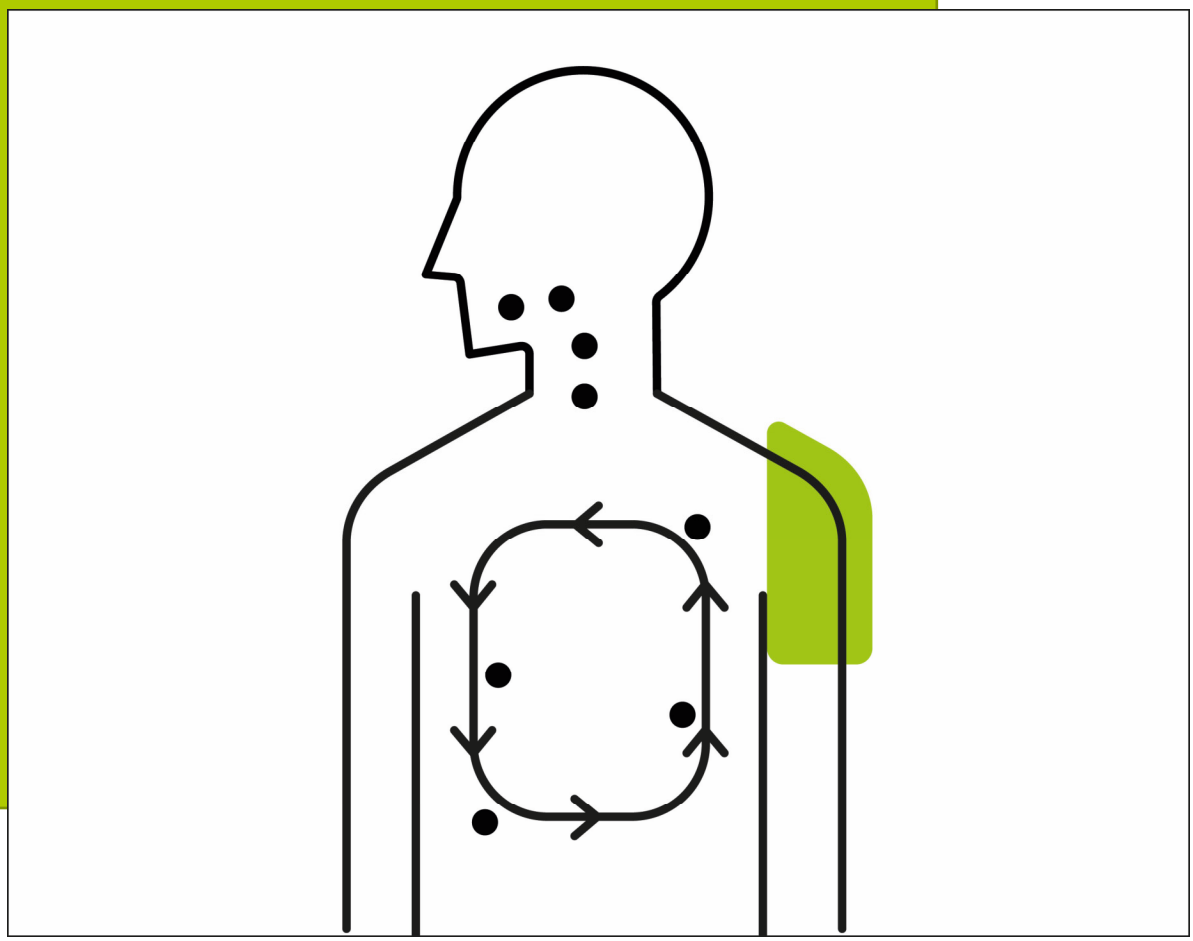
Poster abstracts



PFAS – Challenges and scientific perspectives in human health risk assessment

International conference

08–10 October 2025, Berlin

BfR
German Federal Institute for Risk Assessment

Preface

Dear colleagues,
dear guests,

The BfR warmly welcomes you to the international conference on "Per- and polyfluoroalkyl substances (PFAS) – Challenges and scientific perspectives in human health risk assessment".

Ten years have passed since the last PFAS meeting at the German Federal Institute for Risk Assessment (BfR) in Berlin – a decade of intensive research, increasing public attention and political and regulatory developments around PFAS. Since then, our scientific understanding about PFAS has advanced considerably in many areas.

However, due to their diversity, persistence, and mobility, PFAS remain a particular challenge for science, politics, industry and society. At the same time, efforts are made worldwide to gain more scientific knowledge on the different substances, for example by improving data on PFAS occurrence and clarifying toxicity, and which will foster the use of new approach methodologies in regulation as well as the development of novel PFAS alternatives.

The international PFAS conference 2025 in Berlin brings together experts, risk assessors and risk managers from all over the world to reflect on the progress made in recent years and to focus on future challenges for consumer health protection.

The first session of the conference will provide an overview on the current status of the PFAS restriction procedure. The state of knowledge on sources and exposure pathways will be presented together with the key health risk assessment challenges as identified in the 2020 EFSA opinion. The second session will focus on the current state of developments in PFAS analysis. Sessions three to five provide an overview on new findings in the field of human exposure, toxicokinetics and the development of digital models that contribute to a better understanding of PFAS in terms of transfer, half-life and health effects. In a double-session, there will be presentations on PFAS toxicity followed by future perspectives, where contributions to alternative assessment and investigation methods will be presented with an outlook on needs for the further development of the risk assessment of PFAS.

What conclusions can we draw from research? What knowledge gaps remain? Which ones need to be closed with higher priority? These are important questions for advancing the risk assessment of PFAS in the context of consumer health protection and which will be explored and addressed in the concluding panel discussion. With this international conference, we are pleased to promote a dialogue that goes beyond the boundaries of disciplines and countries with the aim of finding ways together to meet the complex challenges posed by PFAS. We would like to express our special thanks to all participants who add to the success of this conference with commitment and scientific exchange.

The BfR and especially all members of the BfR working group PFAS wish you a pleasant stay and a successful conference in Berlin.

Berlin, October 2025

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1 Programme

Wednesday, 08 October 2025	
Opening	
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12:30–13:00	**Background and state of play on the PFAS restriction** Dr Christian Unkelbach, Federal Institute for Occupational Safety and Health, Germany
13:00–13:30	**PFAS everywhere?! Sources and pathways of human exposure** Prof. Dr Stuart Harrad, University of Birmingham, United Kingdom
13:30–14:00	**EFSA 2020: risk assessment and related challenges** Dr Ron Hoogenboom, Wageningen University & Research, The Netherlands
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09:35–10:05	**Kinetics in humans** PD Dr Klaus Abraham, BfR, Germany
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11:30–12:00	**Understanding half-life variability using mechanistic kinetic modelling** Dr James Chan, Agency for Science, Technology and Research, Singapore
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14:30–15:00	**C8 health study and Veneto cohort study: exposure and health impacts assessment** Prof. Dr Tony Fletcher, London School of Hygiene and Tropical Medicine, United Kingdom
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17:00–17:30	**Mode of action on immunotoxicity** Dr Macon Carroll, Oregon State University, United States of America
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Panel discussion on challenges and advances in human health risk assessment Session chair: Dr Frederic Müller, BfR	
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12:00–12:10	**Panel discussion in pictures** Lorna Schütte, Illustrator, Germany
12:10–12:30	**Wrap up and closure** Dr Tewes Tralau, Vice-President of the BfR, Germany

2 Targeted and untargeted analytical methods

2.1 Trifluoroacetic acid in fruits and vegetables – monitoring results from official food control

Luzia Buchstab, Leonie Moser, Anne Benkenstein, Cristin Wildgrube, Ellen Scherbaum

Chemisches- und Veterinäruntersuchungsamt Stuttgart, Germany

Trifluoroacetic acid (TFA) is a highly polar, water-soluble, and persistent compound belonging to the group of per- and polyfluorinated alkyl substances (PFAS). It is formed among other pathways, as a degradation product of fluorinated chemicals used in pesticides, refrigerants, propellants, and fluoropolymers, and is increasingly recognized as a ubiquitous environmental contaminant. Due to its high mobility, TFA is widely distributed via the water cycle, enters soils and plants, and thus the food chain.

As part of the official food monitoring, a total of 5739 fruit and vegetable samples were analysed for TFA in 2016/17 and 2024/25 (Q1). In 20 % of the samples, TFA findings at or above the reporting limit of 0.02 mg/kg were detected – regardless of cultivation method (organic/conventional) or country of origin. The highest mean residue levels were found in leafy vegetables and exotic fruits, while pome, stone, and citrus fruits had the lowest average levels. There was no significant difference between the two monitoring periods.

The provisional acceptable daily intake (ADI) set by EFSA (0.05 mg/kg bw) was not exceeded even at the highest levels measured. However, the ubiquitous presence and the high number of positive samples require that risk assessments take into consideration not only specific fruits or vegetables, but the entire diet across different consumer groups. The current lack of a legal maximum residue level makes regulatory assessment difficult.

Our results give an overview of the current TFA findings in fruits and vegetables and underline the need for critical evaluation of TFA inputs into the food chain – both analytically and regulatory.

