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Validation of dietary assessment methods for PFOS exposure using biomonitoring data in a senior population with metabolic syndrome

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ABSTRACT

Perfluorooctanesulfonic acid (PFOS) exposure through diet represents an escalating public health concern due to its bioaccumulation and potential adverse health effects. This study aimed to validate the assessment of dietary PFOS intake by examining the relationship between estimated dietary intake and plasma PFOS concentrations in 196 participants with overweight or obesity and metabolic syndrome from Spain. PFOS dietary intake was assessed using validated food frequency questionnaires (FFQs) and using EFSA's updated PFOS food concentration data. Plasma PFOS levels were measured using Ultra-High-Performance Liquid Chromatography and Mass Spectrometry in Tandem (UHPLC-ESI-MS/MS). Statistical analyses included Spearman correlation, independent t-tests, Kappa agreement, and partial regression models, all adjusted for demographic and lifestyle variables. The results demonstrated a statistically significant correlation and a fair agreement between estimated dietary PFOS intake and plasma PFOS concentrations, supporting the modest reliability of FFQs for estimating dietary exposure. While these findings suggest that FFQs could provide a practical, non-invasive approach for dietary PFOS exposure assessment, the fair agreement observed in Kappa analysis indicates that FFQs alone may not fully capture individual exposure levels. Future research should focus on refining dietary exposure assessment tools, improving validation methods to enhance the accuracy of PFOS exposure estimates in epidemiological studies.

1. Introduction

Per- and Polyfluoroalkyl Substances (PFAS) are synthetic chemicals widely used in industrial and consumer products for their water and oil-resistant properties (Glüge et al., 2020; Paul et al., 2009). Their extensive use, combined with their high environmental persistence, make PFAS ubiquitous contaminants and contributing to widespread human exposure across the globe. Among the several PFAS congeners, the European Food Safety Authority (EFSA) has particularly evaluated Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS) for their health risks. The EFSA has progressively reduced the tolerable weekly intake (TWI) for PFAS compounds, reflecting growing concerns about their safety. In 2020, a combined TWI of 4.4 ng/kg of body weight/week for four major PFAS compounds (PFOS, PFOA, PFNA and PFHxS) was set, significantly lowering previously set thresholds. Chronic exposure to PFOS, with its long biological half-life, leads to its accumulation in human tissues, raising significant concerns about its potential health effects and prompting the need for reliable exposure assessment (Lau et al., 2007). However, PFOS stands out as one of the most pervasive and persistent (Schrenk et al., 2020), it is an endocrine disruptor that has attracted considerable attention, leading to rapidly evolving regulations surrounding their use and monitoring (Schrenk et al., 2020).

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PFOS is commonly found in products such as water-repellent textiles, nonstick cookware, and food packaging materials (Glüge et al., 2020). Due to its high stability, bioaccumulative properties, and biomagnification, PFOS enters and persists in the food chain (Schrenk et al., 2020; Olsen et al., 2007). Dietary intake has been identified as the primary route of human exposure, particularly through the consumption of animal-based products and water (Costopoulou et al., 2022; Kedikoglou et al., 2019; Domingo et al., 2012).

Assessing PFOS exposure through dietary intake estimations presents a feasible and cost-effective alternative to human monitoring, particularly for large epidemiological studies. Human monitoring, which involves measuring PFOS concentrations directly in biological samples such as plasma, not only imposes a financial burden but may also requires invasive procedures that may deter participant involvement if it is not taken, for example, in conjunction with a general check-up to reduce the inconvenience of the procedure especially in an elderly population. Reliable assessment of dietary PFOS intake is therefore crucial. Nevertheless, while dietary intake estimations using food frequency questionnaire (FFQ) are logistical advantageous and less invasive than biomonitoring, their validity remains underexplored. This study aims to address this critical gap in the literature by validating the FFQ assessment of PFOS intake from diet through a comparison with plasma PFOS concentrations.

