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Perfluorinated alkyl substances in Spanish adults: Geographical distribution and determinants of exposure

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are widely found in humans and the environment. Their persistence, bioaccumulation and toxicity make them a source of increasing public health concern. In this study, we analyzed the concentrations and geographical distribution of six PFAS in the serum of 755 Spanish adults aged 18–65.

The geometric mean concentrations (and P95 values) for PFOS (perfluoroctane sulfonate), PFOA (perfluorooctanoic acid), PFHxS (perfluorohexane sulfonate), PFNA (perfluorononanoic acid) and PFDA (perfluorodecanoic acid) were 7.67 (19.3), 1.99 (5.48), 0.91 (2.84), 0.96 (2.44) and 0.42 (0.99) μg/L, respectively. *N*-Methylperfluorooctane sulfonamide (*N*-MeFOSAA) was detected in only 3.3% of samples.

Residents in northeast (Catalonia) and northwest of Spain (Galicia) were found to have the highest serum values, whereas residents in the Canary Islands had the lowest values for almost all PFAS. Men presented higher levels than women, and we confirm that lactation (breastfeeding) contributes to a reduced body burden for all PFAS in women.

Our data provide new information on exposure to PFAS in a national cross section sample of Spanish adults, thus providing a proxy for reference values for the Spanish population and forming the base for following temporal trends in the future.

Keywords: Perfluorinated alkyl substances; Exposure Spanish adults; Human serum samples; Human biomonitoring; PFOS; PFOA

~~1.~~1 Introduction

Perfluoroalkyl substances (PFAS) are a class of chemicals with both hydrophobic and oleophobic properties. They are resistant to thermal, chemical, and biological degradation due to their strong carbon–fluorine (C–F) bonds. These characteristics make them ideal for use in a variety of consumer products and industrial processes (Schultz et al., 2003). Their ubiquity, persistence and bioaccumulation result in their widespread presence in the environment as a result of direct emissions during manufacturing, use, or disposal of products as well as transformation of other precursors into PFAS (Armitage et al., 2009).

PFAS have been found in air, dust and drinking water, and in food items such as fish and dairy products. The contribution of individual pathways and sources to the body burden appears to depend on age, dose and substance. Food, drinking water and house dust are considered to be the main exposure sources for adults, while hand-to-mouth contact with consumer products and house dust is the most important source for children (Freberg et al., 2010; Jain, 2014; Mak et al., 2009). Furthermore, dermal absorption of PFAS occurs as a result of direct contact with consumer products, for example from all-weather clothing and other textiles (Trudel et al., 2008).

Four PFAS, namely perfluoroctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexane sulfonate (PFHxS), are commonly detected in humans (Calafat et al., 2007a; Fromme et al., 2007). The substances are metabolized slowly, and particularly long-chain PFAS are accumulating in the body (Perez et al., 2013). Elimination is species- and gender-dependent and particularly humans are slow eliminators of PFAS compared with other species (Olsen et al., 2009). For example, the half-life of PFOS, PFOA, and PFHxS in human serum is 5.4, 3.8, and 8.5 years respectively (Olsen et al., 2009). In general, the elimination is faster with decreasing carbon chain length (Freberg et al., 2010). The compounds have an affinity for proteins such as albumin and liver fatty acid-binding protein (Han et al., 2003). The highest concentrations in exposed animals are found in blood, liver, kidney and gall bladder (Peng et al., 2010).

There is evidence that perfluorinated compounds may pose a risk to human health. In September 2016, the Persistent Organic Pollutants Review Committee (POPRC) to the Stockholm Convention on Persistent Organic Pollutants reached a consensus agreement that PFOA is likely to lead to significant adverse human health and environmental effects such that global action is warranted. Current regulatory actions within the European Union, and elsewhere, mainly concern PFOS and PFOA, while other widely used PFAS are still under evaluation. PFOA has been added to the European Candidate List for authorization within REACH (ED/69/2013) as a substance of very high concern (Kennedy et al., 2004). PFOS and PFOA are classified as suspected carcinogenic (WHO-IARC, 2016), presumed reprotoxic, harmful to breast-fed children and specifically toxic to target organs. It is currently not known if non-regulated PFAS exert similar toxicity. SAICM (Strategic International Chemicals Management Actions, http://www.saicm.org) recognizes PFAS as an emerging policy issue.