2.2 Detecting PFAS beyond the current regulative request: a comprehensive overview of the contamination in Dutch water by UPHLC-ion mobility-HRMS

David Liwara[1], Philippe Diederich[2], Carsten Baessmann[2], Arnd Ingendoh[2], Birgit Schneider[2], Ilona Nordhorn[2], Sicco Brandsma[1], Pim Leonards[1]

[1] Vrije Universiteit Amsterdam, The Netherlands
[2] Bruker Daltonics GmbH & Co KG, Bremen, Germany

The US EPA estimates 15,000 PFAS as manufactured compounds, precursors, and degradation products. With this high number and the lack of reference standards or spectral libraries and plenty of isomers, a systematic and comprehensive monitoring becomes an extremely challenging task. Here, the intention was to sample and analyse wastewater and surface water at 30 sites in the Netherlands to create a map of the PFAS distribution by a non-target, unbiased analysis. UHPLC-HRMS combined with trapped ion mobility (TIMS) was used to identify the PFAS compounds with "4-dimensions" of criteria: mass accuracy, isotope pattern fit, MS/MS, CCS (collisional cross sections).

Samples were prepared by SPE according to EPA 1633 with a 2000x preconcentration for surface water and 500x for wastewater. Data acquisition was done with timsTOF Pro 2 (Bruker) in PASEF and bbCID modes. Kendrick mass defect (KMD) filters PFAS compounds from background, based on the fluorine content (repeating CF2 units). The identification workflow comprised of several steps using library searches, suspect list screening and denovo determination.

In total more than 20,000 features were detected from all effluents and surface water samples of the 30 sampling sites. About 500 potential PFAS candidates were left after the KMD filtering. For the wastewater, in total 188 features could be annotated as PFAS, and for the surface water samples, 137 features could be annotated. The number of detected PFAs varied at the different sampling sites, as expected from their local conditions and infrastructure. A couple of PFAS precursors and degradation products have been found which are not screened yet in any legal directives. These would have been overlooked in a targeted approach. I.e., while the targeted compounds may have been quantified then at actual low levels, the non-targeted degradation products would be present at high levels still posing potential hazard and health risks. An example is H2-U-PFOS which was identified by denovo determination. This unsaturated PFOS has already been found before in the environment, but a standard is not commercially available yet. Compounds like this one cannot be found with any current target or library search approach. In the presented non-targeted approach, the PFAS analysis is clearly extended beyond those limits since it enables the detection of a virtually unlimited number of PFAS incl. isomers in complex matrices.

2.3 Unveiling the PFAS fingerprint in biota leveraging the technique LC-VIP HESI(-)-TIMS-HRMS and untargeted workflows

Georgios Gkotsis[1], Philippe Diederich[2], Dimitrios Damalas[1], Reza Aalizadeh[1], Ilona Nordhorn[2], Carsten Baessmann[2], Nikolaos S. Thomaidis[1]

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PFAS identification presents significant analytical challenges. The U.S. Environmental Protection Agency (EPA) estimates that over 9,000 different PFAS exist, including both parent compounds and their metabolic and degradation products. This vast number of individual PFAS compounds, combined with the lack of commercially available reference standards, molecular databases, and spectral libraries, hinders PFAS identification. Furthermore, these compounds exhibit varied chemical properties and exist alongside an even larger number of transformation products (TPs). Analysing complex matrices poses additional challenges. PFAS are often present in low concentrations, and the presence of isomeric and isobaric PFAS, along with matrix interferences, complicates identification. This can lead to inefficiencies in MS/MS spectra acquisition, especially in data acquired using DIA modes, which often generate complex spectra [3]. Given these complexities, it is essential to employ analytical techniques capable of identifying not only well-known PFAS but also identifying the PFAS "chemical fingerprint" in the environment. The primary objective of this study was to evaluate the capabilities of ion mobility spectrometry (IMS) in the established LC-HRMS workflows for the in-depth monitoring of PFAS in complex environmental matrices, such as biota.

A dedicated PFAS sample preparation protocol, validated for 56 PFAS congeners, was carried out aiming to enrich the final extracts with PFAS covering a wide range of physicochemical properties, making them suitable for untargeted post-acquisition data treatment workflows. The IMS-HRMS analysis was conducted using optimized broad mass and mobility transfer modes.

The features, included in the feature table were prioritized using Kendrick Mass Defect (KMD). An extensive PFAS suspect list containing 4,967 fluorinated substances was used in the framework of the present study for the annotation of the prioritized features. This suspect list was available through NIST public data depository and uploaded in MetaboScape as a CSV file. The structural information originated from InChI enable retention time, CCS prediction and in-silico fragmentation of each PFAS included in the suspect list.

A comprehensive feature table was created afterwards peak picking algorithm, including tens of thousands of features, due to the complexity of biota samples. By applying the KMD for the CF_2 repeating unit the list of untargeted features was significantly reduced, containing hundreds of features, and the number of true mass matches in a prior suspect screening approach was enhanced. Each annotated feature was thoroughly evaluated based on the precursor ion's mass error, isotope profile, retention time, MS/MS spectrum, and CCS value matching. Thus, the ion mobility-derived CCS values further enhanced the identification confidence by serving as an additional identification criterion. Some features were annotated with more than one fluorinated compound. To address this, a detailed investigation of the MS/MS spectra matching was conducted. Ion mobility filtering contributed to cleaner MS/MS spectra, while the data-dependent acquisition mode, PASEF, provided extensive MS/MS spectra coverage, which is often a challenge in untargeted workflows. These approaches enabled the confident

identification of PFAS congeners. Additional chemometric tools were employed to support the non-targeted identification of fluorinated compounds and their transformation products. This comprehensive IMS-HRMS-based untargeted workflow significantly enhanced the identification confidence of PFAS in biota samples.

2.4 Advanced GC×GC-TOFMS strategies for detection and characterization of semi-volatile PFAS in water samples

Maria Chiara Corviseri[1], Dmitrii Rakov[2], Anaïs Rodrigues[2], Allan Polidoro[1], Marco De Poli[1], Claudia Stevanin[1], Luisa Pasti[1], Sebastiano Panto[2], Flavio A. Franchina[1]

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[2] LECO Instrument GmbH, Berlin, Germany

Clean air, water, and soil are essential for sustaining life, and growing concern about environmental contamination has intensified the need for advanced screening of harmful substances. Persistent Organic Pollutants (POPs), including poly- and perfluoroalkyl substances (PFAS), represent a critical challenge due to their structural diversity, wide concentration ranges, and complex environmental matrices. Despite their relatively high molecular masses (often >600 Da), many PFAS possess sufficient volatility to allow analysis by gas chromatography–mass spectrometry (GC–MS).

In this study, we present a dual analytical strategy combining one-dimensional gas chromatography (1D-GC) and comprehensive two-dimensional gas chromatography (GC×GC) coupled with both low- and high-resolution time-of-flight mass spectrometry (TOFMS) for the detection and characterization of semi-volatile PFAS. Four major subclasses were targeted: fluorotelomer alcohols (FTOHs), fluorotelomer acrylates (FTAc), and sulfonamide-based derivatives (N-MeFOSA, N-EtFOSA, N-MeFOSE, and N-EtFOSE). Multiple ionization modes, including electron ionization (EI), positive chemical ionization (PCI), and negative chemical ionization (NCI), were applied to investigate fragmentation pathways and ionization behaviour.

A novel workflow integrating dynamic headspace (DHS) extraction with thermal desorption (TD) and GC×GC-TOFMS was developed for water samples. This approach provides a robust platform enabling both targeted quantification and untargeted screening of fluorinated contaminants. The results highlight the potential of GC×GC-TOFMS to improve environmental monitoring of PFAS and other (semi-)volatile persistent pollutants.

2.5 Gas chromatography and high-performance time-of-flight mass spectrometry for the advanced characterization of PFAS in anti-fog solutions

Anaïs Rodrigues[1], Dmitrii Rakov[1], Lena Dubois[1], Sebastiano Panto[1], David E. Alonso[2]

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Research into the prevalence of Per- and polyfluoroalkyl substances (PFAS) continues to grow due to concerns about their impact on human health. These materials are used in a wide variety of consumer products, including non-stick cookware, anti-stain solutions, fast food packaging, paints, and fire-fighting foams. PFAS accumulate in the environment, where they can undergo degradation to produce a wide array of fluorinated materials with different physicochemical properties. Unfortunately, regulatory control of these compounds has focused on the targeted analysis of a narrow quantity of PFAS classes. In this study, a comprehensive non-targeted analysis of anti-fog solutions was conducted using gas chromatography with time-of-flight mass spectrometry (GC-TOFMS).

Several PFAS compounds found in the anti-fog solutions, including fluorotelomer alcohols (FTOH), fluoroalkyl sulfonamides (PASA), and fluorotelomer ethoxylates (FTEOs), were not in standard databases. PFAS standards were purchased and analysed to create an internal library to supplement NIST and Wiley databases. Data processing included peak finding with deconvolution, spectral database matching, retention index filtering, and mass delta calculations for molecular and fragment ions. Data were also acquired on a high-resolution TOFMS using a multi-mode ion source (MMS) to identify unknowns. The MMS was used to generate complementary EI and CI high-resolution data, and the examination of mass accuracy values (1 PPM) and isotopic fidelity for the ion clusters helped in the confident annotation of emerging PFAS compounds in anti-fog solutions. In addition, scaled mass defect plots were used to target other unknown PFAS compounds in the solutions.

