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Distribution of poly- and perfluoroalkyl substances in pig liver and muscle: implications for food safety

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants with high mobility and potential health risks. Despite growing regulatory attention, data on tissue-specific distribution in pigs remain scarce. This study aimed to assess PFAS occurrence in pig liver and muscle (longissimus dorsi, semimembranosus) to support risk-based monitoring strategies. A liquid chromatography-tandem mass spectrometry method was developed and validated for 13 PFAS, following European Union Reference Laboratory for Persistent Organic Pollutants and Regulation (EU) 2022/1428 guidelines. Liver and muscle samples from pigs reared under controlled dietary conditions were analyzed. The method showed excellent linearity, precision, and trueness; the limit of quantification (LOQ) was 0.04 µg/kg for all matrices. The perfluorooctanesulfonic acid and perfluorooctanoic acid (PFOA) were consistently detected in liver samples, whereas PFAS concentrations in the two muscle cuts remained mostly below the LOQ, with only occasional PFOA quantification in a subset of samples. None of the samples exceeded current EU maximum levels [Regulation (EU) 2023/915]. Our findings confirm a marked hepatic accumulation of PFAS and negligible contamination in muscle, showing the liver as a priority matrix for biomonitoring. Consumer exposure through pork muscle appears minimal, while offal consumption requires attention. These preliminary data contribute to defining risk-based control strategies in the pork production chain.

Key words: PFAS, pig liver, pig muscle, LC-MS/MS, food safety.

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Introduction

Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals widely used in industrial and consumer products (*e.g.*, nonstick cookware, waterproof clothing, grease-resistant food packaging, cosmetics) because of their water- and oil-repellent properties and high stability. Their application in non-stick coatings, food-contact materials, water-repellent textiles, and firefighting foams has contributed to their global dissemination (EFSA, 2020; Baker and Knappe, 2022). Due to their exceptional resistance to chemical and biological degradation, PFAS are often referred to as “forever chemicals”, raising increasing concern for both environmental integrity and human health (Peritore *et al.*, 2023). Environmental persistence has resulted in widespread PFAS contamination of soil, surface and ground waters, and ultimately the food chain, with evidence reported across Europe, Asia, and the Americas (Domingo and Nadal, 2017; Jha *et al*., 2021). Major emission sources include industrial activities, firefighting training and production sites, military areas, wastewater treatment plants, and agricultural practices involving the application of sewage sludge and biosolids (Jha *et al*., 2021). Consequently, dietary intake of contaminated food is recognized as a relevant exposure pathway for the general population (Death *et al*., 2021).

PFAS can bioaccumulate in animals, with tissue-dependent distribution patterns and a notable tendency to concentrate in the liver, partly explained by binding to serum proteins and liver fatty acid-binding proteins (Peng *et al*., 2024; Birchfield *et al*., 2025). The most frequently detected compounds in biomonitoring and food surveys are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), which have been classified by the International Agency for Research on Cancer as carcinogenic to humans (Group 1) and possibly carcinogenic to humans (Group 2B), respectively (Zahm *et al*., 2023).

In its 2020 opinion, the European Food Safety Authority (EFSA) set a tolerable weekly intake of 4.4 ng/kg body weight for the sum of four perfluoroalkyl substances [PFOS, PFOA, perfluorononanoic acid (PFNA), and perfluorohexane sulfonate (PFHxS)] to limit potential adverse health effects from dietary exposure (EFSA, 2020). To reduce consumer exposure, the European Union (EU) subsequently introduced maximum levels for these compounds in selected foodstuffs, including fish, eggs, meat, and shellfish (European Commission, 2022a). In addition, Recommendation (EU) 2022/1431 calls on Member States, working in cooperation with food business operators, to strengthen monitoring activities for PFAS by extending surveillance beyond regulated compounds to include structurally related PFAS, thereby improving the evidence base for exposure assessment and risk management (European Commission, 2022b).

Despite the central role of pigs in the European food supply chain, information on PFAS occurrence and tissue-specific distribution in porcine matrices remains limited. This gap constrains the interpretation of monitoring results and the refinement of risk-based control strategies, particularly for edible offal such as liver, which may represent a critical contributor to dietary exposure.

This study aims to quantify PFAS in pig liver and muscle using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method in order to characterize tissue-specific distribution and evaluate implications for food safety. The study is designed in accordance with Recommendation (EU) 2022/1431, contributing to the enhanced monitoring of PFAS, including compounds structurally related to those currently regulated, and generating robust data to support risk-based surveillance. Its novelty lies in the controlled experimental design and the paired, tissue-specific assessment (liver *vs.* muscle), which minimizes variability by using samples from animals raised under identical dietary and water conditions. This approach provides new evidence on PFAS bioaccumulation in pigs and strengthens the scientific basis for European monitoring programs and policy development.