2. Methodology

2.1. Study design and participants

This cross-sectional study was conducted in a subsample of male and female participants with overweight/obesity and metabolic syndrome (mean age: 64.8 ± 5.11), using baseline data from the PREDIMED-Plus cohort. Additional information about this cohort study can be accessed at https://www.predimedplus.com, and has been described in prior publication (Sayón-Orea et al., 2019). The current analysis is conducted in a sub-sample of participants (n = 196) from Reus center with available PFAS plasma information at baseline and with available data from food frequency questionnaire (FFQ). Participants included in the present study showed no differences in sociodemographic characteristics compared to the remaining randomized PREDIMED-Plus participants (n = 460) in the Reus center (Supplementary Material, Table S1).

2.2. Dietary PFOS assessment

To assess dietary exposure to PFOS, participants in the study completed a validated FFQ administered face-to-face by trained dietitians (Fernández-Ballart et al., 2010). The participants reported their average consumption in terms of frequency and quantity for 143 food and beverage items during the previous year. The frequency of consumption was shown through nine categories ranging from never or almost never to more than six times a day, and a commonly used portion size was specified (e.g., slices, glass, and teaspoons) to indicate serving sizes for each of the items (Supplementary material, Table S2). The nutrient intake was calculated as the sum of the frequency of daily consumption of each item multiplied by the nutrient composition of the specified serving size according to the Spanish food composition database. Additionally, a validated fluid-specific questionnaire was used to evaluate water and beverage consumption (Ferreira-Pêgo et al., 2016) (Supplementary material, Fig. S1).

Dietary PFOS exposure was assessed using updated PFOS concentrations in food, based on the 2020 EFSA data (Schrenk et al., 2020) and was calculated in ng/kg of body weight/week. PFOS levels for each food group were gathered from EFSA studies conducted across Europe (2012–2020), and we prioritized the most recent data from 2020 (Supplementary material, Table S3). Furthermore, two case scenarios were applied using the average PFOS levels in food for the lower bound (LB) and upper bound (UB). The LB represents a best-case scenario (assuming non-detects are zero), while the UB represents a worst-case scenario (assuming non-detects are at the limit of detection, LOD). This approach, recommended by EFSA, offers a realistic and cautious estimation of PFOS exposure. Consequently, dietary PFOS exposure was estimated following the methodology previously published (Khoury et al., 2024).

2.3. Plasma PFAS analysis

A total of 196 fasting plasma samples were analyzed for 4 perfluoroalkyl substances, PFOS, PFOA, PFNA and PFHxS by the isotope dilution method. The analytical method involved plasma sample extraction and clean-up procedures followed by instrumental analysis using an ultra-high-performance liquid chromatography system coupled to tandem mass spectrometry (UHPLC-ESI-MS/MS; Waters Corporation, Milford, MA, USA). The method was validated in compliance with EURACHEM guidelines.

The analysis of PFAS compounds (PFOS, PFOA, PFNA, and PFHxS), was conducted using solvents of LC grade and chemicals of analytical grade. Standards of PFOS, PFOA, PFNA, PFHxS, as well as stable isotope-labeled standards $^{13}C_8$-PFOA, $^{13}C_9$-PFNA, $^{13}C_8$-PFOS, $^{13}C_3$-PFHxS were purchased from Wellington Laboratories. Ammonium acetate, acetonitrile, methanol (LC grade), sodium chloride (NaCl), magnesium sulfate ($MgSO_4$), and C18 (RP-18) were obtained from Merck (Darmstadt, Germany). Envi-carb was supplied by Supelco, and ultrapure water was generated using a Nanopure system (Barnsted/Thermolyne, USA).