When assessing human exposure, the measurement of PFAS levels in body fluids is the most reliable tool for establishing individual exposure associated with environment and lifestyle. In this regard, the human biomonitoring studies that have been performed since the early 2000s (Calafat et al., 2007a; Schroeter-Kermani et al., 2013) have demonstrated that populations worldwide are exposed to several PFAS and that these chemicals are accumulating in the body (Sturm and Aherns, 2010; Vestergren and Cousins, 2009). The findings have demonstrated how different regulatory and non-regulatory actions have affected human exposure. For example, the voluntary restrictions on PFOA and PFOS resulted in decreasing levels in human samples, while the presence of non-regulated PFAS, used to replace PFOS and PFOA increased (Sturm and Aherns, 2010; Vestergren and Cousins, 2009).

In 2008, the Spanish Ministry of Agriculture, Food and the Environment promoted a national Human Biomonitoring program (HBM). The purpose of this program was to enhance the current understanding of the distribution of priority environmental pollutants, such as metals, pesticides, flame retardants, perfluorinated compounds, and polychlorinated biphenyls (PCB), in the Spanish population and to establish reference values (Bartolomé et al., 2015; Cañas et al., 2014; Huetos et al., 2014; López-Herranz et al., 2016; Ramos et al., 2016). In this regard, we designed BIOAMBIENT.ES project, a nationwide cross-sectional study, aimed at obtaining a representative sample of the Spanish occupied population. A subsample of Bioambient.es was selected to analyze PFAS. Here we present baseline serum values for six PFAS in a national cross-section of Spanish occupied adults.

~~2.~~2 Materials and methods

~~2.1.~~2.1 Study ~~P~~population

BIOAMBIENT.ES is a nationwide cross-sectional epidemiological study with a stratified cluster sampling designed to cover all geographical areas, sex and occupational sectors, and aimed to obtain a representative sample of the Spanish active workforce. The complex design of the study is detailed elsewhere (Perez-Gomez et al., 2013). In brief, volunteers had to be consecutively selected among occupied people older than 16 years, residents in Spain for 5 years or more, which underwent their annual occupational medical check-up between March 2009 and July 2010 in the health facilities of the Societies for Prevention of IBERMUTUAMUR, MUTUALIA, MC-PREVENCIÓN, MUGATRA, UNIMAT PREVENCIÓN, and PREVIMAC, which were distributed across Spain.

A total amount of 113 Prevention Health Centers were available for the project. Those centers are distributed across the whole country; they provide their services to ~~more than~~ ≥ 436,000 companies in Spain in all activity sectors, with 3,600,000 workers employed within a large spectrum of occupations and occupational categories, and perform ~~more than~~ ≥ 650,000 occupational health exams per year. This high number of annual surveys, as well as its wide geographical coverage could allow us to obtain a fairly representative sample of the Spanish workforce. A total of 38 Health Prevention Centers were randomly selected across 12 previously pre-defined geographical areas,

following a proportional distribution according to data from the Spanish Active Population Survey 2007 (Instituto Nacional de Estadística, 2012). Sampling was undertaken in four quarterly recruitment periods to take into account seasonal variability and, was also stratified by sex and by economical sector (two groups: "Service activities" and "Agriculture, Industry & Construction" as defined on the National Classification of Economic Activities for 2009 (Instituto Nacional de Estadística, 2011). Individual information on socio-demographic aspects, lifestyle and environmental conditions, including specific questions on tobacco exposure, diet and food frequency, was obtained using a self-administered questionnaire. Of the total 1880 blood samples collected within Bioambient.es, a subset of 755 serum samples was used to study PFAS exposure. They were obtained by random sampling within each area-gender-occupational sector combination. A set of weights was defined, assigning each participant the inverse of his probability of selection relative to the distribution of occupied people in Spain by autonomous community, sex, and economic activity sector, in accordance with the information provided by the last Spanish Active Population Survey (Instituto Nacional de Estadística, 2009).

~~2.2.~~2.2 Ethical approval

The study was performed in accordance with legal/ethical principles and regulations concerning research involving individual information and biological samples, including the Organic Law 15/1999 on Personal Data protection and its Regulations, Law 41/2002 on the Autonomy of Patients and rights and obligations relating to health information and documentation, and General Health Law 14/1986. Ethical approval was given by the Scientific Ethics Committee and the Legal Department of IBERMUTUAMUR and, as blood samples were collected, the principles of the Declaration of Helsinki and those contained in the UNESCO Universal Declaration on the Human Genome and Human Rights were taken into consideration.

~~2.3.~~2.3 Biological ~~S~~sampling and ~~S~~storage

Fasting blood specimens were collected in S-Monovette® neutral tubes (Sarstedt, Nümbrecht, Germany) by trained healthcare staff. Blood samples were centrifuged within 5 h of collection and transported at 4 °C within 96 h post-sampling (Esteban et al., 2013). In the laboratory, samples were left to rest for approximately 60 min at room temperature then centrifuged again. Serum aliquots (0.5 mL) were stored at ~~-~~ 20 °C until analysis.