Materials and Methods

Chemicals and reagents

Analytical-grade PFAS standards, including the corresponding isotopically labelled internal standards, were obtained from Wellington Laboratories (Guelph, Ontario, Canada). The solvents and reagents required for sample extraction and cleanup: acetonitrile, methanol, formic acid, ammonium hydroxide, and sodium hydroxide were purchased from Merck (Milan, Italy). Solid-phase extraction (SPE) was carried out using Oasis WAX cartridges (6 cc, 150 mg; Waters, Milford, MA, USA). Nylon syringe filters (0.22 µm) were supplied by FAVS (Bologna, Italy).

For LC-MS/MS analysis, ammonium acetate, methanol and ultrapure water were sourced from Sigma Aldrich (Milan, Italy).

Working solution

Individual PFAS standard mixtures were serially diluted in methanol to obtain the working solutions required for calibration and sample fortification. A first working solution (WS-B) was prepared by transferring 50 µL of the mixed native standards into a volumetric flask and bringing the final volume to 5 mL with methanol, yielding a concentration of 20 ng/mL. A second working solution (WS-A) was subsequently obtained by diluting 500 µL of WS-B to 5 mL with methanol, resulting in a final concentration of 2 ng/mL.

The working solution of isotopically labeled internal standards was prepared by diluting the EPA-537IS reference mixture 1:100 in methanol, producing concentrations in the 10-40 ng/mL range, depending on the specific labelled compound.

All standard solutions were stored at -20°C until use to ensure stability and prevent degradation.

Sampling

Samples of liver, *longissimus dorsi* muscle, and *semimembranosus* muscle were collected from 15 pigs reared at the Department of Veterinary Medical Sciences, University of Bologna. At the beginning of the study, the animals were 70 days old and were subsequently fed for a period of 204 days, reaching a final live weight of approximately 160 kg and a minimum age of 9 months at slaughter.

Throughout the trial, the pigs received a commercial feed formulated in compliance with the requirements for Protected Designation of Origin (PDO) production. The diet included soybean meal and animal fats at levels not exceeding 2%, with the latter characterized by a melting point above 40°C, as established for PDO-certified supply chains.

A total of 15 samples were collected and analyzed for each anatomical district of every animal. The samples were collected from three matrices: liver, *longissimus dorsi* muscle, and *semimembranosus* muscle. All collected tissues were stored at -20°C and thawed overnight at 4°C prior to sample preparation and extraction.

Sample preparation

Sample preparation was carried out following a workflow suggested by Organtini *et al*. (2022), with substantial adaptations specific to the present study. Liver, *longissimus dorsi*, and *semimembranosus* muscle samples were manually chopped using stainless-steel scissors and transferred into 50 mL polypropylene centrifuge tubes. Tissue homogenization was performed using an Ultra-Turrax T25 at a moderate speed to prevent heat build-up during processing. For each matrix, aliquots of 2 grams were spiked with isotopically labelled internal standards prior to extraction. The extraction procedure consisted of several sequential steps: liquid extraction of the fortified samples; conditioning of the SPE cartridges; loading of the extract onto the SPE sorbent, followed by washing and elution of the PFAS-containing fraction; and final sample preparation for LC-MS/MS analysis.

After extraction, the purified eluates were evaporated to dryness under a gentle nitrogen stream and reconstituted in a mixture of methanol and ultrapure water (50:50, v/v) before instrumental determination. To minimize analyte losses and prevent adsorption phenomena commonly associated with fluorinated compounds, all sample handling steps were performed exclusively using polypropylene materials. To avoid potential interactions between PFAS and glass, all procedural steps were conducted using polypropylene materials.

Liquid chromatography-mass spectrometry conditions

The analyses were performed on a Waters Acquity UPLC® system (Waters Corporation, Milford, MA, USA) configured with a binary pump and equipped with a dedicated perfluorinated compound isolator kit to reduce background interference from fluorinated contaminants. Chromatographic separation was achieved using an Acquity UPLC® BEH Shield C18 column (50×2.1 mm, 1.7 µm), preceded by a VanGuard™ pre-column containing the same stationary phase (Waters Corporation, Milford, MA, USA). Both the analytical and guard columns were maintained at 35°C throughout the process.

The mobile phases consisted of 5 mM ammonium acetate in water (phase A) and methanol (phase B). Separation was performed under a linear gradient starting from 50% A/50% B and progressing to 10% A/90% B within 8.0 minutes at a constant flow rate of 0.3 mL/min. This high-organic composition was held for 2.0 minutes, after which the system was returned to the initial mobile-phase conditions over 0.5 minutes, followed by a further 1.5 minutes equilibration step before proceeding with the subsequent injection.