Plasma samples (0.5 mL) were transferred to 15 mL Falcon tubes and spiked with 10 ng of isotope-labeled internal standards. Subsequently, 10 mL of acetonitrile was added. The samples were vortexed for 1 min and sonicated for 15 min. After centrifugation at 5000 rpm for 15 min, the supernatant was transferred to clean Falcon tubes containing 2 g $MgSO_4$, 0.5 g NaCl, 0.1 g C18, and 0.1 g of Envi-carb. The mixture was shaken for 2 min and centrifuged again at 5000 rpm for 15 min. The resulting supernatant was concentrated to 1 mL using a rotary evaporator, dried under a gentle stream of nitrogen, and reconstituted in 200 μL of LC mobile phase. The analysis was performed using a Waters Acquity UHPLC-ESI-MS/MS system, equipped with an Acquity BEH C18 column (1.7 μm, 2.1 mm × 100 mm). The flow rate used during UHPLC-ESI-MS/MS analysis was 0.3 mL/min. The mobile phase was composed of (A) 2 mM ammonium acetate in 90:10 water: methanol and (B) 2 mM ammonium acetate in methanol. The elution gradient started at 100 % A, transitioned to 100 % B at 17 min, and remained at 100 % B until 19 min. A10 μL injection volume was used, and detection was carried out in negative electrospray ionization (ESI) mode. Parent ions, product ions, and collision energies for target PFAS are detailed in Supplementary Material (Table S4).

The method was validated for specificity, repeatability, reproducibility, recovery, and sensitivity following the EURACHEM guidelines using repeated analyses of spiked plasma samples. The relative standard deviation (RSD) met the acceptance criterion of ≤ 20 %, and recovery ranged between 80–120 % using isotopically labeled internal standards. Specificity was confirmed by matching analyte retention times to labeled standards within ± 0.2 s. Repeatability and reproducibility were evaluated through multiple analyses of spiked samples. Recovery rates, evaluated using isotopically labeled internal standards, ranged from 60 to 90 %. The limit of detection (LOD) was established as the lowest concentration with an acceptable signal-to-noise ratio, while the limit of quantitation (LOQ) was defined as the lowest concentration with an ion abundance ratio within ± 15 % of the theoretical value and a relative response factor deviation ≤ 20 % from the mean. The calculated LOD and LOQ were 0.03 ng/mL and 0.1 ng/mL, respectively. The limit of detection (LOD) was defined as the concentration yielding a signal-to-noise (S/N) ratio ≥ 3, and the limit of quantification (LOQ) was defined by an S/N ratio ≥ 10.

Additionally, the laboratory is accredited by EN/ISO 17025 for PFASs analysis in blood serum/plasma. The reliability of the analysis

method is also confirmed by participation in three inter-laboratory trials per year, such as the AMAP Ring Test for Persistent Organic Pollutants in Human Serum, organized by the Quebec National Institute of Public Health.

We specifically assessed PFOS in our study due to its major role in contributing to the overall dietary intake of PFAS, as well as its widespread presence in food sources. In addition, in our study population, PFOS contributed the most to the plasma PFAS exposure when we conducted the biomonitoring. This decision was made to address the specific concern regarding PFOS exposure.

2.4. Covariates

General questionnaires and anthropometry were collected about the study participants. These questionnaires included items related to personal and medical data, such as socioeconomic status, education level, marital status and adherence to Mediterranean diet measured by the validated 14-point MEDAS score (Range 0–14) (Schröder et al., 2011). Body weight was measured twice with light clothes using high-quality electronic calibrated scales and the mean of both measurements was used. Height was measured twice using a wall-mounted stadiometer. BMI was then calculated by dividing weight in kilograms by height in meters squared.

2.5. Statistical analysis

Descriptive analysis was conducted using independent *t*-test to compare the means and standard deviation (SD) of plasma PFAS concentrations and chi-squared test to compare sociodemographic categorical variables number (percentage) between groups with low and high dietary exposure using the median cut-off value. For those variables following a non-normal distribution, the values were presented as median and interquartile ranges (IQR), and differences were tested using the Mann-Whitney *U* test.

Spearman correlation coefficients were calculated to examine the relationship between dietary PFOS estimates and plasma PFOS concentrations. Correlation strength was interpreted following established guidelines with coefficients exceeding 0.50 in absolute value as indicative of a high correlation (Hemphill, 2003). Kappa agreement analysis was performed to assess the concordance between quintiles of dietary PFOS exposure (considering both, LB and UB) and quintiles of plasma PFOS concentrations (Rigby, 2000).