~~2.4.~~2.4 Analysis

PFAS analysis was described in detail previously (Bartolomé et al., 2016). Briefly, 100 µL of serum, 100 µL of ACN for protein precipitation and 25 µL of labelled standard solution were mixed. The final volume of 270 µL was achieved using MeOH. The mixed sample was vortexed for 15~~-~~20~~ seconds~~s and centrifuged for 10~~ minutes~~min at 13,500 rpm and 4 °C. The supernatant organic layer was injected into the on-line SPE apparatus with LC-MS/MS.

The limit of quantification (LQ) was established using labelled standards. All PFAS were quantified between 0.16 and 0.34 µg/L (Bartolomé et al., 2016). Internal quality control was performed with PFAS Standard and PFAS labelled Standard. Quality in the analytical procedure was assured by participating in G-EQUAS 51/2013 (German External Quality Assessment Scheme, Erlangen, Germany). All values were within the established range of tolerance, z-score < ~~-~~2|.

~~2.5.~~2.5 Data analysis

The complex design of the study, including strata (geographic zones), primary sampling units (health examination centers), and population weights was taken into account in all the analyses.

A descriptive analysis was carried out for each PFAS, obtaining the arithmetic (AM) and geometric means (GM). The 10th, 25th, 50th, 75th, 90th and 95th percentiles, along with their 95% confidence intervals (CI), were also obtained for the whole set of 755 serum samples and by gender, age, occupational sector, smoking habit, geographical area and sampling trimester. Based on the symmetry in the distribution of the natural logarithm of the variables we choose GM for analysis and their confidence intervals.

The serum levels of PFAS were tested for their normal or log-normal distribution by the Kolmogorov-Smirnov test~~: A~~ (a)nd correlation coefficients were executed by Pearson test in data from a normal distribution and Spearman rank test in data with a non-~~-~~normal distribution. Wilcoxon matched pairs ranks test was used for comparison between various groups.

Means were not calculated when the proportion of subjects with levels below the limit of quantification was above 60%. The problem of censoring imposed by the limits of quantification was overcome by imputing censored values with the limit of quantification divided by the square root of two (Perez-Gomez et al., 2013). Inter-group differences were tested using a simple interval regression model (Conroy, 2005).

A multiple interval regression model was also used to study other interactions. Significant factors were included in new models along with different variables from the questionnaire in order to identify exposure factors (years in Spain, environment, type of house, breastfed children, diet), and interactions with gender, age, occupational sector, geographical area and sampling trimester were investigated.

All statistical analyses were performed using Stata IC v12 (StataCorp LP, USA) and maps were prepared using R and ArcView.

3.3 Results

This study is based on a sub-sample from the Bioambient.es survey, which totally included 1892 volunteers covering the whole area of Spain (Esteban et al., 2013; Perez-Gomez et al., 2013). The current sample includes 404 women and 351 men, all together 755 samples, divided in four age groups (18–29, 30–39, 40–49 and 50–65 years) and separated on basis of area of residence and sampling time of the year (Esteban et al., 2013).

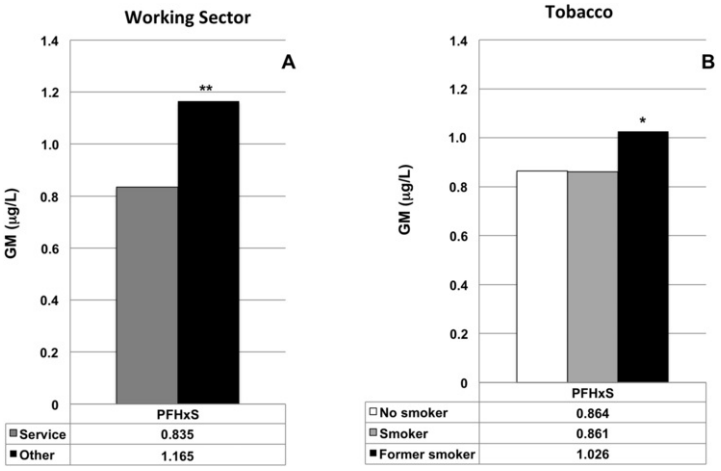
PFOA, PFOS and PFNA were detected in 100%, 99.7% and 99.9% of the samples respectively, while PFHxS and PFDA were detected in 84.8% and 86.4% of samples. Only 3.3% of the samples contained *N*-MeFOSAA. The arithmetic (AM) and geometric means (GM), and selected percentiles of PFAS at national level are summarized in Table 1, whereas the other descriptive variables, age, sex, occupational sector and geographical information are available in Tables A1 to A6 as Appendix A.