Detection was carried out using a Waters Xevo® TQ-S micro triple-quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA) operating in negative electrospray ionization mode, with the capillary voltage set to 0.50 kV. A complete list of the instrumental MS parameters is reported in *Supplementary Table 1*. Quantification was based on multiple reaction monitoring, employing two transitions for each analyte and one transition for each isotopically labelled internal standard. The monitored transitions, together with the cone voltages, collision energies, and the full list of analytes and internal standards, are detailed in *Supplementary Table 2*. Data acquisition and processing were performed using MassLynx™ v4.2 (Waters Corporation, Milford, MA, USA).

Method performance assessment

Before proceeding with the analysis of the samples, the analytical method underwent a comprehensive performance assessment, as method validation is essential to ensure the reliability and robustness of the results.

The method was evaluated in accordance with the guidelines of the European Union Reference Laboratory for Persistent Organic Pollutants (EURL POPs, 2024) for the determination of PFAS in food and feed, and in compliance with Commission Implementing Regulation (EU) 2022/1428 (European Commission, 2022c). Within this framework, the principal performance characteristics of the method were verified as detailed below.

Selectivity was evaluated by analyzing blank samples processed with the same analytical procedure and verifying the absence of interfering peaks co-eluting at the retention times of the target analytes.

Linearity was evaluated based on the back-calculated concentrations, obtained by applying the calibration curve equation to the measured response at each calibration level. Six-point calibration curves, including a zero point, were constructed for each anatomical matrix (liver, *semimembranosus* muscle, and *longissimus dorsi* muscle) by fortifying samples with working solutions to achieve concentrations of 0, 0.040, 0.100, 0.200, 0.500, and 1.000 µg/kg.

Intermediate precision (intra-laboratory reproducibility) and trueness were evaluated using quality control samples prepared by fortifying 2 g of each matrix with the target analytes at two concentration levels (0.040 µg/kg and 0.200 µg/kg). Both parameters were assessed under intra- and interday conditions by extracting each level in triplicate and analyzing the samples on three separate days to characterize accuracy within the laboratory.

The limit of quantification (LOQ) was established using the minimum validated level approach, as the lowest concentration at which the analyte fulfilled the predefined accuracy and precision criteria. The limit of detection (LOD) was defined as the lowest concentration yielding a signal with a signal-to-noise ratio of 3.

Results

Method performance

The method performance evaluation yielded satisfactory results, confirming its suitability for the determination of the 13 target PFAS. For each analyte, matrix-matched calibration curves exhibited excellent linearity, with back-calculated concentrations at individual calibration levels deviating by less than 15% from the nominal values (*Supplementary Table 3*), *i.e.*, well within the ±20% acceptance criterion proposed by the European Union Reference Laboratory for Persistent Organic Pollutants (EURL POPs, 2024).

The method showed excellent precision and trueness across all analytes and matrices. Intermediate precision ranged from 1% to 18% for all compounds in the three investigated matrices, and trueness, expressed as percent bias, was within -20 to +19 for all target PFAS (*Supplementary Table 3*).

These performances comply with the acceptance criteria set by the EURL POPs guidelines for regulatory compliance testing, which require intermediate precision ≤20% and trueness within ±20%, and are therefore well within the wider tolerances permitted for monitoring (reproducibility ≤25%, trueness within ±35%).

In this study, a LOQ of 0.04 µg/kg was achieved for all matrices, thereby complying with and exceeding the most stringent indicative LOQs stipulated in Commission Recommendation (EU) 2022/1431 for PFOS, PFOA, PFNA, and PFHxS in meat from fish and terrestrial animals (0.10 µg/kg) and in edible offal from terrestrial animals and fish oil (0.50 µg/kg). This performance supports the reliability of the developed analytical method for the determination of regulated PFAS in the investigated matrices.

The LOD was established at 0.02 ng/kg for all PFAS, based on the observed instrumental performance

Per- and polyfluoroalkyl substances levels in the liver and muscles of swine

Among the 13 PFAS investigated, 6 were detected within the validated working range of the method. The occurrence and concentrations of these compounds in the three tissues are summarized in Table 1, and none of the analyzed samples exceeded the maximum regulatory levels currently in force.

Table 1. Frequency of quantified per- and polyfluoroalkyl substances, their concentrations, and the regulatory maximum levels established for the three tissues.

