screening and for prioritizing samples for target and non-target analysis was suggested. However, standardization is crucial for consistency, and while non-target screening can offer comprehensive data and help to detect emerging PFAS, it requires harmonization and capacity building.

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Appendix 3. Scientific literature

TABLE A4. PFAS reported in environmental, food, drinking water, and human samples in the literature review (32 scientific articles, references in brackets).

PFAS	Environmental								Food & drinking water							Human			
Abbrev. (CAS reg. no.)	Air	Dust (In/Out)	Snow	Rain	Surface water	Groundwater	Soil / Sediment	Biota	Drinking water	Vegetables / Fruit	Fish	Shellfish	Seaweed	Eggs	Contacting material	Blood / Serum / Plasma	Serum/ Urinary	Cerebrospinal fluid	Tissue samples
TFA (76-05-1)				[1]	[2]														
TMS (1493-13-6)					[2]														
PFPrA (422-64-0)					[2,3]														
PFBA (375-22-4)	[4]	[4,5]		[1,4,6]	[2,3,7–9]	[10–12]	[13]	[13–15]	[16]							[17]			[18]
PFPeA (2706-90-3)	[4]	[4,5]		[1,4,6]	[2,3,7,9]	[11]	[13]	[13]	[16]							[17]	[19]		[18]
PFHxA (307-24-4)	[4,20]	[4,5]		[1,4,6]	[3,7–9,21]	[11,12]	[13,22]	[13]	[16]			[23]	[24]		[24]	[17]	[19]	[25]	[18]
PFHpA (375-85-9)	[4,20]	[4,5]		[1,4,6]	[3,7–9,21]	[11,12]	[13,22]	[13]	[16]			[23]	[24]		[24]	[17]	[19]	[25]	[18]
PFOA (335-67-1)	[4,20]	[4,5]		[1,4,6]	[3,7–9,21]	[11,12]	[22]	[13,15]	[16]			[23]	[24]		[24]	[17]	[19]	[25]	[18]
PFNA (375-95-1)	[4,10,20]	[4,5]	[26]	[1,4,6]	[3,7–9,21]	[10–12]	[13,22]	[13–15,27]	[16]	[10]	[23]	[23]	[24]	[24]	[24]	[14,17,28,29]	[19]	[25]	[18]
PFDA (335-76-2)	[4,20]	[4,5]	[26]	[1,4,6]	[3,8,9]	[11,12]	[13,22]	[13–15,27]	[16]		[23]	[23]	[24]	[24]	[24]	[14,17,29]	[19]	[25]	[18]
PFUnDA (2058-94-8)	[4,20]	[4]	[26]	[1,4,6]	[3,7–9]	[11,12]	[13,22]	[13,15,27]			[23]	[23]	[24]	[24]	[24]	[14,17]	[19]	[25]	[18]
PFDoDA (307-55-1)	[4,20]	[4,5]	[26]	[4]	[3,8,9]	[11]	[13,22]	[13,15,27]			[23]	[23]	[24]		[24]	[14,17]	[19]	[25]	[18]
PFTrDA (72629-94-8)	[4,20]	[4,5]		[4]		[11]	[22]	[13,27]			[23]	[23]		[24]	[24]	[17]		[25]	[18]
PFTeDA (376-06-7)		[5]			[8]	[11,12]	[13,22]	[13–15,27]			[23]	[23]			[24]	[17]		[25]	[18]
PFPeDA (141074-63-7)	[20]	[5]			[8]						[23]	[23]			[24]				[18]

PFHxDA (67905-19-5)								[32]											
PFODA (16517-11-6)						[11]													[18]
PFBS (375-73-5)					[8]												[19]		[18]
PFEtS (354-88-1)	[4,20]	[4,5]		[4,6]	[2,3,7–9,21]	[11,12]	[13]	[13]	[16]			[23]				[17]	[19]		[18]
PFPrS (423-41-6)					[2]														
PFPeS (2706-91-4)					[2]	[12]													
PFHxS (355-46-4)		[5]		[6]	[7,9,21]	[12]			[16]							[17]			
PFHpS (375-92-8)	[4,10,20]	[4,5]		[4,6]	[3,8,9]	[10–12]	[13]	[13–15,27]	[16]	[10]		[23]	[24]	[24]	[24]	[14,28,29]	[19]	[25]	[18]
PFOS (1763-23-1)		[5]		[6]	[8]	[12]		[15]	[16]							[17]			
PFNS (68259-12-1)	[4,10,20]	[4,5]	[26]	[1,4,6]	[3,7–9]	[10–12]	[13,22]	[13–15,27]	[16]	[10]	[23]	[23]	[24]	[24]	[24]	[14,17,28,29]	[19]	[25]	[18]
PFDS (335-77-3)		[5]		[6]	[9]											[17]		[25]	
PFUdS (749786-16-1)	[20]	[5]			[8]	[11]		[32]								[17]		[25]	[18]
PFDoS (79780-39-5)																			
PFTrS (749786-16-1)																			
PFOSA (754-91-6)																			
1:3 FTCA (406-93-9)	[20]	[5]			[8,9]		[13]	[27]								[17]	[19]		
2:3 FTCA (3637-31-8)					[2]														
3:3 FTCA (356-02-5)					[2]														
6:2 FTCA (53826-12-3)					[2]	[12]													
7:2 FTCA (812-70-4)																			[18]
8:2 FTCA (27854-31-5)																			
10:2 FTCA (53826-13-4)																			[18]
6:2 FTUCA (161094-75-3)																			[18]
8:2 FTUCA (70887-84-2)	[20]																		