Table 1. Table 1 Arithmetic (AM) and geometric means (GM) for PFAS, and selected percentiles for all samples.

alt-text: Table 1

PFAS	N	AM (µg/L) IC 95%	GM (µg/L) IC 95%	p10	p25	p50	p75	p90	p95	< LQ (%)
PFOS	755	9.16 8.33–9.98	7.67 7.17–8.19	3.76	5.30	7.55	11.10	16.05	19.33	0.3
PFOA	755	2.35 2.19–2.51	1.99 1.89–2.09	1.09	1.40	2.03	2.99	4.37	5.48	0.0
PFHxS	755	1.18 0.97–1.39	0.91 0.84–0.99	< LQ	0.52	0.82	1.27	2.27	2.84	15.2
PFNA	755	1.11 1.01–1.21	0.96 0.89–1.03	0.51	0.65	0.92	1.34	1.89	2.44	0.1
PFDA	755	0.49 0.43–0.55	0.42 0.39–0.46	< LQ	0.25	0.36	0.55	0.78	0.99	13.6
N-MeFOSAA	719			< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	96.7

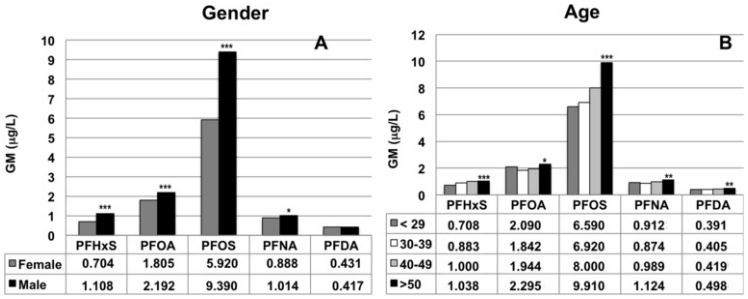
The results of the univariate (unadjusted) analysis suggest no differences in PFAS exposure as a result of occupational sector or smoking habits, except for PFHxS, which is found at lower levels in workers from the service sector ($p = 0.005$) and at higher levels in former smokers ($p = 0.012$) (Fig. 1A and B).



~~Fig. 1.~~Fig. 1 Differences in serum levels of PFHxS according to: **A** occupational sector (n = 243) (two groups: "Service" and "Other: Agriculture, Industry & Construction" as defined on the National Classification of Economic Activities for 2009 ~~(INE, 2009)~~) and **B** smoking habit (n = 162) after univariate analysis. Geometric means (GM) expressed in µg/L. * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

alt-text: Fig. 1

There were differences in serum PFAS levels by sex and age too. The concentrations of all PFAS are higher among men than women (Fig. 2A) except for PFDA (GM = 0.41 µg/L male, GM = 0.43 µg/L female()). The biggest differences ~~e~~ were observed for PFOS and PFHxS, with geometric means increasing from 6.59 to 9.91 µg/L and 0.71 to 1.04 µg/L respectively from younger to older aged. (Fig. 2B).



~~Fig. 2.~~Fig. 2 Differences in serum levels of PFAS according gender and age after univariate analysis. Geometric means (GM) expressed in µg/L. * = p < 0.05; ** = p < 0.01; *** = p < 0.001. **A**. Gender differences in serum levels of PFHxS (n = 351), PFOA (n = 351), PFOS (n = 351) and PFNA (n = 351). The concentrations of all PFAS are higher among men than women, except for PFDA. **B**. Serum levels of PFAS according four groups of age: 18–29, 30–39, 40–49 and 50–65 years old. Differences between the youngest and the oldest group were statistically significant for all substances. The biggest increase was observed for PFOS and PFHxS, with geometric means increasing from 6.59 to 9.91 µg/L and 0.71 to 1.04 µg/L respectively from younger to older aged. PFHxS (n = 749), PFOA (n = 749), PFOS (n = 749), PFNA (n = 749) and PFDA (n = 749).

alt-text: Fig. 2

Multiple regression analysis show significant age influence for PFOS, PFOA, PFHxS (p < 0.001) and PFNA (p < 0.043), but this influence was not significant for PFDA (p = 0.57) (Table 2). Men aged ~~less than~~ ≤ 30 have higher levels of all PFAS than women of the same age group. The levels in males increased in every successive age group, with the differences between the first and last group being significant for all substances (Table 2). In women the situation is different. PFAS levels remained practically stable across all age ranges. Significant p values only appeared in the age group 30–39 for PFOA (p = 0.01) and PFNA (p = 0.031), and in the > 50 group for PFHxS (p < 0.001).