Tissue	PFAS	Quantifiable samples	Concentration range (µg/kg)	Mean (µg/kg)	Median (µg/kg)	Standard deviation	Maximum levels*(µg/kg)
Liver	PFOA	100%	0.045-0.167	0.071	0.061	±0.032	0.70
	PFOS	100%	0.044-0.150	0.104	0.115	±0.034	0.30
	PFNA	40%	0.040-0.138	0.026	0.000	±0.040	0.40
	PFHpA	13%	0.055-0.063	0.008	0.000	±0.021	/
	PFDA	7%	0.126	0.008	0.000	±0.033	/
	PFUdA	7%	0.114	0.008	0.000	±0.029	/
	Σ PFAS	100%	0.097-0.645	0.225	0.180	±0.150	/
	Reg. PFAS	100%	0.097-0.456	0.201	0.180	±0.094	1.3
Semimembranosus muscle	PFOA	13%	0.041-0.045	0.005	0.000	±0.015	0.80
Longissimus dorsi muscle	PFOA	47%	0.040-0.050	0.020	0.000	±0.022	0.80

PFAS, poli- and perfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFNA, perfluorononanoic acid; PFHpA, perfluoroheptanoic acid; PFDA, perfluorodecanoic acid; PFUdA, perfluoroundecanoic acid; Σ PFAS, sum of all quantified PFAS; Reg. PFAS, sum of regulated PFAS. *Commission Regulation (EU) 2022/2388 [the values of the four regulated PFAS (PFOS, PFOA, PFNA, PFHxS) and their sum are reported].

Discussion

Although the preferential accumulation of PFAS in the liver is well documented, systematically quantified data for pork meat, particularly for specific cuts, remain scarce, and information for non-hepatic tissues is still fragmented.

A large survey on 2929 porcine liver samples collected across 30 regions in China and analyzed by LC-MS/MS reported detection frequencies of approximately 97% for 17 PFAS, with a mean total concentration of 1.80 µg/kg and identified PFOS and PFOA as the predominant contributors to hepatic burden (Zhang *et al.*, 2021). These findings underline the importance of medium-chain PFAS as key contaminants in the liver and show that measurable residues occur even in animals from regions with relatively low apparent anthropogenic impact, consistent with the high environmental mobility and long-range transport potential of these substances.

In line with this evidence, the present study revealed a distinct tissue-specific distribution pattern. PFOA and PFOS were quantified in all liver samples, whereas concentrations of most PFAS in *semimembranosus* and *longissimus dorsi* muscles were below the LOQ. Left-censored ("non-detect") data were handled using the lower-bound approach recommended by EFSA (EFSA, 2020), assigning a value of zero to results <LOQ to obtain conservative, health-protective exposure estimates and to minimise the risk of overestimating dietary intake.

The results presented in this study showed a clear and toxicologically relevant difference between tissues: liver showed a substantially higher contaminant load, with a more complex profile including PFOA, PFOS, and PFNA, while skeletal muscles exhibited negligible contamination, restricted to sporadic traces of PFOA. This heterogeneous distribution reflects PFAS toxicokinetics in mammals, as these compounds exhibit high affinity for serum proteins (*e.g*., albumin) and hepatic fatty-acid binding proteins (Peng *et al*., 2024; Birchfield *et al*., 2025), making the liver a primary site of sequestration where concentrations can exceed those in blood or muscle by several orders of magnitude, whereas skeletal muscle, due to its different structural and metabolic properties, does not provide comparable binding or retention capacity.

Conclusions

The present study, conducted in accordance with Recommendation (EU) 2022/1431, provides novel data on the occurrence and tissue-specific distribution of PFAS in pigs, thereby addressing a critical knowledge gap within the animal production chain.

The integration of existing literature with the present findings reveals a consistent and tissue-specific pattern of PFAS bioaccumulation. The liver distinctly emerges as the principal site of accumulation, exhibiting high uptake sensitivity, a complex congener profile, and consistent detection even under low environmental exposure conditions. In contrast, PFAS concentrations in skeletal muscle (*semimembranosus* and *longissimus dorsi*) were negligible. This pronounced organ-specific disparity underscores a fundamental concept in food toxicology: tissue distribution is a major determinant of internal exposure and subsequent human health risk. Accordingly, the liver can be regarded as a reliable biomonitoring matrix for environmental PFAS and should be prioritized in surveillance and source apportionment studies. Conversely, the low contaminant levels observed in muscle tissue indicate that pork meat constitutes a minor dietary exposure route for PFAS.

The outcomes underscore the need for continued surveillance and targeted research to better elucidate PFAS accumulation dynamics across the food production chain.

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Online supplementary material:
Supplementary Table 1. Tandem mass spectrometry instrumental conditions.
Supplementary Table 2. Analyte identification and tandem mass spectrometry transition parameters for target per- and polyfluoroalkyl substances.
Supplementary Table 3. Method performance assessment (BCC-back-calculated concentration, trueness, intermediate precision).

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