4:2 FTS (757124-72-4)	[20]																		
6:2 FTS (27619-97-2)								[32]											
8:2 FTS (149724-40-3)								[32]											
10:2 FTS (108026-35-3)								[32]											
12:2 FTS (1034143-66-2)								[32]											
14:2 FTS (1377603-17-2)								[32]											
4:2 FTSA (757124-72-4)								[32]											
6:2 FTSA (27619-97-2)		[5]		[6]	[7]	[11]													
8:2 FTSA (39108-34-4)	[10,20]	[5]		[6]	[7–9,21]	[10–12]	[10]			[10]								[25]	
4:2 FTOH (2043-47-2)	[10]	[5]		[6]	[8,9,21]	[10,11]	[10]			[10]								[25]	
6:2 FTOH (647-42-7)	[20]																		
8:2 FTOH (678-39-7)	[20,30,31]		[26]																
10:2 FTOH (39239-77-5)	[20,30,31]		[26]																
12:2 FTOH (865-86-1)	[20,30,31]		[26]																
PFECHS (646-83-3)	[20,31]		[26]																
6:2-diPAP (57677-95-9)						[11,12]													
8:2-diPAP (678-41-1)					[9]														
6:2/8:2-diPAP (943913-15-3)					[9]														
N-Me-FOSA (31506-32-8)					[9]														
N-Et-FOSA (4151-50-2)	[20,30,31]	[5]	[26]													[17]	[19]		
N-Me-FOSE (24448-09-7)	[20,30,31]	[5]	[26]		[8]											[17]	[19]		
N-Et-FOSE (1691-99-2)	[20,30,31]		[26]																
PFOSAA (754-91-6)			[26]																
MeFOSAA (2355-31-9)					[9]			[32]											

EtFOSAA (2991-50-6)					[9]													[25]	
HFPO-DA/ GenX (or acid form) (13252-13-6 / 62037-80-3)					[9]			[32]											
ADONA (or acid form) (958445-44-8)				[1,6]	[3,7,9]	[11]													
DONA (114873-37-9)					[3]														
NaDONA (2250081-67-3)					[7,9]														
PFMoPrA (377-73-1)					[3]														
C6O4 (151772-58-6)				[6]		[11]													
9Cl-PF3ONS (756426-58-1)					[3]														
F53B (acid form) (73606-19-6)																			
OBS (87-56-8)				[6]	[9]	[11]													
Capstone A (80475-32-7)																			
Capstone B (34455-29-3)						[11]													

References

[1] K.A. Pike, P.L. Edmiston, J.J. Morrison, J.A. Faust, Correlation Analysis of Perfluoroalkyl Substances in Regional U.S. Precipitation Events, Water Res 190 (2021). https://doi.org/10.1016/j.watres.2020.116685.

[2] Y. Wang, M. Liu, S. Vo Duy, G. Munoz, S. Sauvé, J. Liu, Fast analysis of short-chain and ultra-short-chain fluorinated organics in water by on-line extraction coupled to HPLC-HRMS, Science of the Total Environment 943 (2024). https://doi.org/10.1016/j.scitotenv.2024.173682.

[3] H. Ulrich, A. Macherius, U. Kunkel, M. Sengl, T. Letzel, Novel PFAS-specific monitoring approach for highly impacted surface waters, Chemosphere 349 (2024). https://doi.org/10.1016/j.chemosphere.2023.140893.

[4] P. Wang, M. Zhang, Q. Li, Y. Lu, Atmospheric diffusion of perfluoroalkyl acids emitted from fluorochemical industry and its associated health risks, Environ Int 146 (2021). https://doi.org/10.1016/j.envint.2020.106247.

[5] A.S. Young, E.H. Sparer-Fine, H.M. Pickard, E.M. Sunderland, G.F. Peaslee, J.G. Allen, Per- and polyfluoroalkyl substances (PFAS) and total fluorine in fire station dust, J Expo Sci Environ Epidemiol 31 (2021) 930–942. https://doi.org/10.1038/s41370-021-00288-7.

[6] M. Guerra de Navarro, Y. Reyna, N. Quinete, It's raining PFAS in South Florida: Occurrence of per- and polyfluoroalkyl substances (PFAS) in wet atmospheric deposition from Miami-Dade, South Florida, Atmos Pollut Res 15 (2024). https://doi.org/10.1016/j.apr.2024.102302.

[7] M. Zarębska, S. Bajkacz, Z. Hordyjewicz-Baran, Assessment of legacy and emerging PFAS in the Oder River: Occurrence, distribution, and sources, Environ Res 251 (2024). https://doi.org/10.1016/j.envres.2024.118608.

[8] A. East, R.H. Anderson, C.J. Salice, Per- and Polyfluoroalkyl Substances (PFAS) in Surface Water Near US Air Force Bases: Prioritizing Individual Chemicals and Mixtures for Toxicity Testing and Risk Assessment, Environ Toxicol Chem 40 (2021) 859–870. https://doi.org/10.1002/etc.4893.

[9] B. Göckener, A. Fliedner, K. Weinfurtner, H. Rüdel, A. Badry, J. Koschorreck, Tracking down unknown PFAS pollution – The direct TOP assay in spatial monitoring of surface waters in Germany, Science of the Total Environment 898 (2023). https://doi.org/10.1016/j.scitotenv.2023.165425.

[10] Y.M. Tefera, S. Gaskin, K. Mitchell, D. Springer, S. Mills, D. Pisaniello, Food grown on fire stations as a potential pathway for firefighters' exposure to per- and poly-fluoroalkyl substances (PFAS), Environ Int 168 (2022). https://doi.org/10.1016/j.envint.2022.107455.

[11] J. Zeng, K. Liu, X. Liu, Z. Tang, X. Wang, R. Fu, X. Lin, N. Liu, J. Qiu, Driving factor, source identification, and health risk of PFAS contamination in groundwater based on the self-organizing map, Water Res 267 (2024). https://doi.org/10.1016/j.watres.2024.122458.

[12] M. Currell, N. Northby, P. Netherway, Examining changes in groundwater PFAS contamination from legacy landfills over a three-year period at Australia's largest urban renewal site, Chemosphere 352 (2024). https://doi.org/10.1016/j.chemosphere.2024.141345.

[13] K. Gao, X. Miao, J. Fu, Y. Chen, H. Li, W. Pan, J. Fu, Q. Zhang, A. Zhang, G. Jiang, Occurrence and trophic transfer of per- and polyfluoroalkyl substances in an Antarctic ecosystem, Environmental Pollution 257 (2020) 113383. https://doi.org/10.1016/J.ENVPOL.2019.113383.