~~Table 2.~~Table 2 Results of multiple regression analysis for PFAS and their associations with selected covariates.

alt-text: Table 2

		PFOS		PFOA		PFHxS		PFNA		PFDA	
Factor	Levels	B CI 95%	p	B CI 95%	p	B CI 95%	p	B CI 95%	p	B CI 95%	p
Gender < 30 (ref. females)	Males < 30	1.51 1.38–1.66	< 0.001	1.28 1.18–1.39	< 0.001	1.91 1.71–2.13	< 0.001	1.11 1.00–1.23	0.043	0.97 0.87–1.08	0.573
Age males (ref. < 30)	30–39	1.21 1.01–1.45	0.042	0.99 0.86–1.14	0.867	1.30 1.04–1.62	0.022	1.04 0.87–1.23	0.686	1.07 0.88–1.30	0.469
	40–49	1.21 1.02–1.44	0.028	1.03 0.93–1.13	0.606	1.42 1.07–1.89	0.017	1.11 0.97–1.26	0.114	1.08 0.91–1.27	0.358
	50–	1.68 1.36–2.68	< 0.001	1.22 1.03–1.44	0.023	1.56 1.33–2.04	0.002	1.29 1.05–1.43	0.020	1.44 1.12–1.86	0.007
Age females (ref. < 30)	30–39	0.87 0.75–1.00	0.055	0.78 0.68–0.89	0.001	1.01 0.79–1.29	0.931	0.86 0.75–0.98	0.031	0.94 0.76–1.15	0.522

	40–49	0.95 0.81–1.12	0.554	1.03 0.93–1.08	0.606	1.11 0.85–1.43	0.430	0.95 0.78–1.15	0.584	1.20 0.94–1.53	0.143
	50–	1.15 0.95–1.44	0.200	1.09 0.90–1.32	0.353	1.73 1.33–2.23	< 0.001	1.20 0.93–1.54	0.154	1.16 0.94–1.44	0.165
Working sector (ref. services)	No services	0.94 0.83–1.06	0.318	0.87 0.79–0.97	0.012	1.06 0.91–1.24	0.459	0.91 0.82–1.01	0.064	0.93 0.82–1.04	0.200
Geographic area (ref. national average)	Galicia	1.42 1.20–1.69	< 0.001	1.03 0.95–1.13	0.432	0.94 0.71–1.24	0.664	1.20 1.02–1.42	0.028	1.31 1.10–1.55	0.003
	Asturias, Cantabria	0.99 0.85–1.16	0.934	0.86 0.74–1.01	0.060	0.74 0.59–0.93	0.013	0.90 0.84–0.95	0.001	0.92 0.58–1.45	0.716
	Basque Country	1.04 0.79–1.38	0.770	0.96 0.83–1.12	0.620	1.16 0.98–1.38	0.090	1.01 0.92–1.11	0.854	0.95 0.78–1.17	0.641
	Navarra, La Rioja Aragón	0.92 0.86–1.00	0.045	1.04 0.88–1.24	0.606	0.86 0.71–1.04	0.110	0.92 0.78–1.09	0.332	0.87 0.70–1.08	0.209
	Catalonia	1.34 1.19–1.52	< 0.001	1.51 1.34–1.71	< 0.001	1.80 1.29–2.50	0.001	1.37 1.18–1.60	< 0.001	1.24 0.95–1.63	0.104
	Castile and León	1.06 0.89–1.26	0.514	1.19 0.92–1.54	0.176	1.23 0.97–1.55	0.083	1.08 0.83–1.39	0.558	1.16 0.89–1.53	0.259
	Madrid	0.96 0.83–1.10	0.504	1.04 0.92–1.19	0.504	1.04 0.88–1.23	0.650	0.93 0.82–1.05	0.213	0.93 0.77–1.12	0.445
	Castile-La Mancha Extremadura	0.81 0.76–0.87	< 0.001	0.86 0.79–0.93	< 0.001	0.90 0.79–1.02	0.092	0.84 0.69–1.02	0.082	0.82 0.70–0.96	0.015
	C. Valenciana Balearic I.	1.07 0.94–1.22	0.299	1.22 1.08–1.38	0.003	0.99 0.84–1.18	0.943	1.11 1.02–1.19	0.011	1.17 1.01–1.36	0.037
	Andalucía. Ceuta	0.98 0.89–1.09	0.742	1.01 0.93–1.10	0.738	1.19 0.94–1.50	0.137	1.06 0.95–1.20	0.296	0.94 0.80–1.09	0.377
	Murcia	0.86 0.74–0.99	0.041	0.61 0.83–1.02	0.101	0.93 0.69–1.26	0.623	0.94 0.85–1.05	0.260	1.06 0.98–1.14	0.153
	Canary Islands	0.74 0.55–0.95	0.019	0.61 0.49–0.75	< 0.001	0.64 0.48–0.84	0.001	0.78 0.60–1.01	0.061	0.77 0.61–0.99	0.040
Sampling trimester (ref. average)	January–March	0.90 0.82–0.98	0.016	0.92 0.86–1.00	0.044	1.14 0.94–1.39	0.163	0.98 0.91–1.06	0.551	0.93 0.85–1.02	0.108
	April–June	1.14 1.07–1.22	< 0.001	1.12 1.05–1.20	0.001	1.05 0.92–1.20	0.472	1.14 1.07–1.22	< 0.001	1.08 0.97–1.21	0.159
	July–September	1.05 0.97–1.13	0.234	1.06 0.96–1.17	0.259	0.89 0.77–1.02	0.094	0.96 0.84–1.10	0.581	1.02 0.89–1.16	0.795
	October–December	0.93 0.85–1.03	0.140	0.91 0.83–1.00	0.047	0.94 0.83–1.06	0.304	0.93 0.83–1.04	0.206	0.97 0.86–1.10	0.666