In addition, partial regression models were used to assess the association between dietary and plasma PFOS concentrations, both in crude analysis and after adjusting by potential covariates selected due the potential association with the exposure or outcome (Supplementary material, Fig. S2): age (continuous), sex (female, male), BMI (continuous), and diet quality (MEDAS 14 points).

P-value < 0.05 was considered as significant. All analyses were conducted using the Stata 14 software program (StataCorp).

3. Results

Table 1 shows the general characteristics of participants included in this cross-sectional study stratified by group of dietary exposure to PFOS. Adherence to the Mediterranean diet was significantly higher ($p < 0.001$) in participants in the high exposure group (8.8 ± 1.6) compared to the low exposure group (7.8 ± 1.7). No significant differences in the remaining sociodemographic characteristics were observed between the groups. However, compared to the low dietary exposure PFOS group, those participants in the higher dietary PFOS exposure group presented higher plasma PFOS levels (4.41 ng/mL vs 5.8 ng/mL, p-value = 0.001). Plasma PFNA levels were also higher in participants with high dietary PFOS exposure (0.80 ng/mL) compared to those with lower exposure group (0.96 ng/mL; $p = 0.011$). No significant differences between groups were observed for plasma PFOA and PFHxS congeners concentrations ($p = 0.371$ and 0.476, respectively); however, a trend towards higher concentrations with higher dietary PFOS exposure was evident.

Table 1
General participant characteristics and plasma PFAS levels (ng/mL) in participants with low and high dietary PFOS exposure (ng/kg $_{\text{of body weight}}$/week).

	Dietary exposure to PFOS (ng/kg $_{\text{of body weight}}$/week) (Low exposure) n = 98	(High exposure) n = 98	p-value
Age	65.0 ± 5.3	64.7 ± 4.9	0.717
Sex			
Women	44 (44.9)	44 (44.9)	–
BMI (kg/m^2)	32.9 ± 3.3	32.1 ± 3.1	0.060
Educational level			
Up to primary	51 (52.0)	52 (53.1)	0.245
Secondary	27 (27.6)	34 (34.7)	
University	20 (20.4)	12 (12.2)	
Smoking status			
Never	47 (48.0)	45 (45.9)	0.613
Former	44 (44.9)	42 (42.9)	
Current	7 (7.1)	11 (11.2)	
Adherence to Mediterranean diet MEDAS score (0–14 points)	7.8 ± 1.7	8.8 ± 1.6	<0.001
Plasma PFAS (ng/mL)			
PFOS (ng/mL)	4.41 [2.8; 5.5]	5.8 [3.96; 6.64]	0.001
PFOA (ng/mL)	2.06 [1.34; 2.36]	2.06 [1.34; 2.36]	0.371
PFNA (ng/mL)	0.80 [0.48; 1.04]	0.96 [0.66; 1.16]	0.011
PFHxS (ng/mL)	1.21 [0.58; 1.44]	1.36 [0.7; 1.58]	0.476

Abbreviations: PFAS: Per- and Polyfluoroalkyl Substances, PFOS: Perfluorooctanesulfonic acid, PFOA: Perfluorooctanoic acid, PFNA: Perfluorononanoic acid, PFHxS: Perfluorohexanesulfonic acid.
Data are expressed as mean ± SD or mean [IQR] for continuous variables and as number (percentage) for categorical variables.

The kappa agreement and Spearman correlation analysis between plasma PFOS levels (ng/mL) and dietary exposure levels (ng/kg $_{\text{of body weight}}$/week) are shown in Table 2. Spearman correlation analysis showed a significant positive relationship between dietary PFOS exposure and plasma PFOS levels. The correlation coefficient was 0.3167 for the UB (p-value < 0.001) and 0.3075 (p-value < 0.001) for LB, indicating a moderate relationship. Kappa agreement analysis indicated a fair concordance between dietary intake estimates and plasma PFOS measurements, with agreement values of (29.08 % (p-value = 0.001) for the UB and 21.43 % for the LB (p-value = 0.309).