[14] S. Palaniswamy, L. Nevala, P. Pesonen, A. Rautio, M.R. Järvelin, K. Abass, D. Charles, Environmental contaminants in Arctic human populations: Trends over 30 years, Environ Int 189 (2024) 108777. https://doi.org/10.1016/J.ENVINT.2024.108777.

[15] M. Duny, A. Cortéjade, L. Wiest, M. Nicolas, E. Vulliet, Single injection LC-MS/MS analytical method for the quantification of diverse families of micropollutants, including PFAS and organotins, in Gammarus fossarum, J Chromatogr A 1720 (2024). https://doi.org/10.1016/j.chroma.2024.464778.

[16] M. Grung, D. Hjermann, T. Rundberget, K. Bæk, C. Thomsen, H.K. Knutsen, L.S. Haug, Low levels of per- and polyfluoroalkyl substances (PFAS) detected in drinking water in Norway, but elevated concentrations found near known sources, Science of The Total Environment 947 (2024) 174550. https://doi.org/10.1016/J.SCITOTENV.2024.174550.

[17] Y.T. Guo, J. bo Huang, J. chuan Xue, S.J. Qin, D. Cai, Q.Z. Wu, C. Chu, C. lan Tang, Y. qi Xie, L.Z. Lin, G.H. Dong, X.W. Zeng, Intrauterine exposure to long-chain perfluorocarboxylic acids (PFCAs) were associated with reduced primary bile acids in three-year-old children: Findings from a prospective birth cohort study, Environmental Pollution 360 (2024). https://doi.org/10.1016/j.envpol.2024.124583.

[18] F. Pérez, M. Nadal, A. Navarro-Ortega, F. Fàbrega, J.L. Domingo, D. Barceló, M. Farré, Accumulation of perfluoroalkyl substances in human tissues, Environ Int 59 (2013) 354–362. https://doi.org/10.1016/j.envint.2013.06.004.

[19] S.M. Eick, Y. Tan, K.R. Taibl, P. Barry Ryan, D.B. Barr, A. Hüls, J.A. Eatman, P. Panuwet, P.E. D'Souza, V. Yakimavets, G.E. Lee, P.A. Brennan, E.J. Corwin, A.L. Dunlop, D. Liang, Prenatal exposure to persistent and non-persistent chemical mixtures and associations with adverse birth outcomes in the Atlanta African American Maternal-Child Cohort, J Expo Sci Environ Epidemiol 34 (2024) 570–580. https://doi.org/10.1038/s41370-023-00530-4.

[20] J.L. Barber, U. Berger, C. Chaemfa, S. Huber, A. Jahnke, C. Temme, K.C. Jones, Analysis of per- and polyfluorinated alkyl substances in air samples from Northwest Europe, Journal of Environmental Monitoring 9 (2007) 530–541. https://doi.org/10.1039/b701417a.

[21] E.L. McFarlan, L.D. Lemke, Per- and polyfluoroalkyl substances (PFAS) fate and transport across a groundwater-surface water interface, Science of the Total Environment 951 (2024). https://doi.org/10.1016/j.scitotenv.2024.175672.

[22] S. Boitsov, A. Bruvold, L. Hanssen, H.K.B. Jensen, A. Ali, Per- and polyfluoroalkyl substances (PFAS) in surface sediments of the North-east Atlantic Ocean: A non-natural PFAS background, Environmental Advances 16 (2024). https://doi.org/10.1016/j.envadv.2024.100545.

[23] W. Young, S. Wiggins, W. Limm, C.M. Fisher, L. Dejager, S. Genualdi, Analysis of Per- and Poly(fluoroalkyl) Substances (PFASs) in Highly Consumed Seafood Products from U.S. Markets, J Agric Food Chem 70 (2022) 13545–13553. https://doi.org/10.1021/acs.jafc.2c04673.

[24] N. Aßhoff, T. Bernsmann, M. Esselen, T. Stahl, A sensitive method for the determination of per- and polyfluoroalkyl substances in food and food contact material using high-performance liquid chromatography coupled with tandem mass spectrometry, J Chromatogr A 1730 (2024) 465041. https://doi.org/10.1016/J.CHROMA.2024.465041.

[25] W. Hu, M.Y. Zhang, L.Y. Liu, Z.F. Zhang, Y. Guo, Perfluoroalkyl and polyfluoroalkyl substances (PFASs) crossing the blood-cerebrospinal fluid barrier: Their occurrence in human cerebrospinal fluid, J Hazard Mater 442 (2023). https://doi.org/10.1016/j.jhazmat.2022.130003.

[26] C.J. Young, V.I. Furdui, J. Franklin, R.M. Koerner, D.C.G. Muir, S.A. Mabury, Perfluorinated acids in arctic snow: New evidence for atmospheric formation, Environ Sci Technol 41 (2007) 3455–3461. https://doi.org/10.1021/es0626234.

[27] N. Pala, K. Vorkamp, R. Bossi, S. Ancora, N. Ademollo, D. Baroni, G. Sarà, S. Corsolini, Chemical threats for the sentinel Pygoscelis adeliae from the Ross Sea (Antarctica): Occurrence and levels of persistent organic pollutants (POPs), perfluoroalkyl substances (PFAS) and mercury within the largest marine protected area worldwide, Science of The Total Environment 947 (2024) 174562. https://doi.org/10.1016/J.SCITOTENV.2024.174562.

[28] T. Saha, M.C.J. Gbemavo, L. Booij, T.E. Arbuckle, J. Ashley-Martin, M. Fisher, G. Muckle, B. Lanphear, E. Asztalos, J. Séguin, M.F. Bouchard, Prenatal exposure to PFAS and the association with neurobehavioral and social development during childhood, Int J Hyg Environ Health 263 (2025). https://doi.org/10.1016/j.ijheh.2024.114469.

[29] C.B. Manzano-Salgado, M. Casas, M.J. Lopez-Espinosa, F. Ballester, M. Basterrechea, J.O. Grimalt, A.M. Jiménez, T. Kraus, T. Schettgen, J. Sunyer, M. Vrijheid, Transfer of perfluoroalkyl substances from mother to fetus in a Spanish birth cohort, Environ Res 142 (2015) 471–478. https://doi.org/10.1016/j.envres.2015.07.020.