B = ratio levels. CI = confidence interval.

The age-related difference between men and women was further investigated by including breastfed children as a variable (zero for men), (Table 3). Women with no breastfeeding history showed no significant differences with

regard to men of the same age group, whereas women who had been breastfeeding their children showed virtually no increase in PFAS levels with age. Significant differences were observed in women who breastfed three or more children in PFOS (p = 0.003), PFHxS (p = 0.004) and PFDA (p = 0.009), and women who breastfed two or more children for PFOA (p = 0.012, p < 0.001) and PFNA (p = 0.028, p = 0.009) (Table 3).

~~Table 3.~~Table 3

Results of multiple regression analysis of PFAS for different exposure factors related to lifestyle. Values have been adjusted for gender, age and sampling trimester. For detailed information see Supplementary material Tables A7–A11.

alt-text: Table 3

		PFOS		PFOA		PFHxS		PFNA		PFDA	
Factor	Levels	B CI 95%	p	B CI 95%	p	B CI 95%	p	B CI 95%	p	B CI 95%	p
Years living in Spain (ref > 35)	5–15	0.45 0.37–0.55	< 0.001	0.63 0.52–0.76	< 0.001	0.46 0.31–0.69	< 0.001	0.74 0.63–0.87	0.001	0.71 0.58–0.87	0.002
	16–25	0.95 0.61–0.92	0.009	0.90 0.73–1.21	0.329	0.77 0.59–1.01	0.058	0.90 0.67–1.19	0.430	0.80 0.64–1.00	0.048
	26–35	0.86 0.77–0.97	0.018	0.93 0.77–1.12	0.429	0.82 0.64–1.06	0.125	0.82 0.82–1.11	0.527	0.88 0.79–0.98	0.019
Breastfed children (ref: none)	One	0.92 0.73–1.15	0.438	0.92 0.76–1.12	0.395	0.82 0.68–1.00	0.051	0.97 0.85–1.11	0.666	1.10 0.92–1.33	0.289
	Two	0.84 0.71–1.01	0.059	0.78 0.65–0.94	0.012	0.90 0.67–1.21	0.468	0.81 0.67–0.97	0.028	0.89 0.72–1.10	0.267
	Three or more	0.68 0.64–0.87	0.003	0.51 0.39–0.67	< 0.001	0.59 0.42–0.83	0.004	0.63 0.46–0.89	0.009	0.61 0.43–0.87	0.009
Fish	1/-week-	1.33 1.16–1.52	< 0.001	1.10 0.97–1.26	0.138	1.16 0.96–1.40	0.120	1.13 1.01–1.28	0.040	1.12 0.97–1.29	0.111
	2–4/-week	1.43 1.25–1.63	< 0.001	1.04 0.93–1.16	0.497	1.26 1.06–1.49	0.011	1.27 1.09–1.46	0.003	1.22 1.07–1.39	0.004
	> 4 week			1.26 1.09–1.46	0.003						
	5–6/-week	1.56 1.25–1.94	< 0.001			1.27 0.97–1.66	0.081	1.42 1.06–1.91	0.020	1.46 1.03–2.06	0.033
	1/-day or more	1.64 1.30–2.08	< 0.001			1.51 1.20–1.89	0.001				
Red wine (ref: < 1/week)	1–6/week	1.15 0.99–1.34	0.064	1.15 1.02–1.30	0.028			1.13 0.96–1.33	0.135	1.06 0.93–1.22	0.374
	1/day or more	1.26 1.07–1.49	0.007	1.24 1.03–1.49	0.022			1.16 0.96–1.33	0.034	1.22 1.09–1.37	0.002
Beer (ref: < 1/week)	1–6/week	1.14 1.02–1.26	0.018	1.11 1.00–1.22	0.043	1.10 0.94–1.29	0.223				
	1/day			1.23 1.06–1.43	0.010	1.33 1.16–1.53	< 0.001				