Partial regression plots are shown in Fig. 1. Significant linear relationship between dietary and plasma PFOS levels were found in the LB (crude model: β coefficient = 0.97 ng/mL, p-value < 0.001; adjusted model: β coefficient = 0.85 ng/mL, p-value = 0.001) as well as in the UB (crude model: β coefficient = 0.01 ng/mL, p-value < 0.001; adjusted model: β coefficient = 0.01 ng/mL, p-value = 0.001).

The Spearman correlation coefficients for plasma PFAS congeners are shown in Supplementary Material, **Table S5**. The correlation

Table 2
Spearman correlation and Kappa agreement between plasma PFOS levels (ng/mL) and dietary PFOS exposure in the upper and lower bound (ng/kg $_{\text{of body weight}}$/week).

	Plasma PFOS	P-value	Kappa agreement	P-value
Dietary PFOS exposure in the upper bound	0.3167	<0.001	29.08 %	**0.001**
Dietary PFOS exposure in the lower bound	0.3075	<0.001	21.43 %	0.309

Abbreviations: PFOS: Perfluorooctanesulfonic acid. N = 196 participants.

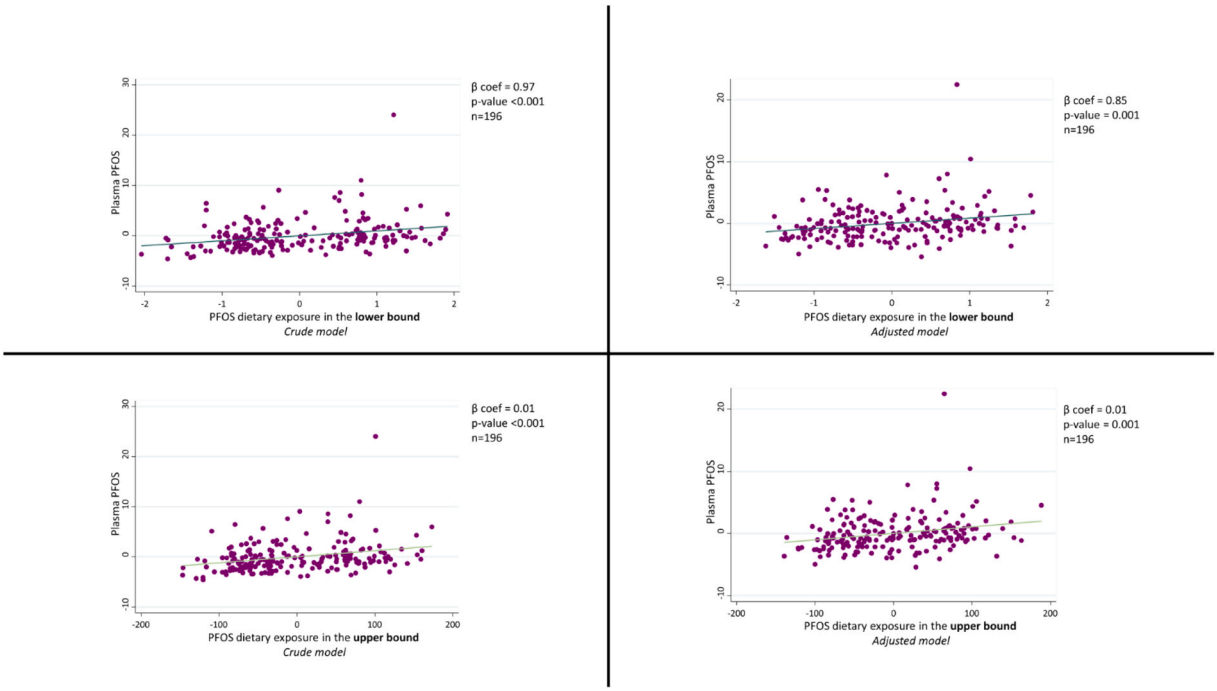

Fig. 1. Partial regression scatter plots showing the association between plasma PFOS and dietary PFOS exposure. **Abbreviations:** PFOS: Perfluorooctanesulfonic acid. Models adjusted for age (continuous), sex (female; male), BMI (continuous) and adherence to the Mediterranean diet (14 points).