[30] A. Jahnke, J.L. Barber, K.C. Jones, C. Temme, Quantitative trace analysis of polyfluorinated alkyl substances (PFAS) in ambient air samples from Mace Head (Ireland): A method intercomparison, Atmos Environ 43 (2009) 844–850. https://doi.org/10.1016/J.ATMOSENV.2008.10.049.

[31] I. Kourtchev, B.G. Sebben, S. Brill, G.G.B. Cybelli, B. Weber, R.R. Ferreira, F.A.F. D'Oliveira, C.Q. Dias-Junior, O.A.M. Popoola, J. Williams, C. Pöhlker, R.H.M. Godoi, Occurrence of a "forever chemical" in the atmosphere above pristine Amazon Forest, Science of The Total Environment 944 (2024) 173918. DOI.

[32] H.A. Langberg, S.E. Hale, G.D Breedveld et al. A review of PFAS fingerprints in fish from Norwegian freshwater bodies subject to different source inputs. Environ Sci Process Impacts 24 (2022):330–342. DOI.

Appendix 4. Method development for non-target screening of neutral PFAS

Fish, shellfish and soil samples are intended to be analysed by GC-QTOF-HRMS at the University of Copenhagen. The following analytical standards of neutral PFAS were obtained from Wellington Laboratories and used to spike soil and biota samples in the method development. Each compound was spiked with approximately 5 ng.

TABLE A5. Standards of neutral PFAS for method development

Compounds	Abbreviation	CAS	Formula	InChI keys	Exact mass
6:2 Fluorotelomer alcohol	6:2 FTOH	647-42-7	C8H5F13O	GRJRKPMIRMSBNK-UHFFFAOYSA-N	364.01328
8:2 Fluorotelomer alcohol	8:2 FTOH	678-39-7	C10H5F17O	JJUBFBTUBACDHW-UHFFFAOYSA-N	464.00689
10:2 Fluorotelomer alcohol	10:2 FTOH	865-86-1	C12H5F21O	FLXYIZWPNQYPIT-UHFFFAOYSA-N	564.00051
N-Methylperfluorobutane sulfonamidoethanol	MeFBSE	34454-97-2	C7H8F9NO3S	DSRUAYIFDCHEEV-UHFFFAOYSA-N	357.00812
N-Methylperfluorooc-tanesulfonamidoethanol	MeFOSE	24448-09-7	C11H8F17NO3S	PLGACQRCZCVKGK-UHFFFAOYSA-N	556.99534
N-Ethyl perfluorooc-tanesulfonamidoethanol	EtFOSE	1691-99-2	C12H10F17NO3S	HUFHNYZNTFSKCT-UHFFFAOYSA-N	571.01099

GC-MS was used in the method development to optimize the chromatographic separation methods for the neutral PFAS and to test the performance of extraction protocols. In addition, GC-MS was used to check the pollution degree of real samples to protect the GC-HRMS. GC-HRMS was used to identify new or unknown neutral PFAS.

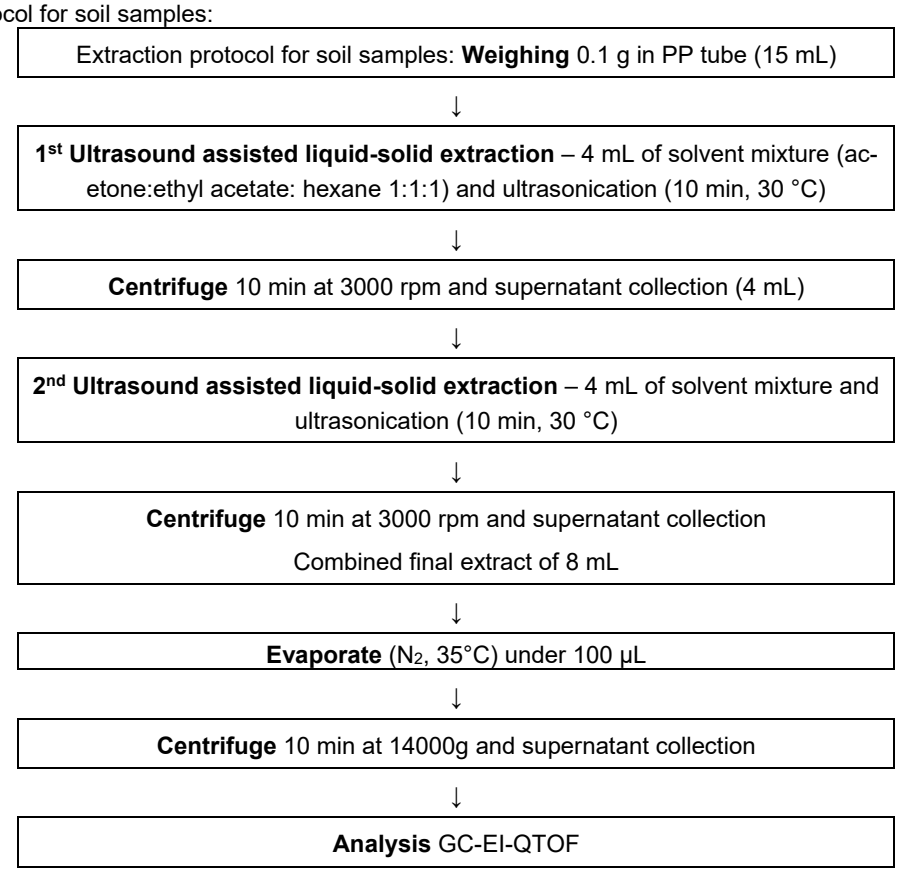
TABLE A6: GC-MS parameters

	GC (6890)-MS(5973)
Injection mode	Splitless
Injection volume	2 µL
Inlet temperature	250 °C
Column	Rxi 5Sil-5, 60 m×0.25 mm× 0.25 µm
Oven program	Held at 40 ◦C for 2 min, ramped up at 25 ◦C/min to 185 ◦C, ramped up at 3 ◦C/min to 230 ◦C, ramped up at 5 ◦C/min to 300 ◦C and held for 10 min.
Ion source	EI (70eV)
Monitoring mode	SIM