2.6 Comprehensive assessment of broad-spectrum PFAS exposure in a mother-child cohort

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Per- and polyfluoroalkyl substances (PFAS) are a group of highly persistent chemicals that are widespread worldwide, raising concerns about their potential adverse health effects. However, due to the scarcity of analytical standards, the large number of compounds and complex biota matrices, many PFAS remain poorly characterized, requiring advanced analytical strategies for comprehensive exposure assessment.

We developed and validated a fast sample preparation and sensitive LC-MS/MS method for trace quantification of diverse PFAS (n=81), ranging from ultrashort to long-chain ones, in mother–child cohort samples. In this pilot, maternal and cord blood serum, and urine from five mother–child pairs of the LINA cohort (Leipzig, Germany; 2006–2023, n = 629 samples) were analysed. Method validation across all matrices confirmed overall sensitive performance, with limits of detection (LODs) ranging from 8.3 to 83 ng/L and limits of quantification (LOQs) ranging from 25 to 208 ng/L for 81 PFAS. Robust recoveries (80–120%) were achieved for 76 of 81 PFAS, covering short-chain carboxylates, sulfonates, and multiple precursors. In total, 39 analytes were detected above their LODs and 27 were quantified above their LOQs in the selected five mother–child pairs of the LINA cohort samples. The median concentrations of PFOA (824 ng/L), PFOS (1039 ng/L), and PFNA (256 ng/L) were detected in the 34 weeks of maternal serum; corresponding were 417, 188, and 102 ng/L, respectively, in the cord serum samples, indicating transplacental transfer from mother to child. At 14 years, both mothers and children still showed PFAS burdens (e.g., PFOS 408 and 202 ng/L), reflecting ongoing exposure. In urine, short-chain PFAS dominated, with PFPrA up to 3.7 ng/mL (maternal, 14 y) and 3.0 ng/mL (child, 14 y), and PFBA 1.1 ng/mL in children. Among emerging PFAS, halogenated 5ClPFPeA was consistently above LOQ in urine (to 69 ng/L).

In a semiquantitative pilot TOP assay, nine PFAS precursors (FOSAA, N-Me-FOSAA, N-Et-FOSAA, DONA, FOSA, N-Me-FOSA, HFPO-DA, 10:2 FTS, 6:2 FTS) were converted nearly quantitatively (98–99 %) in water, with lower conversion for HFPO-DA (61–82 %) and 10:2 FTS (71–92 %). Product formation was dominated by PFOA (35 %) and PFOS (34 %). In spiked serum, peak areas of end-products increased 6.8-fold, driven by PFBA (68 %) and PFPrA (22 %), with median precursor peak area decreases of 58 % (5 ng) and 39 % (10 ng). These pilot results show that the TOP assay can be used to reveal hidden PFAS precursors in serum.

This pilot provides the first integrated view of legacy, short-chain, and emerging PFAS in the LINA cohort. The workflow will next be applied to identify broad-spectrum PFAS in all matched mother-child pairs (n = 629) from birth/age 1 year and after 14 years and complemented by quantitative TOP assays and suspect/non-target screening for comprehensive exposure assessment.

2.7 Rethinking PFAS analysis: how far can GC-MS take us?

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PFAS (per- and polyfluoroalkyl substances) represent a broad and complex class of synthetically produced chemicals with estimated 10,000 different compounds. Due to their persistence, toxicity, and widespread occurrence, PFAS have been a major focus of scientific research over the past decade, particularly in the areas of identification, quantification, toxicity, and potential pathways into the environment.

Effective monitoring of PFAS requires highly sensitive analytical techniques. Currently, liquid chromatography (tandem) mass spectrometry (LC-MS(/MS)) remains the gold standard for PFAS detection, offering a significantly extended detection range compared to gas chromatography-mass spectrometry (GC-MS). However, to expand accessibility GC-MS-based approaches are being actively developed and optimized. Although several analytical methods exist for detecting fluorinated contaminants in food, improving their reliability, sensitivity, and applicability remains a major challenge. Further optimization of GC-MS methodologies is essential to align with or extend beyond LC-MS techniques, enhancing their effectiveness for food and packaging analysis. Ultimately, these advancements will contribute to improved food safety.

This research is part of the EU project 23IND13 ScreenFood, which aims to develop sensitive analytical methods for improved identification and quantification of various PFAS – both currently regulated and emerging. Various analysis techniques are to be tested and compared for the comprehensive identification and sensitive quantification of PFAS used in food contact materials (FCM).

This poster presents improved GC-MS methods for the detection and quantification of multiple PFAS classes and provides initial insights into the analysis of FCM, such as paper-based packaging.

Funding

The project (23IND13, ScreenFood) has received funding from the European Partnership on Metrology, co-financed from the European Union's Horizon Europe Research and Innovation Programme and by the Participating States.

2.8 Quantification of branched PFASs in hay of a contaminated area in Germany

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Introduction

Branched (br-) PFASs are a side-product of the electrochemical fluorination (ECF) process, which was used from the early 1950s to the early 2000s as one of the major methods to produce PFOS and PFOA. Complex mixtures with various ratios of linear and branched isomers were formed, containing for example 20 – 30% br-PFOA and br-PFOS [1]. Linear and br-PFASs vary in their physicochemical properties and therefore, the occurrence in the environment, in living beings and the toxicologic behaviour differs [2, 3]. Currently, only a few data are available on the presence of br-PFASs in feed of plant origin. Furthermore, analysing the PFAS profile containing br-PFASs in feed is of interest with respect to a possible transfer to livestock and subsequent to humans through food of animal origin. Therefore, the aim of this study was to develop a LC-MSMS method for the simultaneous quantitation of linear and br-PFASs in feed. Representative for feed, hay was chosen. It is one of the most important feed materials for livestock. The developed method was further applied to a hay sample from a contaminated area in North Rhine-Westphalia, Germany.

Materials and methods

Hay samples were extracted using a modified QuEChERS protocol with formic acid after addition of isotopically labelled internal standards and clean up via graphitized carbon black (GCB). For analysis, an Agilent UHPLC 1290 Infinity II coupled to an Agilent 6495B triple quadrupole tandem mass spectrometer (Agilent, Santa Clara, CA) interfaced with an electrospray ion source working in the negative ion mode was used. Thirteen linear perfluorinated carboxylic acids (PFCA) with chain-lengths of 4 to 18 C-atoms and eight linear perfluorinated sulfonic acids (PFSA) with chain-lengths of 3 to 11 C-atoms were determined. The chromatographic separation was achieved using an Agilent InfinityLab Poroshell 120 EC-C18 analytical column (2.1 × 150 mm, 2.7 µm particle size) with a corresponding guard column and delay column.

Results

The chromatographic system was optimized to get base line separation of linear and br-PFASs. For quantification of br-PFASs the respective linear standard was used. Two MRM transitions were identified for each br-PFAS. Analysis of the hay sample of a contaminated area in Germany revealed several branched isomers of the sulfonates and carboxylates (C5 to C14). The branched Isomers of perfluorononanesulfonic acid (br-PFNS) and perfluorodecanesulfonic acid (br-PFDS) were detected with highest amounts (117 µg/kg and 86.5 µg/kg, respectively). The ratio of the branched to linear form is 2.4 for PFNS and 0.9 for PFDS. Perfluorobutanoic acid (PFBA) was the dominant PFAS found in the hay sample. Due to a lack of standards, the content varies depending on the transition used for quantification.

Discussion and conclusion

We detected linear and branched PFASs in a hay sample of a contaminated area in Germany. Interestingly, also branched isomers of long-chain PFASs like br-perfuorotetradecanoic acid (br-PFTeDA) were found. But, the quantification of br-PFASs needs further harmonization as given to wide-ranging results by calculating the amount of PFASs using each of the two different mass transitions.

References

[1] Buck et al, 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr. Environ. Assess. Manag. 7, 513–541

[2] Schulz K. et al.; 2020. Distribution and effects of branched versus linear isomers of PFOA, PFOS, and PFHxS: a review of recent literature. Sci. Total Environ. 773

[3] Schrenk, D., et al.; 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal. 18(9): p. e06223.

2.9 Transfer of selected PFASs in yellow mealworm larvae (*Tenebrio molitor*)

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Introduction

The larvae of the yellow mealworm beetle (*Tenebrio molitor*) can be a dietary protein source for animals and humans. While consumed worldwide, in the EU it was initially authorised as feed for use in aquaculture [1] and recently as feed for poultry and pigs [2]. Furthermore, dried *Tenebrio molitor* larvae were authorised in the EU in 2021 as novel food after the European Food Safety Authority (EFSA) assessed the larvae as safe for human consumption [3, 4]. Nevertheless, EFSA stated that only limited data were available on the possible transfer of chemical contaminants from contaminated substrate into larvae [4]. The aim of the project was to investigate and determine the possible transfer of different pollutants like dioxins, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and PFASs from contaminated oat-bran to yellow mealworm larvae. The present work shows the findings of the investigations with PFASs.