between different plasma PFAS congeners presented significant positive correlations, all with p-values < 0.001. Specifically, plasma PFOS levels showed a strong correlation with total PFAS congeners (Rho = 0.895; $p < 0.001$) and moderate correlations with PFOA (Rho = 0.4272; $p < 0.001$) and PFNA (Rho = 0.6198; $p < 0.001$), suggesting overlapping exposure sources or metabolic pathways. PFOA levels showed a moderate positive correlation with total PFAS congeners (Rho = 0.6624; $p < 0.001$) and a weaker association with PFNA (Rho = 0.5961; $p < 0.001$). As for PFNA, positive correlation with PFAS (Rho = 0.7147) was shown. For PFHxS, this congener presented weaker correlations, with its highest correlation being with PFAS (Rho = 0.542; $p < 0.001$).

4. Discussion

This study contributes to the limited scientific evidence available (Haug et al., 2011, 2010; Poothong et al., 2020) that assesses the validity of dietary intake assessment by comparing them with biological measurements. The results of our study showed a statistically significant correlation and a fair agreement between estimated dietary PFOS intake and plasma PFOS concentrations, highlighting the modest reliability of FFQs for estimating dietary exposure. While these findings suggest that FFQs can provide a practical, non-invasive approach for dietary PFOS exposure assessment, the fair agreement observed in Kappa analysis indicates that FFQs alone may not fully capture individual exposure levels and this probably makes it difficult to find potential epidemiological associations between PFOS exposure and health outcomes.

Since PFOS biomagnifies throughout the food chain, it is highly abundant in animal-derived products and, due to widespread soil and surface water contamination, it is also found in other foods such as fruits, vegetables, and drinking water. For this reason, it is anticipated that the main human exposure to PFOS comes from dietary intake and that a certain agreement between PFOS intake and blood levels exist as we have observed in our study. In fact, previous studies have identified diet as the primary contributor to total PFAS exposure (Schrenk et al., 2020). The significant differences observed in PFOS concentrations between low- and high-exposure groups in our study support the hypothesis that dietary sources are a major route of PFOS exposure in the population.

It is important to comment that in our study those individuals with higher adherence to Mediterranean diet tend to be more exposed to dietary PFOS and have higher levels of PFOS in plasma due to the higher consumption of fish, seafood and, fruits and vegetables in those participants adhering to this food pattern, which are primary dietary sources of PFOS (Augustsson et al., 2021; Haug et al., 2010; Vestergren et al., 2012). For this reason, to determine the association between PFOS intake and plasma levels using partial regression, we have additionally adjusted the models by the MEDAS scores in order to establish if the association was independent of this variable.

The observed Spearman correlations in our study align with previous research indicating dietary exposure contributes meaningfully to overall PFOS burden (Vestergren et al., 2012). However, the Kappa agreement values, which indicate modest agreement, suggest variability in the dietary estimates potentially influenced by factors related to the surveyed person, the heterogeneity and variations in PFOS concentrations across food sources and contamination from other non-dietary sources. Lower Kappa values suggest that while FFQs provide a useful estimate of PFOS intake, their precision in categorizing individuals into exposure groups remains modest. Despite these limitations, the statistically significant agreement at the upper bound suggests that dietary estimates still provide meaningful, albeit imperfect, insights into PFOS exposure. Future research should focus on refining dietary exposure assessment tools, improving validation methods to enhance the accuracy of PFOS exposure estimates in epidemiological studies.