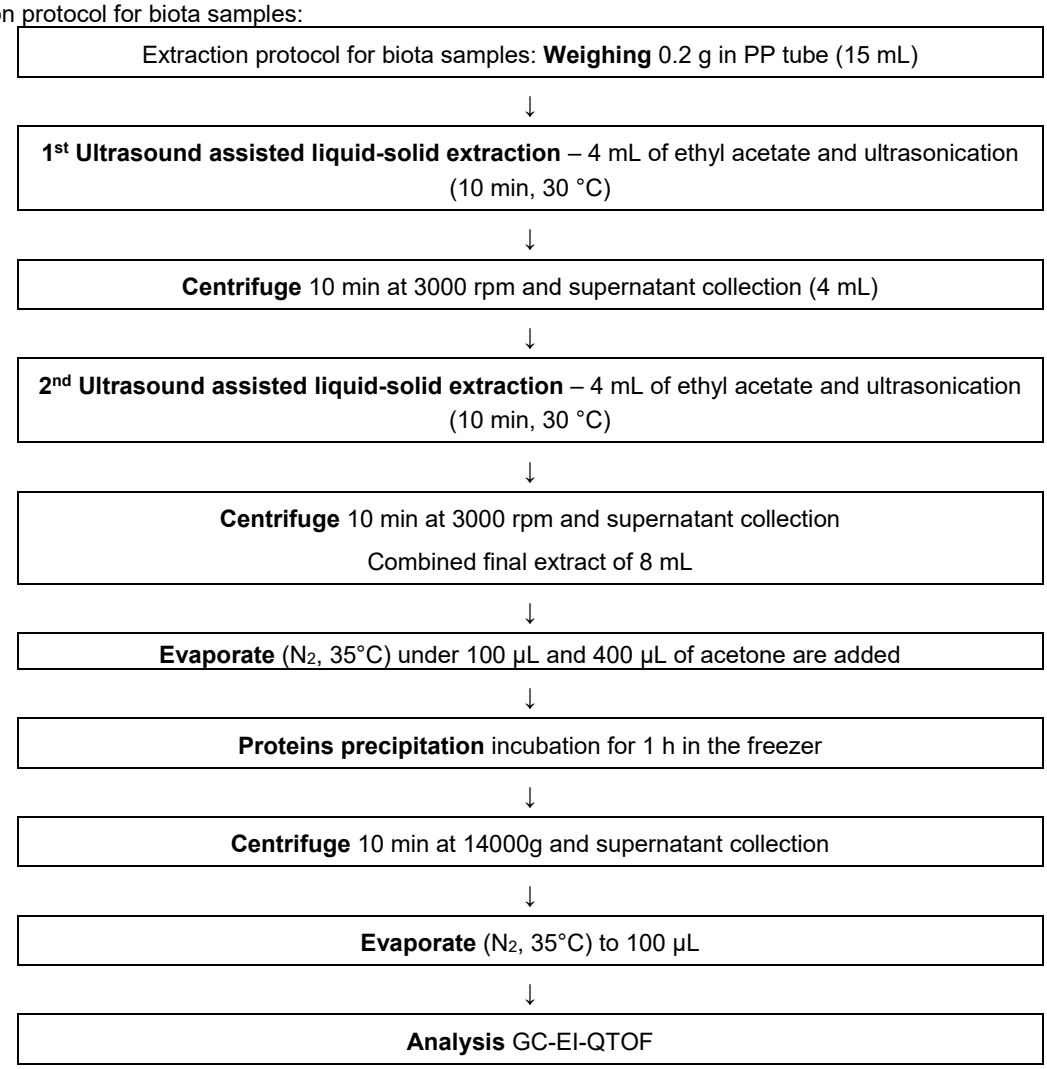
TABLE A7. GC-QTOF-HRMS parameters

	GC(7890B)-QTOF(7200)
Injection mode	Splitless
Injection volume	1 µL
Inlet temperature	250 °C
Column	Rxi 5Sil-5, 60 m×0.25 mm× 0.25 µm
Oven program	Held at 40 ◦C for 2 min, ramped up at 25 ◦C/min to 185 ◦C, ramped up at 3 ◦C/min to 230 ◦C, ramped up at 5 ◦C/min to 300 ◦C and held for 10 min.
Ion source	EI (70eV)
Monitoring mode	SCAN
Acquisition rate	2 Hz

Extraction protocol for soil samples:



Extraction protocol for biota samples:



Appendix 5. Results for PFAS target analysis

The individual PFAS vary between samples because some of the samples were analysed in the past, when the PFAS method included fewer individual substances.

Freshwater fish (perch liver); PFAS concentrations in ng/g wet weight.

Sample Type	Freshwater fish 1	Freshwater fish 2	Freshwater fish 3
AU I.D. number	2023-22135	2023-22139	2023-22145
PFBA	<0.10	<0.10	<0.10
PFPeA	<0.10	<0.10	<0.10
PFHxA	<0.10	<0.10	<0.10
PFHpA	<0.07	<0.07	<0.07
PFOA	<0.09	<0.09	1.37
PFNA	0.35	0.27	1.84
PFDA	2.62	1.99	5.65
PFUnDA	2.00	4.80	1.22
PFDoDA	0.94	2.19	0.61
PFTrDA	1.86	17.41	2.37
PFTeDA	0.32	2.16	0.59
PFODA	<0.10	<0.10	<0.10
PFBS	<0.10	<0.10	<0.10
PFPeS	<0.10	<0.10	<0.10
PFHxS	<0.16	<0.16	<0.16
PFHpS	<0.01	<0.01	<0.01
PFOS	32.29	21.38	450.33
PFDS	<0.08	<0.08	<0.08
PFOSA	<0.09	<0.09	<0.09
6:2 FTUCA	<0.10	<0.10	<0.10
8:2 FTUCA	<0.10	<0.10	<0.10
ADONA	<0.10	<0.10	<0.10
HFPO-DA	<0.10	<0.10	<0.10

Blue mussels; PFAS concentrations in ng/g wet weight.