Materials and methods

Yellow mealworm larvae were exposed to oat-bran ad libitum over a period of six weeks under conditions controlled for temperature and air humidity. The contaminated oat-bran was fortified with 10 µg/kg PFASs. Weekly, larvae were taken and analysed for concentrations and results compared to control larvae reared on blank oat-bran. At the same time, larvae were weighed, counted and feces was removed. For sample preparation, larvae were spiked with isotopically labelled internal standards and extracted using a modified QuEChERS protocol with formic acid. The extracts were further cleaned via graphitized carbon black (GCB) prior to analysis using an Agilent 1260 Infinity LC coupled with an Sciex 6500 Triple quadrupole tandem-mass spectrometer (Agilent, Santa Clara, CA) interfaced with an electrospray ion source (ESI) operating in the negative ion mode. Thirteen linear perfluorinated carboxylic acids (PFCAs) with chain-lengths of 4 to 18 C-atoms and eight linear perfluorinated sulfonic acids (PFSAs) with chain-lengths of 3 to 11 C-atoms were determined. The chromatographic separation was achieved using an Agilent InfinityLab Poroshell 120 EC-C18 analytical column (2.1 × 150 mm, 2.7 µm particle size) with corresponding guard and delay columns.

Results

Yellow mealworm larvae kept on contaminated oat-bran developed similarly to control larvae reared on blank oat-bran regarding feed intake, weight and mortality. Furthermore, we observed a transfer of all compounds present in contaminated oat-bran into yellow mealworm larvae. After four weeks of exposure a steady-state was achieved.

Discussion and conclusion

We could demonstrate a transfer of different PFASs from feed into yellow meal larvae. A possibility to reduce the amounts might be fasting the larvae before processing into feed and food. Therefore, further investigations on the impact of fasting for 24 h and 48 h on contamination levels are on-going.

References

[1] Commission Regulation (EU) 2017/893, OJ L 138 pp. 92-116

[2] Commission Regulation (EU) 2021/1925, OJ L 393 pp. 4-8

[3] Commission Implementing Regulation (EU) 2021/882, OJ L 194 pp. 16-21

[4] EFSA Journal, 19(1):6343

2.10 New PFAS reference materials for environment and consumer products

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Per- and polyfluoroalkyl substances (PFAS) are a large group of emerging organic pollutants that contaminate the environment, food, and consumer products. They have a wide range of applications due to their water- and oil-repellent properties, as well as their chemical and thermal stability. However, the use of PFAS has raised concerns due to their persistence in the environment and their adverse health effects, leading to regulations aimed at controlling their use and minimizing exposure. Substances such as PFOS, PFHxS, and PFOA are listed in the Stockholm Convention on Persistent Organic Pollutants (POP Regulation, EU 2019/1021). Maximum levels have been set for PFAS in environmental matrices such as soil and water, but also for textiles as a relevant area of circular economy.

Reliable PFAS analysis leads to an increasing global demand for certified reference materials (CRM). However, CRMs for PFAS in soils and textiles are rare or currently not available. To improve the metrological infrastructure and support PFAS measurements, two CRMs for PFAS in soil (BAM-U027) and PFAS in textiles (BAM-B003) based on ISO 17034 and ISO 33405 were developed. This poster provides an overview of the different steps for the preparation and characterization of these two reference materials. The assignment of the certified mass fractions for relevant PFAS targets (18 PFAS compounds for BAM-B003) is based on isotope dilution HPLC-MS/MS at three independent workplaces. The results of the BAM in-house study on BAM-U027 were supported by an interlaboratory comparison study.

2.11 Interlaboratory comparison study for PFAS analysis in soils and eluates

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PFAS (per- and polyfluoroalkyl substances) comprise more than 10,000 compounds that have been used in various industrial and consumer products for decades. However, PFAS represent a growing problem because of their persistence in the environment and their adverse health effects. Therefore, reliable PFAS analysis of environmental compartments is indispensable but poses a complex challenge due to the large number of individual compounds in widely varying concentrations. The investigation and assessment of PFAS contamination in soil, as well as the determination and monitoring of necessary remediation measures, require comparable and high-quality standards in chemical analysis.

Interlaboratory comparison studies (ILCs) are suitable tools to ensure these quality standards. Thus, an ILC involving 17 participating laboratories in Germany was organized and conducted by BAM, SenMVKU, and BImA in 2024 to determine PFAS in real soil samples (solid matter and eluates). Essential results of the ILC will be presented focusing on analytical issues related to the HPLC-MS/MS determination of the PFAS target compounds based on current regulations but also alternatives such as GenX and Capstone A/B. Furthermore, unexpectedly observed effects interfering with the quantification of specific PFAS will be discussed.

2.12 PFAS determination in wastewater – monitoring and quality assurance

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Per- and polyfluoroalkyl substances (PFAS) are well-known as "forever chemicals" and persistent pollutants. The potential release of PFAS from different anthropogenic sources has gained increasing attention in the past years. This creates a need for the monitoring of PFAS in environmental matrices. Due to their high polarity and mobility, PFAS determination in surface-, ground- and wastewater is of particular interest for monitoring purposes. The investigation of PFAS contaminations in real wastewater samples is extremely challenging because of their high and varying matrix loads, and the high requirements for PFAS target-analysis regarding sensitivity according to current regulations (e.g., Drinking Water Ordinance, TrinkwV).

Since there is hardly any quality-assured measurement data available for PFAS quantified in wastewater, a monitoring project was conducted in 2024/2025 to determine PFAS levels from various wastewater sources in the state of Brandenburg (Germany). The main results will be presented and discussed with emphasis on analytical issues related to the HPLC-MS/MS determination. While investigations were focused on the 20 PFAS targets based on TrinkwV, also several other relevant PFAS compounds, e.g., trifluoroacetic acid (TFA) and HFPO-DA (GenX), were included into the monitoring.

2.13 A human biomonitoring method for quantification of ultra short-chain PFAS in urine for exposure analysis

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In addition to classic PFAS, the importance of ultra short-chain carboxylic and sulfonic acids with a chain length of C1 – C3 is steadily increasing. Recent studies indicate that trifluoroacetic acid (TFA) makes up the largest proportion of PFAS in the environment globally. In contrast, knowledge about human exposure to these substances is scarce. The aim of the study was the development of a human biomonitoring method for quantitation of ultrashort-chain PFAS in urine to assess occupational and environmental exposures.

An analytical method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) using isotope-labelled internal standards was developed. For quantification of five ultra short-chain PFAS in urine the samples were diluted with 0.1 % formic acid in water, the internal standard solution was added, and chromatography was then performed on an Ultra IBD column.

Linearity ranged from 0.05 to max. 75 µg/L. The limit of quantification (LOQ) of the method was between 0.05 µg/L and 1.5 µg/L, respectively. The precision at two different spiked concentration levels was less than 3 % and 15 % within the series and from day to day, respectively. Relative recovery in five different spiked urine samples was in the range of 85 – 115 %. The method was applied successfully to 90 urine samples from the German National Cohort (NAKO). TFA was detected in 100 % of the samples with concentrations ranging from 2.96 to 249 µg/L. The other analytes were detected below the LOQ.

The developed method enables the reliable determination of ultra short-chain PFAS in urine. However, one mass transition for TFA is a limiting factor in LC-MS/MS-determination. First data of urine samples reveal a ubiquitous internal burden of the general population to TFA. To gain a comprehensive overview of the extent of ultra short-chain PFAS exposure within the population, this method is now being applied to analyse a larger sample from the Hamburg study centre of the NAKO.

3 External and internal exposure

3.1 Systematic review on internal exposure to per- and polyfluoroalkyl substances (PFAS) and risk of ischemic heart diseases

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Per- and polyfluoroalkyl substances (PFAS) are highly persistent chemicals showing ubiquitous environmental contamination since several decades. Human exposure to PFAS is largely determined by the four substances perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorohexanesulfonic acid (PFHxS). Previous epidemiological research suggests that PFAS elevates blood lipid levels, which are established risk factors of coronary atherosclerosis, up to an internal exposure level of about 40 ng/ml. The present systematic review aimed to investigate whether internal exposure to PFOA, PFOS, PFNA, PFHxS and/or the sum of these PFAS influence the risk of ischemic heart diseases (IHD) in humans.

The databases MEDLINE and EMBASE were systematically searched, most recently on January 8, 2025. Only studies investigating individual blood PFAS exposure and risk of IHD in adults were eligible. Risk of bias was assessed with the OHAT Risk of Bias Rating Tool.

In total, sixteen observational studies comprising 79,313 participants were included. In six longitudinal studies, no associations of PFOA exposure and IHD risk were reported. Among eight cross-sectional studies, three reported significant positive associations of PFOA exposure and IHD risk. Two of three available longitudinal studies on PFOS reported significant associations with IHD risk in opposite directions. Most cross-sectional studies investigating PFOS, PFHxS, and PFNA reported small effect estimates.

Based on this systematic review cannot be sufficiently clarified whether there is an association between PFAS exposure and IHD. Future studies are needed, especially longitudinal investigations with exposure levels in the relevant range with respect to changes in blood lipids.

3.2 Effects of feeding per- and polyfluoroalkyl substances (PFAS) contaminated hay on the liver of dairy goats

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Small ruminants are often used to graze areas along rivers or industrial wasteland. As these areas pose an increased risk of PFAS contamination these animals are likely exposed. Besides concerns about the transfer of such chemicals from animal feed to human food of animal origin, the effects on animal health are largely unexplored in the affected species. Therefore, the present investigation gathered data on animal health in a feeding experiment with dairy goats on the transfer of PFAS into milk. Special attention was laid on the liver, as investigations on laboratory animals identified the liver as target organ.