Cooking water (ref: tap water)	Private well			1.22 1.05–1.40	0.009	0.72 0.59–0.89	0.003	1.21 1.06–1.39	0.006	1.45 1.20–1.75	< 0.001
	bottled water			1.18 1.00–1.39	0.045	1.19 0.99–1.44	0.063	1.15 0.99–1.34	0.067	1.11 0.95–1.31	0.188
	Others			0.99 0.73–1.34	0.924	0.85 1.66–1.09	0.195	1.00 0.82–1.21	0.969	1.03 0.88–1.21	0.704

B = ratio levels. CI = confidence interval.

The region of residence has an impact on PFAS serum levels (for detailed information see Tables A1–A5 in Supplementary information). Subjects residing in the northeast of Spain (Catalonia) and in the northwest (Galicia) had higher geometric means than in other areas. Catalonia inhabitants are the most exposed for PFOA (2.72 μg/L, p < 0.001), PFHxS (1.26 μg/L, p < 0.001) and PFNA (1.28 μg/L, p = 0.003), whereas subjects from Galicia had slightly higher levels of PFOS (10.46 μg/L, p = 0.041) and PFDA (0.50 μg/L, p = 0.012) than those from Catalonia. It proved impossible to make any comparisons for *N*-MeFOSAA since only a few samples had levels above the limit of quantification, with this substance only being detected in a few individuals from Catalonia, the Basque country, Aragon and Madrid. The lowest levels for all PFAS were found in residents of the Canary Islands. As the model includes the quarter of the year as covariate, these regional results are not affected by seasonal influences because of the design and timing of the survey.

Multiple regression analysis including gender, age, occupational sector, geographic area and time of the year confirmed the non-significance of the occupational sector as an exposure source for all PFAS (Table 2). In a multiple regression analysis in which gender, age, occupational sector and geographic area were held constant, additional exposure factors for different PFAS could be identified (Table 3 and Supplementary information in Tables A7 to A11). The serum concentrations of all the studied PFAS increased with the number of years of living in Spain, suggesting that current levels have been acquired as a result of exposure in Spain.

Lifestyle-related environmental factors were not common for all the substances studied. The type of water used in the household appeared to be important with respect to PFAS exposure: when municipal tap water is taken as a reference, consumers of water from private wells had significant higher levels of PFOA and PFDA, but lower levels of PFHxS (Table 3) which follows the general trend of PFHxS exposure being lower in rural than in urban areas (Table A9).

Data from the food frequency questionnaire allowed us to assess the possible influence of fish, beer and wine consumption on PFAS exposure (Tables 3 and A7–A11 in the ~~suplemnent~~Supplement). Fish consumption (including canned fish) showed a positive association with PFHxS, PFOA, PFOS, PFNA and PFDA levels, and the strength of the association increased with consumption frequency irrespective of fish species. Beer and wine consumption also influence PFAS levels. Therefore, regular beer drinkers (1 to 6/week) showed an association with PFOA and PFOS, but not significant with PFHxS (p = 0.223). On the other hand, consumers of wine also had a positive association with the levels of PFOA, PFOS, PFNA and PFDA. However, regular red wine consumers (1 to 6 times/week) did not show a significant association with PFNA (p = 0.135) and PFDA (p = 0.374).

~~4.~~4 Discussion

This nationwide Human Biomonitoring survey present data on exposure to six PFAS in a sample of Spanish workers aged from 18 to 65 years as a proxy of the exposure of Spanish general occupied population. Thus, it should be taken into account that unemployed, disabled and homeworkers are not included by study design.

Our findings show that Spanish adults in general are exposed to PFAS since the most relevant PFAS (PFOS, PFOA and PFNA) were detected in almost all the 775 samples and PFHxS and PFDA in ~~more than~~ ≥ 85% of samples. Exposure to *N*-MeFOSAA appears to be minor as judged from serum levels (3.3% of the samples were positive). However, it cannot be excluded that this substance has a different accumulation pattern than the other PFAS and this remains to be elucidated.