Sample Type	Blue mussel 1	Blue mussel 2	Blue mussel 3
AU I.D. number	2024-22849	2024-23082	2024-23083
PFBA	<0.10	<0.10	<0.10
PFPeA	<0.10	<0.10	<0.10
PFHxA	<0.10	<0.10	<0.10
PFHpA	<0.10	<0.10	<0.10
PFOA	<0.09	<0.09	<0.09
PFNA	<0.18	<0.18	<0.18
PFDA	<0.13	<0.13	<0.13
PFUnDA	<0.10	<0.10	<0.10
PFDoDA	0.19	<0.07	<0.07
PFTrDA	<0.19	<0.19	<0.19
PFTeDA	<0.08	<0.08	<0.08
PFHxDA	<0.10	<0.10	<0.10
PFODA	<0.10	<0.10	<0.10
PFBS	<0.10	<0.10	<0.10
PFPeS	<0.10	<0.10	<0.10
PFHxS	<0.16	<0.16	<0.16
PFHpS	<0.01	<0.01	<0.01
PFOS	0.06	<0.05	<0.05
PFNS	<0.04	<0.04	<0.04
PFDS	<0.08	<0.08	<0.08
PFUdS	<0.03	<0.03	<0.03
PFDoS	<0.03	<0.03	<0.03
PFTrS	<0.03	<0.03	<0.03
PFOSA	0.31	0.09	<0.09
6:2 FTUCA	<0.10	<0.10	<0.10
8:2 FTUCA	<0.10	<0.10	<0.10
ADONA	<0.10	<0.10	<0.10
HFPO-DA	<0.10	<0.10	<0.10
4:2 FTSA	<0.15	<0.15	<0.15
6:2 FTSA	<0.15	<0.15	<0.15
8:2 FTSA	<0.15	<0.15	<0.15
6:2 FTCA	<0.05	<0.05	<0.05
7:3 FTCA	0.13	<0.05	<0.05
8:2 FTCA	<0.05	<0.05	<0.05
10:2 FTCA	<0.05	<0.05	<0.05
PFECHS	0.02	0.02	0.02
9-Cl-PF3ONS	<0.02	<0.02	<0.02
11-Cl-PF3OUdS	<0.02	<0.02	<0.02

Crabs; PFAS concentrations in ng/g wet weight.

Sample Type	Crab 1	Crab 2	Crab 3
AU I.D. number	2022-21717	2022-21537	2022-21536
PFOS	0.11	0.07	0.07
PFOSA	<0.09	<0.09	<0.09
PFHxS	<0.16	<0.16	<0.16
PFDA	<0.13	0.47	<0.13
PFNA	<0.18	0.43	<0.18
PFOA	<0.09	1.21	<0.09
PFUnDA	0.12	0.19	<0.10

Marine fish (muscle); PFAS concentrations in ng/g wet weight.

Sample Type	Marine fish muscle 1 (Eelpout)	Marine fish muscle 2 (Plaice)	Marine fish muscle 3 (Eelpout)	Marine fish muscle 4 (Flounder)	Marine fish muscle 5 (Flounder)
AU I.D. number	2023-22384	2023-22386	2023-22388	2023-22409	2024-22507
PFBA	<0.10	<0.10	<0.10	<0.10	<0.10
PFPeA	<0.10	<0.10	<0.10	<0.10	<0.10
PFHxA	<0.10	<0.10	<0.10	<0.10	<0.10
PFHpA	<0.10	<0.10	<0.10	<0.10	<0.10
PFOA	<0.09	<0.09	<0.09	<0.09	<0.09
PFNA	<0.18	<0.18	<0.18	<0.18	<0.18
PFDA	<0.13	<0.13	<0.13	<0.13	<0.13
PFUnDA	0.13	<0.10	<0.10	<0.10	<0.10
PFDoDA	<0.07	<0.07	0.17	<0.07	<0.07
PFTrDA	<0.19	<0.19	<0.19	<0.19	<0.19
PFTeDA	<0.08	<0.08	<0.08	<0.08	<0.08
PFHxDA	<0.10	<0.10	<0.10	<0.10	<0.10
PFODA	<0.10	<0.10	<0.10	<0.10	<0.10
PFBS	<0.10	<0.10	<0.10	<0.10	<0.10
PFPeS	<0.10	<0.10	<0.10	<0.10	<0.10
PFHxS	<0.16	<0.16	<0.16	<0.16	<0.16
PFHpS	<0.01	0.01	0.01	<0.01	0.01
PFOS	0.27	0.50	0.53	0.26	0.39
PFNS	<0.04	<0.04	<0.04	<0.04	<0.04
PFDS	<0.08	<0.08	<0.08	<0.08	<0.08
PFUdS	<0.03	<0.03	<0.03	<0.03	<0.03
PFDoS	<0.03	<0.03	<0.03	<0.03	<0.03
PFTrS	<0.03	<0.03	<0.03	<0.03	<0.03

PFOSA	<0.09	0.16	0.10	<0.09	<0.09
6:2 FTUCA	<0.10	<0.10	<0.10	<0.10	<0.10
8:2 FTUCA	<0.10	<0.10	<0.10	<0.10	<0.10
ADONA	<0.10	<0.10	<0.10	<0.10	<0.10
HFPO-DA	<0.10	<0.10	<0.10	<0.10	<0.10
4:2 FTSA	<0.15	<0.15	<0.15	<0.15	<0.15
6:2 FTSA	<0.15	<0.15	<0.15	<0.15	<0.15
8:2 FTSA	<0.15	<0.15	<0.15	<0.15	<0.15
6:2 FTCA	<0.05	<0.05	<0.05	<0.05	<0.05
7:3 FTCA	<0.05	0.05	0.10	<0.05	<0.05
8:2 FTCA	<0.05	<0.05	<0.05	<0.05	<0.05
10:2 FTCA	<0.05	<0.05	<0.05	<0.05	<0.05
PFECHS	0.02	0.02	0.02	0.02	0.02
9-Cl-PF3ONS	<0.02	<0.02	<0.02	<0.02	<0.02
11-Cl-PF3OUdS	<0.02	<0.02	<0.02	<0.02	<0.02

Wild birds (muscle); PFAS concentrations in ng/g wet weight.