A feeding trial was conducted with eight lactating German Improved White dairy goats assigned to two groups: a control group, fed with uncontaminated hay and an experimental group, fed with PFAS-contaminated hay (mean total of 1310±536µg PFAS/kg hay at 88%DM with 75% mainly long-chain perfluorosulfonic acids (chain lengths of C8–13) and 24% mainly shorter-chain perfluorocarboxylic acids (C4–C8)) for the first eight weeks, followed by a 12-week depuration period. Blood samples for health monitoring were collected weekly. At the end of the experiment, the animals were euthanized and dissected and tissue samples were obtained for further analysis. In liver, the tissue composition (protein, RNA, DNA, total lipids), histology and transcriptome (directional mRNA-Seq, Illumina, 2x 150bp, 30M pe reads/sample) were analysed.

Feeding the contaminated hay resulted in significantly decreased concentrations of total RNA in liver tissue resulting in decreased RNA:DNA ratio as an indicator of transcriptional activity. Concentrations of DNA, protein and lipids were not affected. However, the transcriptome analysis revealed a majority of upregulated genes which enriched the hematopoietic cell lineage, cell adhesion molecules and chemokine signalling pathway in the KEGG pathway -enrichment analysis (clusterProfiler 4.10.1, DAVID). Gene ontology analysis revealed inflammatory response and immune response as the top terms in biological processes while transmembrane signalling receptor activity and integrin binding were identified as the top molecular functions affected. The external side of plasma membrane and plasma membrane were the top cellular components. Therefore, the observed alterations seem to be driven by blood cells present in the liver tissue. However, PFAS contamination of the hay is not the only possible cause of the described effects. Differences in nutritional value of the hay of both feeding groups and further contaminants may have contributed to the observations. Further investigations under controlled exposure including sufficient replicates are needed to clarify causality.

3.3 PFAS in the hive: results from a controlled transfer study in bees and apicultural products

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Although the environmental distribution of per- and polyfluorinated alkyl substances (PFAS) is well documented, the extent to which PFAS are transferred into bee products and whether this can result in significant human exposure remains unclear. This study investigated the uptake and accumulation of PFAS in bee products at environmentally relevant concentrations.

For this purpose, honey bees were kept in mating units and fed sugar syrup enriched with 19 PFAS compounds of different classes and chain lengths over a period of seven days. Two exposure levels (low and high, n = 4 each) represented possible environmental pollution scenarios Uncontaminated syrup served as a negative control (n = 2). Upon exposure, samples of bee-stored nectar substitute were taken. All samples were analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

While all 19 fortified PFAS were detected in the nectar substitute stored by the bees, the focus here is on the four EFSA-evaluated compounds (PFOA, PFNA, PFHxS, PFOS). For these substances, feed concentrations in the low-dose group ranged from 6.4–8.7 µg/kg, whereas mean concentrations in the stored nectar substitute were 1.2–2.6 µg/kg, indicating a reduction of ~60–85 % during transfer. The measured PFAS levels in the stored nectar substitute were used alongside consumption data for honey among adults and children to estimate the weekly intake of these substances. These intakes were then compared with the Tolerable Weekly Intake (TWI) for the sum of PFOA, PFNA, PFHxS and PFOS (4.4 ng/kg body weight/week), as established by the European Food Safety Authority (EFSA). Depending on the amount consumed and the level of contamination, modelled intakes can reach or exceed the TWI.

This study provides a first well-founded assessment of the potential risks to food safety posed by PFAS in bee products and highlights the suitability of bees and their products as bioindicators of environmental PFAS contamination.

3.4 Distribution of per- and polyfluoroalkyl substances (PFAS) in goat milk fractions

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According to data on human biomonitoring, human exposure to PFAS occurs mainly through the consumption of contaminated food, which is why maximum levels for PFAS in food of animal origin have been set to protect health, with the exception of milk. A reduced antibody response in children after vaccination was identified as the most critical toxicological endpoint. Since children consume high amounts of milk and are a vulnerable population group, knowledge about the transfer of PFAS and their distribution in milk/milk products is important for consumer health protection.

In order to improve the data available, the Federal Institute for Risk Assessment conducted a transfer study on dairy goats in 2023. For this purpose, German White Noble goats were fed hay containing PFAS for eight weeks and contaminated raw milk was then separated into cream and skimmed milk at the Max Rubner-Institut and further processed into butter and ultrafiltration retentate and permeate (polysulfone-membrane cut-off 5000 dalton). To investigate the distribution of PFAS in milk products, the PFAS contents were analysed using HPLC-MS/MS.

PFAS were detected in almost all milk products, especially in products that are rich in both protein and fat. Solely by ultrafiltration, PFAS in skim milk were retained in the retentate together with milk proteins, among other macromolecules, and separated from the smaller molecules that remained in the permeate (e.g., lactose, minerals, small peptides, water). Further studies will investigate on how PFAS bind in milk components.

3.5 PFAS exposure from homegrown eggs: a health risk assessment across dietary patterns in a Polish pilot study

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Per- and polyfluoroalkyl substances (PFAS) are widespread contaminants posing health risks through bioaccumulation in the food chain. As dietary habits evolve, exposure levels may shift with food choices. Eggs, a common dietary component, can accumulate PFAS, becoming a notable exposure source. Thus, monitoring and reducing dietary PFAS intake is vital to stay within safe exposure thresholds (EFSA, 2020).

This study analysed PFAS content in homegrown eggs from an agricultural region of Poland and assessed related health risks. Concentrations of PFTriDA, EtFOSAA, PFUnDA, PFOS, PFNA, PFHpS, PFOA, THPFOS, PFHxS, PFHpA, PFHxA, PFBS, PFPeA and PFBA were determined by ultra-high-performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS). Only PFHpA and PFHxA levels were below the detection limit, while PFOS, PFNA, PFOA, and PFHxS exceeded the Maximum Level (ML) for eggs set in Commission Regulation (EU 2023/915).

Dietary exposure was estimated using statistical egg intake for a general diet and recommended consumption for ketogenic and carnivore diets in adults. EFSA's (2020) Tolerable Weekly Intake (TWI) was applied. Human Health Risk Assessment followed USEPA (1989) methodology, with Monte Carlo simulation performed in @RISK software (Lumivero) for PFAS with established reference dose (RfD) values, namely PFOS, PFOA, and PFBS.

References

EC 2023/915. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in foodstuffs and repealing Regulation (EC) No 1881/2006. Official Journal of the European Union, L 119.

EFSA 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal, 18(9), 6223). https://doi.org/10.2903/j.efsa.2020.6223

USEPA 1989. Risk assessment guidance for Superfund, Volume I: Human health evaluation manual (Part A, Interim final) (EPA/540/1-89/002). Office of Emergency and Remedial Response.

3.6 Results of the national feed monitoring 2022–2023 on representative PFAS levels in feed

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In 2020, the European Food Safety Authority (EFSA) re-assessed the risk of poly- and perfluoroalkyl substances (PFAS) present in food to human health. They concluded that PFAS exposure of humans is mainly contributed by consumption of contaminated food and drinking water, whereas the European food monitoring data exhibit highest PFAS levels in food of animal origin. The accumulation of PFAS in the food chain is a result of their persistent and bioaccumulative properties, whereby the latter increases with chain length. This has been demonstrated by feeding studies on farm animals investigating the transfer of PFAS into various edible tissues (e.g., meat and offal) and products (e.g., milk and eggs). As increased internal exposure to PFAS in humans is associated with health risks, the presence of PFAS in the food chain is undesirable.

Safe feed is fundamental for safe food. Accordingly, in close collaboration with the competent authorities in its federal states, Germany has performed a national monitoring programme for PFAS in feed in 2022 and 2023 and subsequently made this data available to the German Federal Institute for Risk Assessment (BfR) for scientific evaluation. For this purpose, a total of 214 feed samples were taken in 13 Federal States (excluding Berlin, Bremen and Hamburg). The feed materials were selected according to harvest yield and proportion in livestock feed. Overall, 52 samples of grass silage, 56 samples of maize silage, 49 samples of rapeseed meal, and 57 samples of wheat grains were collected. The samples were collected by the competent authorities in accordance with Regulation (EC) No 152/2009. All feeds were tested for a total of 16 perfluoroalkyl acids (PFAAs) (C4–C12; C13 and C14 optional) using HPLC-MS/MS analysis in the official monitoring laboratories. The test results from the federal states were compiled by the Federal Office for Consumer Protection and Food Safety (BVL) and evaluated by the BfR.

Of the 214 feed samples analysed, one to a maximum of four PFAS were quantified in 40 samples, resulting in a high proportion of left-censored data (82%). PFAS were most frequently detected in grass silage (22 samples) and maize silage (10 samples). The highest levels in the feed samples were of the short-chain compounds PFBA and PFPeA. Additionally, the compound PFOA was detected in grass and maize silage. Of the 40 samples with PFAS levels above the limits of quantification, 34 had a sum of PFAS levels below 1 µg/kg (88% dry matter (DM)). For the risk assessment, the mean lower bounds (LB) of PFOS and PFOA were determined to be 0.01 ± 0.04 µg/kg and 0.02 ± 0.06 µg/kg, respectively, and 0.002 ± 0.005 µg/kg for PFNA (88% DM). Maize silage had mean lower bound (LB) levels of PFOA and PFHxS of 0.01 ± 0.03 µg/kg and 0.001 ± 0.009 µg/kg, respectively. None of the EFSA-4-PFAS (PFOA, PFOS, PFNA and PFHxS) were detected in either rapeseed meal or wheat grains. Using the ConTrans prediction tool, the BfR concluded that, due to the low PFAS levels in the examined feed, the maximum levels in food of animal origin set out in Regulation 2023/915 were unlikely to be exceeded.