In our study, the significant correlations observed among plasma PFAS compounds suggest their co-occurrence in environmental exposure settings, and the strong correlation observed between PFOS and other PFAS congeners suggests shared pathways of exposure and potential bioaccumulation. These findings emphasize the need to consider cumulative exposure to multiple PFAS compounds and their potential synergistic effects on health outcomes. Moreover, given that all correlations were significant, it is important to explore whether findings related to PFOS could also be relevant when considering the broader PFAS congeners. Future studies are warranted to validate reliability of FFQs in assessing the exposure to these other congeners.

This study has several strengths that contribute to its significance and validity. First, it is the first to try to validate dietary PFOS exposure assessments using FFQs by comparing them with plasma PFOS concentrations, bridging an important gap in the literature. The use of advanced UHPLC-MS/MS techniques for plasma PFAS analysis ensured highly accurate and reliable quantification, meeting rigorous EURACHEM guidelines. Additionally, the application of EFSA-referenced PFOS concentrations in food for both lower bound (LB) and upper bound (UB) scenarios provided robust and realistic dietary exposure estimates that align with international regulatory standards. By incorporating a well-characterized subsample from the PREDIMED-Plus cohort, our study benefits from detailed dietary and clinical data, enabling a comprehensive assessment of PFOS exposure.

Despite these strengths, our study has some limitations that should be acknowledged. One limitation of our study is the reliance on FFQ that was not specifically designed and validated to assess PFOS dietary exposure, which may introduce measurement errors (Thompson and Subar, 2017). Future research should focus on developing and validating dietary assessment tools tailored to PFOS-rich food sources to improve accuracy. Moreover, PFOS exposure from non-dietary sources, such as dermal contact, household dust, or inhalation, was not accounted for, which may also influence plasma concentrations (DeLuca et al., 2022). This could partially explain the relatively modest correlation observed between dietary intake and plasma levels. In addition, EFSA's Europe-wide PFOS exposure data incorporate PFAS concentrations from multiple countries, including Spain, providing a robust reference for dietary exposure estimation with the most up-to-date data on extensive food groups. However, national variations in food consumption patterns, sourcing, and preparation methods may still lead to differences in actual exposure levels. While EFSA data include Spanish measurements, they may not fully reflect region-specific dietary habits or localized contamination sources. Future studies with more Spain-specific PFOS data would help refine exposure assessments for this population.

5. Conclusion

In conclusion, this study demonstrates a modest correlation between dietary PFOS intake estimated using FFQs, and plasma PFOS concentrations. While these findings suggest that FFQs can provide a practical, non-invasive approach for dietary PFOS exposure assessment and confirm that food intake is a major contributor to PFOS bioaccumulation in humans, the fair agreement observed in Kappa analysis indicates that FFQs alone may not fully capture individual exposure levels. The study does not account for other potential PFOS exposure routes, such as inhalation or dermal exposures, or differences in PFOS metabolism and excretion. Future research should focus on refining dietary exposure assessment tools, integrating additional exposure pathways, and improving validation methods to enhance the accuracy of PFOS exposure estimates in epidemiological studies.

CRediT authorship contribution statement

Nadine Khoury: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Nancy Babio:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **María Ángeles Martínez:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Eleni Serafeim:** Writing – review & editing. **Danae Costopoulou:** Writing – review & editing. **Lucía Iglesias-Vazquez:** Writing – review & editing. **Leondios Leondiadis:** Writing – review & editing, Data curation, Conceptualization. **Jordi Salas-Salvadó:** Writing – review & editing, Methodology, Data curation, Conceptualization.

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Author contributions

NK, MÁ.M, NB, and JS-S designed and performed the study. JS-S, ES, DC and LL led data acquisition. NK, MÁ.M, NB, and JS-S ran statistical analyses. NK, MÁ.M, NB, ES, DC, LL, LI-V and JS-S interpreted the results. NK, MÁ.M, NB, ES, DC and LL, LI-V and JS-S elaborated the first draft. All authors were involved in reviewing and approving the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2025.109599.

Data availability

Data will be made available on request.

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