Sample Type	Wild bird 1 (Greylag goose)	Wild bird 2 (Eurasian teal)	Wild bird 3 (Eurasian teal)	Wild bird 4 (Mallard)	Wild bird 5 (Mallard)
AU I.D. number	**2023-22438**	**2023-22452**	**2024-22558**	**2024-22571**	**2024-22574**
PFBA	<0.10	<0.10	<0.10	<0.10	<0.10
PFPeA	<0.10	<0.10	<0.10	<0.10	<0.10
PFHxA	<0.10	<0.10	<0.10	<0.10	<0.10
PFHpA	<0.07	<0.07	<0.07	<0.07	<0.07
PFOA	0.18	1.70	1.11	0.57	8.93
PFNA	0.39	1.11	25.56	3.12	8.82
PFDA	1.07	0.34	6.74	0.44	0.70
PFUnDA	0.54	0.13	1.80	0.16	0.39
PFDoDA	0.61	<0.07	0.30	<0.07	0.12
PFTrDA	0.93	0.21	0.21	<0.19	<0.19
PFTeDA	0.40	<0.08	0.06	<0.08	<0.08
PFODA	<0.10	<0.10	<0.10	<0.10	<0.10
PFBS	<0.10	<0.10	<0.10	<0.10	<0.10
PFPeS	<0.10	<0.10	<0.10	<0.10	<0.10
PFHxS	0.27	0.30	2.17	2.83	4.27
PFHpS	0.06	0.38	2.51	1.71	1.36
PFOS	9.28	27.57	183.03	36.91	38.24
PFNS	<0.04	<0.04	<0.04	<0.04	<0.04
PFDS	<0.08	<0.08	<0.08	<0.08	<0.08
PFUdS	<0.03	<0.03	<0.03	<0.03	<0.03
PFDoS	<0.03	<0.03	<0.03	<0.03	<0.03

PFTrS	<0.03	<0.03	<0.03	<0.03	<0.03
PFOSA	0.44	<0.09	<0.09	<0.09	<0.09
6:2 FTUCA	<0.10	<0.10	<0.10	<0.10	<0.10
8:2 FTUCA	<0.10	<0.10	<0.10	<0.10	<0.10
ADONA	<0.10	<0.10	<0.10	<0.10	<0.10
HFPO-DA	<0.10	<0.10	<0.10	<0.10	<0.10
4:2 FTSA	<0.15	<0.15	<0.15	<0.15	<0.15
6:2 FTSA	<0.15	<0.15	<0.15	0.23	<0.15
8:2 FTSA	<0.15	<0.15	<0.15	<0.15	<0.15
6:2 FTCA	0.81	<0.05	<0.05	<0.05	<0.05
8:2 FTCA	<0.05	<0.05	<0.05	<0.05	<0.05
10:2 FTCA	<0.05	<0.05	<0.05	<0.05	<0.05
PFECHS	0.02	0.07	0.13	0.18	0.37
9-Cl-PF3ONS	<0.02	<0.02	<0.02	<0.02	<0.02
11-Cl-PF3OUdS	<0.02	<0.02	<0.02	<0.02	<0.02

Sediment; PFAS concentrations in ng/g dry weight.

The samples were air-dried prior to analysis.

Sample Type	Marine sediment 1	Marine sediment 2	Marine sediment 3
AU I.D. number	2024-23579	2024-23581	2024-23582
PFBA	0.081	<0.004	<0.004
PFPeA	0,097	<0.004	<0.004
PFHxA	<0.002	<0.002	<0.002
PFHpA	<0.004	<0.004	<0.004
PFOA	0.062	0.033	6.32
PFNA	0.055	0.033	0.055
PFDA	0.111	0.032	0.043
PFUnDA	0.099	0.032	0.048
PFDoDA	0.179	<0.001	0.102
PFTrDA	<0.001	<0.001	1.308
PFTeDA	<0.001	<0.001	<0.001
PFHxDA	<0.001	<0.001	<0.001
PFODA	<0.001	<0.001	<0.001
PFBS	<0.002	<0.002	<0.002
PFPeS	<0.001	<0.001	<0.001
PFHxS	<0.001	<0.001	<0.001
PFHpS	<0.001	<0.001	<0.001
PFOS	0.318	0.023	4.370
PFNS	<0.001	<0.001	<0.001
PFDS	<0.001	<0.001	<0.001
PFUdS	<0.001	<0.001	<0.001
PFDoS	<0.001	<0.001	<0.001
PFTrS	<0.001	<0.001	<0.001
PFOSA	<0.020	<0.020	<0.020
6:2 FTUCA	<0.020	<0.020	<0.020
8:2 FTUCA	<0.020	<0.020	<0.020
ADONA	<0.001	<0.001	0,024
HFPO-DA	<0.001	<0.001	<0.001
4:2 FTSA	<0.001	<0.001	<0.001
6:2 FTSA	<0.180	<0.180	<0.180
8:2 FTSA	<0.002	<0.002	<0.002
6:2 FTCA	<0.08	<0.08	<0.08
7:3 FTCA	0.029	0.015	0.024
8:2 FTCA	<0.001	<0.001	<0.001
10:2 FTCA	<0.001	<0.001	<0.001
PFECHS	<0.001	<0.001	0,039
9-Cl-PF3ONS	<0.001	<0.001	<0.001
11-Cl-PF3OUdS	<0.001	<0.001	<0.001