Acknowledgment

The BfR would like to thank the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) and the Federal Office of Consumer Protection and Food Safety (BVL) for their planning and support in implementing the PFAS feed monitoring programme,

as well as all the competent authorities of all federal states in Germany for their work in sampling feed and capturing metadata, as well as performing PFAS analyses.

Reference

EFSA 2020. Scientific Opinion on the risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal 2020;18(9):6223, 391 pp. https://doi.org/10.2903/j.efsa.2020.6223

3.7 PFAS accumulation in wild boars – a risk for consumers?

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Wild boars accumulate PFAS through their omnivorous diet and environmental exposure. Consequently, consuming wild boar may result in a higher PFAS intake than consuming meat and offal from livestock. Since the associated risk is directly linked to PFAS intake, it is crucial to obtain comprehensive data on PFAS levels in wild boar populations across Germany and in various edible tissues.

The German Federal Institute for Risk Assessment (BfR) has been investigating patterns of PFAS accumulation in wild boars for over eight years. This research comprises ongoing field sampling of wild boars for PFAS analysis, evaluation of wild boar liver as an indicator of terrestrial PFAS contamination, and assessment of consumer risks from consuming their meat and offal.

One of the latest BfR studies examined eleven PFAS in wild boars (n = 82), investigating their individual distribution in various tissues (liver, kidney, lung, spleen, heart muscle, and skeletal muscle) and plasma. The most prevalent PFAS relevant to internal exposure, i.e., PFOA, PFNA, PFHxS, and PFOS, were detected in all tissues and plasma samples. In edible tissues, the median level of the sum (∑4PFAS) was highest in liver (90.2 µg/kg), followed by kidney (9.45 µg/kg), lung (6.84 µg/kg), heart muscle (2.60 µg/kg), and skeletal muscle (1.03 µg/kg). In a 70-kg person, consuming a single 125-g liver portion with the median ∑4PFAS level would exceed EFSA's tolerable weekly intake (TWI) 36.6-fold, whereas skeletal muscle consumption would remain below the TWI. These data underline the variable risks for consumers of wild boar meat and offal.

To foster the exchange of knowledge on consumer safety related to game meat at a European level, the network "Safety in the Game Meat Chain" (CA22166) has been established under the leadership of the BfR in 2023. This publication is partially based upon work from COST Action SafeGameMeat, CA22166, supported by COST (European Cooperation in Science and Technology).

3.8 Two years (2023 – 2024) of perfluoroalkyl substances (PFASs) results from Italian official food control plans: what are the raising issues?

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Perfluoroalkyl substances (PFASs) are a family of persistent, bioaccumulative, highly fluorinated aliphatic compounds widely synthesized and used in multiple industrial applications [1]. Diet is the main exposure route. In 2020 EFSA established a cumulative Tolerable Weekly Intake (TWI) of 4.4 ng/kg bw for PFOA, PFOS, PFNA, and PFHxS, and in Jan 2023 maximum levels (MLs) for these PFASs and their sum in food entered into force. Italian Official Labs were requested to monitor food, and our laboratory analysed 224 samples from various commodities sampled in north/centre of the peninsula. 11 perfluoroalkyl carboxylic acids and 8 perfluoroalkane sulfonic acids were analysed by LC-MS/MS [1]. Σ19PFASs in food ranged from <LOQ to 6.6 µg/kg (mean 0.30 ug/kg). They were not quantified in milk and in most of the meat, in ovine meat had the higher PFAS levels. Relevant concentrations were found in offal of terrestrial animals confirming their great affinity for hepatic proteins and phospholipids. However, none of the sample exceeded the MLs. Non-negligible were the levels in chicken eggs (mean 0.17 µg/kg), and one sample showed a concentration very close to MLs (PFOS: 0.97 µg/kg; Σ4PFASs: 1.3 µg/kg). Fish and fish products showed the highest levels. ML for PFOA was exceeded in one cooked yellow clam from Vietnam (5.2 µg/kg).

The contamination patterns of the different matrices were different. PFOS was the dominant in eggs, meat, and offal, (48%, 70%, 83% of total PFASs). In clams (*C. gallina*, *T. semidecussatus*) PFOS and PFOA contributed almost equally (27% and 26%), followed by PFCAs C9-C14. In mussels and marine fish, PFOS and PFTrDA (C14) were most abundant, with long-chain PFCAs (C11–C14) significant. In vegetables, PFOA, short-chain PFCAs, and PFBS were most frequent. The Σ4 regulated PFASs represented 64–66% of total in meat, offal, eggs, and clams, but only ~30% in fish and mussels due to long-chain PFCAs.

3.9 Linking forever chemicals detected in prospective Janus Serum Bank cohort with kidney and testicular cancers

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Objective

Per- and polyfluoroalkyl substances (PFAS), also known as, "forever chemicals", are widely used since the 1940s in industrial and consumer products such as non-stick cookware, waterproof coatings, fire suppression foams, and even cosmetics. PFAS are recognized as persistent and bioaccumulating and are also possibly carcinogenic based on limited evidence for kidney and testicular cancer. Our aim is to assess the evidence connecting PFAS exposure to kidney and testicular cancer.

Material and methods

We are performing a nested case-control study of 1000 kidney cancer and 1000 matched controls, and 69 testicular cancer cases and 111 controls within the Janus Serum Bank (JSB) cohort, examining levels of > 24 different PFAS in pre-diagnostic serum samples. Cases and controls were matched by sex, birth-year, and residential county at blood draw. JSB enrolment questionnaires have been requested, including information on anthropometric and lifestyle factors. PFAS levels are being assessed using high-performance LC-MS/MS (limit of quantification: 0.015–0.2 ng/ml serum).

Statistical analysis

Logistic regression will be used to calculate adjusted odds ratios (95 % CI) of the association between PFAS exposure and cancer risk. Samples from cases and controls are currently undergoing analysis for PFAS levels, including longitudinal samples. Sources for high exposure, such as residential area or occupation, and the association between PFAS levels and kidney and testicular cancer risk, are being examined.

4 Toxicokinetics

4.1 Transfer and elimination of per- and polyfluoroalkyl substances (PFAS) in growing lambs

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A broad spectrum of toxicological effects has been demonstrated for several PFAS substances. Some of them are suspected to have even carcinogenic properties. Their potential to bioaccumulate increases with chain length, which leads to accumulation along the food chain. Sheep can be exposed through grass, soil, and drinking water, which may adversely affect the safety of animal-derived products.

A feeding trial was conducted using 4 x 4 male Suffolk lambs to assess the uptake, distribution, and elimination of 9 PFAS (PFBA, PFPeA, PFHxA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFOS) in blood plasma, liver, kidney, and different meat cuts (chop, leg and flank). Two groups were orally exposed to PFAS before euthanasia (10 and 20 weeks, respectively), while a control group was fed PFAS-free. In order to study the depuration phase, another group was exposed to PFAS for 10 weeks and then fed PFAS-free for further 10 weeks.

All PFAS were detected in blood plasma and tissue samples. Significant differences in the transfer kinetics of short- and long-chain PFAS were observed. In all examined compartments, the less polar long-chain PFAS showed enhanced accumulation compared to the short-chain variants. PFAS patterns of muscle and blood plasma were dominated by PFHxS, whereas PFNA, PFOS and PFDA were the main factors in liver. PFAS concentrations were highest in liver followed by kidney and muscle tissue. In the depuration phase, the elimination half-lives of short-chain PFAS in blood plasma were less than one week, whereas those of long-chain PFAS extended over several weeks. In tissues PFAS reduction was less pronounced for long-chain PFAS during the depuration phase. Furthermore, the percentage reduction of long-chain PFAS was higher in meat compared to liver and kidney. The data will be shared with the BfR to develop transfer prediction models for risk management authorities and to derive management recommendations for sheep farmers, thereby contributing to consumer protection.

4.2 Physiologically-based toxicokinetic model of the transfer 1 of branched and linear perfluoroalkyl acids in dairy goats

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Introduction

Perfluoroalkyl acids (PFAAs), a group of per- and polyfluoroalkyl substances (PFAS), are extremely persistent and often bioaccumulative, and have been associated with adverse health effects. Because of their bioaccumulative nature, some PFAAs can concentrate along food chains, making food of animal origin a major route of human exposure. This, in turn, has led to the establishment of maximum levels for PFAAs in animal-derived food in the European Union. Understanding PFAA toxicokinetics in livestock, and subsequently assessing the transfer of these substances into edible animal products, is critical from a human health perspective and for ensuring safe livestock production systems. This study aims to develop a physiologically based toxicokinetic (PBTK) model to simulate the absorption, distribution, metabolism, and excretion (ADME) of different PFAAs in dairy goats and their edible products. The primary focus is to study experimentally the effect of the chain length and functional group of a total of 30 branched (br-) and linear (n-) PFAAs on their respective kinetics and accumulation in five different tissues and in excreta (e.g., milk). The PBTK model is intended to integrate and extrapolate the experimental toxicokinetic dataset, with a view to predicting PFAA levels across the goat's lifespan at different growth and lactation stages.