Surface water and groundwater; PFAS concentrations in ng/L

Sample	Surface water (Brande Å)	Surface water (Søby Å)	Surface water (Elkjær Bæk)	Surface water (Isen Bæk)	Groundwater (Flyvestation Skrydstrup)
AU I.D. number	2024-23970	2024-23972	2024-23973	2024-23974	2024-24001
PFBA	1.36	<0.07	2.72	0.16	52.75
PFPeA	0.40	0.77	6.79	<0.06	268.58
PFHxA	0.63	2.51	8.88	0.11	117.42
PFHpA	0.55	0.41	1.52	0.12	138.63
PFOA	1.40	0.97	1.55	0.26	95.30
PFNA	0.06	0.04	0.11	<0.05	2.08
PFDA	0.06	<0.06	0.09	<0.06	<0.06
PFUnDA	<0.05	<0.05	<0.05	<0.05	<0.05
PFDoDA	0.04	0.04	0.04	0.04	<0.01
PFTrA	0.08	1.08	2.08	<0.01	<0.01
PFTeDA	<0.01	<0.01	<0.01	<0.01	<0.01
PFHxDA	<0.01	<0.01	<0.01	<0.01	<0.01
PFODA	<0.01	<0.01	<0.01	<0.01	<0.01
PFBS	0.29	0.75	0.96	0.10	0.21
PFPeS	0.09	0.09	0.12	0.06	0.10
PFHxS	0.38	0.28	0.52	0,12	1.13
PFHpS	0.04	<0.01	0.05	<0.01	0.19
PFOS	0.72	0.34	0.64	<0.01	0.77
PFNS	<0.01	<0.01	<0.01	<0.01	<0.01
PFDS	<0.01	<0.01	<0.01	<0.01	<0.01
PFUdS	<0.01	<0.01	<0.01	<0.01	<0.01
PFDoS	<0.01	<0.01	<0.01	<0.01	<0.01
PFTrS	<0.01	<0.01	<0.01	<0.01	<0.01
6:2 FTCA	<0.01	<0.01	<0.01	<0.01	<0.01
8:2 FTCA	<0.01	<0.01	<0.01	<0.01	<0.01
10:2 FTCA	<0.01	<0.01	<0.01	<0.01	<0.01
6:2 FTUCA	<0.01	<0.01	<0.01	<0.01	0.14
8:2 FTUCA	<0.01	<0.01	<0.01	<0.01	<0.01
HFPO-DA	<0.03	<0.03	<0.03	<0.03	<0.03
ADONA	<0.01	<0.01	<0.01	<0.01	0.03
PFOSA	<0.06	<0.06	<0.06	<0.06	<0.06
11-Cl-PF3OUdS	<0.01	<0.01	<0.01	<0.01	<0.01
9-Cl-PF3ONS	<0.01	<0.01	<0.01	<0.01	<0.01
PFECHS	<0.01	<0.01	<0.01	<0.01	<0.01
4:2 FTSA	<0.01	<0.01	<0.01	<0.01	0.25
6:2 FTSA	0.06	0.07	0.08	0.03	30.92
8:2 FTSA	0.01	<0.01	<0.01	<0.01	0.04

Human serum; PFAS concentrations in ng/mL.

The sample was analysed in triplicate. The recovery of the internal standard was relatively low for the first sample, resulting in fewer compounds detected in this sub-sample than in the two other replicates.

Sample Type	Human serum 1	Human serum 2	Human serum 3
AU I.D. number	2024-23631-1	2024-23631-2	2024-23631-3
PFBA	<0.07	<0.07	<0.07
PFPeA	<0.07	<0.07	<0.07
PFHxA	<0.03	<0.03	<0.03
PFHpA	<0.05	0.05	0.05
PFOA	0.84	0.70	0.66
PFNA	0.49	0.38	0.36
PFDA	0.19	0.16	0.15
PFUnDA	0.16	0.14	0.13
PFDoDA	<0.14	<0.14	<0.14
PFTrDA	<0.14	<0.14	<0.14
PFTeDA	<0.14	<0.14	<0.14
PFHxDA	<0.14	<0.14	<0.14
PFODA	<0.14	<0.14	<0.14
PFBS	<0.07	<0.07	<0.07
PFPeS	<0.07	<0.07	<0.07
PFHxS	<0.08	0.35	0.33
PFHpS	<0.11	<0.11	0.11
PFOS	3.80	3.24	3.06
PFNS	<0.03	<0.03	<0.03
PFDS	<0.03	<0.03	<0.03
PFUdS	<0.03	<0.03	<0.03
PFDoS	<0.03	<0.03	<0.03
PFTrS	<0.03	<0.03	<0.03
PFOSA	<0.07	<0.07	<0.07
6:2 FTUCA	<0.03	<0.03	<0.03
8:2 FTUCA	<0.03	<0.03	<0.03
ADONA	<0.03	0.03	0.03
HFPO-DA	<0.03	<0.03	<0.03
4:2 FTSA	<0.03	<0.03	<0.03
6:2 FTSA	<0.03	<0.03	<0.03
8:2 FTSA	<0.03	<0.03	<0.03
6:2 FTCA	<0.03	<0.03	<0.03
7:3 FTCA	<0.03	<0.03	<0.03
8:2 FTCA	<0.03	<0.03	<0.03
10:2 FTCA	<0.03	<0.03	<0.03
PFECHS	<0.03	0.05	0.05
9-Cl-PF3ONS	<0.03	0.04	0.04
11-Cl-PF3OUdS	<0.03	<0.03	<0.03

Further development of analytical methods for the monitoring of PFAS in environmental, food and human samples

PFAS is typically analysed as a group of 20-30 individual compounds in monitoring programmes, using liquid chromatography – mass spectrometry (LC-MS/MS). The Drinking Water Directive of the European Union also includes ”PFAS Total”, a sum parameter for extractable organic fluorine (EOF) determined by Combustion Ion Chromatography (CIC). This project included EOF analyses of environmental, food and human samples, including liver of freshwater fish, muscle of marine fish, shellfish (mussels, crabs), wild birds (muscle of geese and ducks), marine sediment, fertilizers, soil and human serum. EOF has higher detection limits than LC-MS/MS analyses of PFAS, resulting in some samples below detection limits for EOF (e.g. most birds and serum samples) although it was possible to detect individual PFAS in these samples. Otherwise, EOF exceeded ΣPFAS (sum of individual PFAS), indicating an unexplained PFAS occurrence.

Based on PFAS lists of different monitoring programmes, a workshop with European PFAS experts and examples from the scientific literature, a monitoring strategy was suggested. It consisted of the same core group of PFAS for different monitoring purposes to allow comparisons across matrices, together with matrix-specific selections of PFAS, such as short-chain PFAS in water and additional long-chain perfluorocarboxylic acids (PFCAs) in biological samples. Furthermore, ideas for combinations of EOF and individual PFAS were developed, for example using EOF in a first screening step, followed by more detailed analyses where relevant. In cases of significant differences between EOF and concentrations of individual PFAS, non-target screening methods could be applied to study the unknown part of EOF.

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