Material and methods

A dynamic, compartmental PBTK model was developed based on experimental data from eight dairy goats fed with hay grown on a PFAS-contaminated site for eight weeks, followed by a 12-week depuration phase with uncontaminated hay. Individual hay intake was recorded daily; hay, feces, urine, milk, and blood serum were periodically sampled and analysed for PFAAs; at the end of the 20-week experiment, the goats were slaughtered, and the muscle tissue, liver, kidney, lung, heart, brain tissue and spleen were analysed for PFAAs. The PBTK model consists of ten interconnected compartments representing tissues and organs playing a key role in ADME processes, including blood, liver, kidney, spleen, mammary gland, gastrointestinal tract (ruman and intestine), as well as three excretion pools (urine, feces, and milk). The model was built in Python [3.14] and enables accelerated computation by solving the differential equations via an analytical matrix solution.

Results and discussion

The results from the in vivo study on feed-to-milk transfer rates (TRs) ranged from less than 1 % (e.g., n- and br-perfluorooctanoic acid, PFOA) to up to 15 % for n-perfluorooctanesulfonic acid (PFOS). Depuration half-lives ranged from less than one day (n-perfluoropentanesulfonic acid, PFPeS) to up to 65 days (br-perfluoroundecanesulfonic acid, PFUnDS). Notably, for all perfluoroalkyl acids (PFAAs) considered, the branched isomers consistently showed somewhat lower TRs than their

linear counterparts, although a comparable trend was not observed for half-lives. For most compounds, the long exposure period (eight weeks) appeared sufficient to reach near steady-state TRs, suggesting that the estimated values are reliable for modelling purposes. Further in vivo results on blood serum, urine, feces, and tissues are expected.

Conclusion and implications

The proposed PBTK model provides a physiologically based tool to simulate n- and br-PFAA kinetics and the excretion, e.g. milk, in dairy goats. With respect to specific substance behaviour, the model aims to provide quantitative predictions of accumulation and depuration of PFAAs in edible products of dairy goats, aiding exposure assessment within human health risk assessments. Its flexible structure allows extrapolation to other goat breeds, different levels of milk yield, and varied exposure or depuration scenarios.

5 Toxicity

5.1 Activation of human PPARα by mixtures of classic and novel (poly-) ether PFAS

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Per- and polyfluoroalkyl substances (PFAS) are a large group of contaminants that are ubiquitously in the environment. Humans are thus exposed to many different PFAS at the same time. Therefore, evaluation of PFAS mixture effects is an important aspect in PFAS toxicity research. For many PFAS, an activating effect on the peroxisome proliferator-activated receptor alpha (PPARα) has been shown. This transcription factor plays an essential role in lipid metabolism and is regarded as an important molecular target of PFAS toxicity.

In the present study, four equimolar PFAS mixtures were analysed with regard to their potential to activate human PPARα in vitro, using a luciferase-based reporter gene assay. The mixtures consisted of (I) classic perfluoroalkyl carboxylic acids (PFCA) and perfluoroalkyl sulfonic acids (PFSA), (II) novel perfluoroalkyl (poly)-ether carboxylic acids (PFECA) and perfluoroalkyl (poly)-ether sulfonic acids (PFESA), (III) PFCA and PFECA, and (IV) PFSA and PFESA. This design allows to compare the effect of a mixture of classic PFAS with a mixture of novel (poly)-ether PFAS as well as the effect of a mixture of carboxy-containing PFAS with a mixture of sulphur-containing PFAS.

The concept of concentration addition was used to calculate the mixture effect for additivity and to further evaluate a potential deviation from additivity. All four mixtures showed a trend towards synergism with increasing PFAS concentrations, indicating that they activated PPARα to a greater extent than the sum of the respective single substances. This trend was more pronounced for the PFECA/PFESA mixture of novel PFAS compared to the classic PFCA/PFSA mixture, and for the PFCA/PFECA mixture compared to the PFSA/PFESA mixture. The PFECA/PFESA mixture of novel PFAS showed the largest deviation from additivity, the PFSA/PFESA mixture of sulphur-containing PFAS showed the smallest deviation from additivity.

5.2 Effects of novel (poly)- ether PFAS on the transcriptome of HepaRG cells

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Due to their high persistence and adverse health effects in humans the use of several "legacy" per- and polyfluoroalkyl substances (PFAS) has been restricted. Thus, novel PFAS are increasingly used for numerous industrial applications, despite the fact that currently limited or no data is available on their toxicity.

This study aims to examine the molecular mechanisms of action of novel PFAS with a focus on the subgroup of mono- and polyether PFAS, with both linear and branched structures, including PFAS congeners with either a carboxylic acid or a sulfonic acid functional group. Differentiated HepaRG cells, a model for human hepatocytes, were incubated for 24h with 33 different PFAS congeners at three non-cytotoxic concentrations each. Total RNA was extracted from the cells and subjected to whole transcriptome analysis.

For most PFAS, a concentration-dependent increase of the number of differentially expressed genes (DEG) was observed. Five of the tested PFAS barely induced alterations in gene expression up to the highest test concentration.

Ingenuity Pathway Analysis (IPA) was employed to identify molecular metabolic and signalling pathways affected by PFAS. The majority of the tested PFAS had an effect on gene expression. They showed comparable effects, pointing to similar molecular mechanism(s) of action in HepaRG cells, despite their structural differences. The tested PFAS activated canonical pathways related to lipid metabolism, regulated by the nuclear receptors PPARα and PPARγ, as well as pathways related to xenobiotic metabolism, regulated by the nuclear receptors PXR and CAR. In addition, a number of PFAS inhibited pathways related to cholesterol biosynthesis. Furthermore, IPA revealed a strong impact of PFAS on a number of hepatocyte-specific global upstream regulators such as HNF4α, HNF1α, and FOXA2. Finally, IPA predicted an association between PFAS exposure and various diseases related to cholestasis, again for all PFAS tested in this study.

5.3 PFAS exposure in Alessandria (Italy): environmental context, population-based assessment, and method validation in the EU SCENARIOS project

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PFAS are highly persistent contaminants widely used in industrial applications for their chemical and thermal stability. Despite these concerns, large-scale biomonitoring is limited by the high cost and complexity of analytical techniques. In the locality of Spinetta Marengo (Piedmont, Italy), a fluoropolymer manufacturing complex has operated since the 1960s. In May 2020, groundwater from Montecastello, 10 km downstream, was found to contain PFOA (0.13 µg/L) and its replacement C6O4 (0.34 µg/L). As a result, on June 11th, 2020, the well supplying water to the town's aqueduct was closed. Within the EU-funded SCENARIOS project, two pilot studies were launched to assess population exposure and validate rapid, cost-effective detection methods, including electrochemical sensors and Raman/SERS spectroscopy, against LC-HRMS reference techniques. The first study involves 160 residents, stratified by gender and age (<18, 18-49, 50-65, >65 years), equally divided between Montecastello ("potentially exposed") and Frugarolo ("likely non-exposed"). Recruitment in Montecastello is complete (n=76), while 60 participants have been enrolled in Frugarolo. The second study extends sampling by distance from the plant (≤3 km, 3–6 km, >6 km) to explore spatial exposure gradients; 37 residents have been recruited so far. All participants provide blood and urine for PFAS quantification and selected clinical-biochemical parameters, urine for microalbuminuria, and household water samples for PFAS determination; trained staff administer a structured questionnaire to each participant, on personal, environmental, occupational, dietary, and water use data. Findings from biochemical and clinical assessments are returned individually, with advice to review them with the participant’s general practitioner. PFAS measurements from biological samples will be reported exclusively in aggregated, pseudo-anonymized form, ensuring full adherence to ethical requirements and data protection regulations.

5.4 FSCJ risk assessment report on PFAS 2024

Tomotaka Sobue and working group on per-and polyfluoroalkyl substances

Food Safety Commission of Japan

Food Safety Commission of Japan (FSCJ) conducted a risk assessment of per- and polyfluoroalkyl substances (PFAS) in food and reported in June 2024. Scientific findings and risk evaluation data regarding PFAS, of international organizations, government agencies in other countries, etc., were reviewed in the current risk assessment. The scientific literature related to three major compounds of PFAS, perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonate (PFHxS), was surveyed and served for the discussion.

Reference doses were derived from two studies for developmental/reproductive toxicity in animals, because 1) dose-response relationships were clearly observed, 2) similar findings were reported from several studies and the level of evidence is strong, and 3) multiple overseas institutions used as points of departure (PODs).

To determine the reference dose, dose estimation models developed by overseas evaluation institutions were adopted for conversion of POD in animal experiments to PODHED (Human Equivalent Dose). Based on the discussions and estimation, the tolerable daily intake (TDI) was set as 20 ng/kg body weight/day (2×10−5 mg/kg bw/d) for PFOS and as 20 ng/kg bw/d (2×10−5 mg/kg bw/d) for PFOA. Insufficient scientific findings precluded the evaluation to specify a reference dose of PFHxS.

The average daily intake in Japan was obtained from the Total Diet Study conducted in a limited number of regions during the fiscal years 2012–2014: PFOS (Lower Bound to Upper Bound (LB–UB) 0.60–1.1 ng/kg bw/d, and PFOA (LB–UB) 0.066–0.75 ng/kg bw/d. These values were lower than the TDIs.

Due to the lack of sufficient data on PFAS concentrations and their distribution in various foods, it is necessary to be aware of these intake estimates carrying considerable uncertainty.

6 In silico methods

6.1 Prediction of PFAS bioaccumulation using KoaLA: a machine learning-based model for predicting the n-octanol/air partition coefficient

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Per and polyfluoroalkyl substances (PFAS) are synthetic compounds extensively used in industrial and consumer products due to their exceptional thermal stability and resistance to chemical degradation. However, their environmental persistence and high potential for bioaccumulation pose serious risks to human and ecological health. Experimental traditional methods for assessing PFAS bioaccumulation are often expensive, time-consuming, and limited by scarce data. The n-octanol/air partition coefficient (Koa) is a key parameter in environmental science, as it correlates strongly with bioconcentration, bioaccumulation, and biomagnification. Due to the difficulty of determining Koa experimentally, there is a growing need for accurate computational models. In this study, we apply KoaLA, machine learning-based tool designed to predict log Koa values from molecular descriptors generated from SMILES representations. We evaluated KoaLA to estimate the bioaccumulation potential of PFAS. This tool offers a novel, efficient, and scalable approach for environmental risk assessment, enabling more informed regulatory and scientific decision-making regarding persistent and hazardous compounds.

6.2 Based computational calculation of binding affinity between proteins and perfluoroalkyl compounds

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Abstract

Proposals have been made to regulate perfluoroalkyl substances (PFAS) as a group; however, it is specific compounds like PFOA, PFNA, and PFOS that are reported to persist in the environment and accumulate in human and ecological systems. Despite this, regulations are being pursued globally on the usage of PFAS. It is logical to regulate chemical substances based on scientific evidence. Given that there are over ten thousand types of PFAS, conducting toxicity tests would be exceptionally time-consuming and labour-intensive. Hence, in silico methods were employed for hazard selection, considering the affinity between proteins and ligands as one of the factors for toxicity manifestation. This study calculates the affinity between several PFAS and proteins, analysing the impact of differences in chain length and terminal functional groups of PFAS on their binding affinity to proteins.

Method

3D structural files of PFAS, centred on perfluoroalkylcarbonic acid, were downloaded from PubChem. Regarding binding with human serum albumin (HSA), site-specific fluorescence probes were used to investigate the binding sites and constants for five types of PFAS with HSA, demonstrating that PFAS chain length and terminal functional groups affect binding affinity. The amber99sb-ildn force field was employed for HSA, and TIP3P was used for water. The structure of HSA was obtained from the Protein Data Bank (PDB ID: 1N5U), where hydrogen was added post-removal of crystal waters, followed by docking with PFAS using AutoDock Vina. PFAS molecules with varying chain lengths and terminal functional groups were prepared. The docking range for HSA specified sites I and II, known as drug binding sites. HSA was treated as a rigid body, allowing rotation around the main axis of PFAS. From 100 docking structure candidates, the one with the highest binding affinity was selected as the initial structure. In creating structures for molecular docking. simulations, HSA amino acids' pKa were calculated to determine protonation states. The initial molecular docking. structure was formed by binding the five PFAS structures obtained from AutoDock Vina to HSA, then adding water molecules and counterions. The simulation box size was 175×157×185, adding 463 sodium ions and 452 chloride ions to achieve an ion concentration of 0.150 M.

The type 2 deiodinase enzyme (D2), which converts thyroid-secreted T4 (thyroxine) into the active T3 (triiodothyronine), was obtained from the Protein Data Bank (PDB ID: 9H48), and the affinity of each PFAS was calculated.

Result

The affinities of several PFAS bound to sites I and II of HSA are compared. Additionally, the affinities of multiple PFAS with the deiodinase enzyme (D2) are discussed.

Reference

Chen, Y.-M., Guo, L.-H.: Fluorescence study on site-specific binding of perfluoroalkyl acids to human serum albumin. Archives of Toxicology 83, 255–261 (2009)

7 New approach methodologies

7.1 In vitro study on effects of novel (poly-) ether-PFAS on key functions of the thyroid hormone system

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Per- and polyfluorinated alkyl substances (PFAS) are a group of persistent industry chemicals and therefore a toxicological long-term burden for the environment. Identification and characterization of possible hazardous effects of PFAS on the ecosystem, and in particular on human health, are part of the risk assessment of these substances. One field of concern are potential interactions of PFAS with key functions of hormonal axis, e.g., the thyroid hormone (TH) system. Compounds interfering with availability and action of THs can impact numerous aspects of body homeostasis, e.g., cardiac function, mood or energy metabolism in the adult organism, but might also affect brain development in utero. In this study, as part of the SCENARIOS project, 33 PFAS were selected with a focus on the subgroup of mono- and polyether PFAS, with both linear and branched structures, including PFAS congeners with either a carboxylic acid or a sulfonic acid functional group. These were tested for interference on certain key functions/molecular initiating events of the thyroid hormone system up to a concentration of 100 µM. Chosen in vitro and in chemico protocols reflect iodide uptake by the sodium-iodide-symporter (NIS), enzymatic function of the thyroid peroxidase (TPO), type 1 deiodinase (DIO1) and dehalogenase (DEHAL) as crucial key activities found within the thyroid gland. Seven PFAS showed an inhibitory effect on DIO1 activity, five PFAS showed an inhibitory effect on DEHAL activity and six of the 33 PFAS showed an inhibitory effect on TPO activity. The relevance of PFAS induced effects on the TH system in humans needs to further be elucidated.

7.2 Socioeconomic analysis as a tool to support the finalization of the uPFAS restriction proposal: the Italian framework

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On January 13, 2023, the competent authorities of Denmark, Germany, the Netherlands, Norway and Sweden submitted to ECHA a uPFAS restriction proposal aimed at reducing environmental emissions. The proposal raised significant concerns, especially in the industrial sector, where uPFAS are widely used and often considered essential.

A PhD project "Socioeconomic analysis as a tool to support the finalization of the uPFAS restriction proposal" developed with CNSC1-ISS2 and UCBM3, applies socioeconomic analysis to assess the impact of the restriction, aiming to balance environmental and health protection with national industrial needs.

The first research phase focused on the TULAC4 sector. A survey was distributed via trade associations to companies involved in this sector to assess their understanding of the concept of essential use as defined by the European Commission and to identify whether any high-risk PFAS applications used by these companies could be considered essential. Results offered a national overview, highlighting both ongoing substitution efforts and concerns, such as the absence of derogations for recycled textiles. A second economically oriented survey is ongoing.

In parallel, the cosmetics sector is being examined through tailored analytical strategies to understand challenges from the EU potential PFAS ban, also considering differences between small and large companies.

The project also contributes to the SEAC Committee's final opinion and the drafting of implementation guidelines. For this purpose, cooperation with the Italian SEAC representative is envisaged with participation at the SEAC meetings since June 2024. The PhD project include also participation in a governmental working group organizing stakeholder hearings on the restriction.

Abbreviations

CNSC: National Centre for Chemicals, Cosmetic Products and Consumer Protection
ISS: National Institute of Health
UCBM: Campus Bio-Medico University of Rome
TULAC: Textiles, Upholstery, Leather, Apparel, Carpets

7.3 Evaluation of real- life PFASs mixture toxicity and impact on 3D placenta spheroid model

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Background

Per- and polyfluoroalkyl substances (PFASs) are abundant and long-persistent organic pollutants that have been associated with many adverse effects including adverse pregnancy outcomes. However, as most of these data are based on single chemicals exposure, here we aim to identify relevant PFASs mixture of concern to study the impact and toxicity on trophoblast spheroids as a proxy of human placenta.

Methods

PFASs concentrations were assessed in 1st trimester placenta tissue obtained from elective terminations of pregnancy with liquid chromatography/ triple quadrupole mass spectrometry. Based on PFASs levels in the placenta, mixture of concern was designed to test in a 3D model of placenta. The trophoblast spheroids were obtained by culturing JEG3 and HTR8/SVneo cell lines in ultra-low attachment plates and consequently exposed to PFASs mixture for 48–96 hrs at concentrations of 0.01–300 µM. Viability was assessed with multiparametric live-cell toxicity assay. Functional placenta properties e.g., invasion and human chorion gonadotropin (hCG) production were assessed with matrix invasion assay and ELISA.

Results

The following PFASs, perfluorononanoic acid (PFNA), perfluorooctane sulfonic acid (PFOS), perfluorobutanoic acid (PFBA), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), and perfluorodecanoic acid (PFDA), were detected at the highest levels in 1st trimester placentas and included to prepare the PFASs mixture of concern. 48 hrs exposure to PFASs mixture affected the viability of JEG3 trophoblast spheroids only at 300 µM, while HTR8/SVneo viability was unaffected. The invasive properties of JEG3 trophoblast spheroids were already increased at 48 hrs due to the exposure to the PFASs mixture at varying concentrations. This was also attributed with decreased hCG production after 48 hrs exposure to the mixture of concern. Additionally, HTR8/SVneo trophoblast spheroids showed decreased invasion protrusions already at 72 hrs of exposure to the PFASs mixture of concern.

Conclusion

This study offers valuable insights into the real-life PFASs concentrations in placenta tissue and the negative impact of PFASs mixtures on placenta functionality by using representative models of human placenta. Collectively, our results call for more stringent pregnancy risk assessment of relevant chemical mixtures.

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