The relationship between diet, age and gender and PFAS exposure has been studied previously in Spain in a small group of adults in Catalonia (Domingo et al., 2012; Ericson et al., 2007; Ericson et al., 2008). Their PFOS and PFHxS concentrations were higher than in our samples from Catalonia, whereas the PFOA, PFNA and PFDA levels were similar to ours. Another, more extended study that also included the socio-economic determinants (Manzano-Salgado et al., 2016) and data from pregnant women from Catalonia, Valencia and the Basque Country reported PFOA, PFOS, PFNA and PFHxS values similar to our samples from the same regions.

We provide the first exposure data for PFAS at a national level in an important sector of the Spanish population, and our results are in line with those previously reported for some regions of the country. They also fall within the ranges found in other countries with similar sampling date with PFOS levels between 3.8 and 13.2 μg/L and PFOA levels of between 1.3 and 4.1 μg/L being reported in Greece (Vassiliadou et al., 2010), Italy (Ingelido et al., 2010), Germany (Schroeter-Kermani et al., 2013), Australia (Toms et al., 2014), Korea (Cho et al., 2015), Canada (Haines and Murray, 2012) and the United States (Kato et al., 2011) (Table 4).

~~Table 4.~~ Table 4 Comparison of PFAS levels for this study and other published studies. To compare PFAS levels in different matrices, a 1:1 ratio for serum plasma and a 2:1 ratio for serum or plasma to whole blood was applied according to Ehresman et al. (2007).

alt-text: Table 4

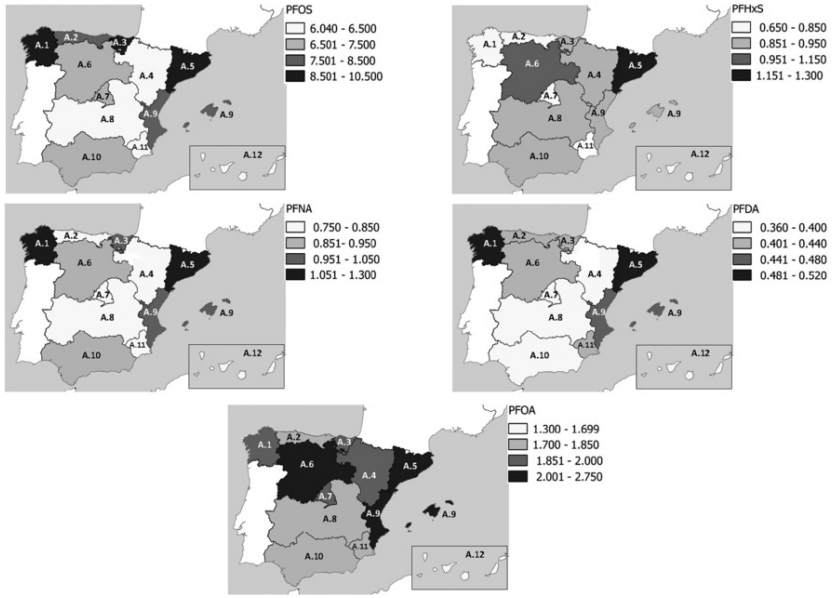
Country	Substance	Age	N	µg/L	Reference
Spain (sampling 2009–2010) (serum)				**GM (CI 95%)**	This study
	PFOS	16–65	755	7.67 (7.17–8.19)	
	PFOA			2.33 (2.19–2.52)	
	PFHxS			0.84 (0.76–0.92)	
	PFNA			0.95 (0.89–1.03)	
	PFDA			0.37 (0.34–0.40)	
Italy (sampling 2008) (serum)				**GM (min-max)**	(Ingelido et al., (2010))
	PFOS	20–65	230	5.77 (0.06–29.6)	
	PFOA			3.32 (0.22–51.9)	
Germany (sampling 2008/2010) (plasma)				**GM (min-max)**	(Schroeter-Kermani et al., (2013))
	PFOS	20–29	20/18	6.1 (2.6–10.2)/3.8 (1.9–12.1)	
	PFOA			4.0 (2.3–6.7)/3.1 (0.8–8.7)	
	PFHxS			1.05(0.25–2.45)/0.82(0.251.39)	
Australia (sampling 2008–2009)/(2010– 2011) (serum)		0–60		**Mean (range)**	(Toms et al., (2014))
	PFOS		2420	14.1 (5.3–19.2)/10.2 (4.4–17.4)	
	PFOA			5.2 (2.8–7.3)/4.5 (3.1–6.5)	
	PFHxS			2.9 (1.2–5.7)/3.3 (1.4–5.4)	
	PFNA			1.2 (0.9–1.6)/0.7 (0.6–0.9)	
	PFDA			0.3(< LOD*–0.4)/0.3 (0.2–0.4) *LOD = 0.2 µg/L	
Canada (sampling 2007–2009) (blood)		20–79		**GM (CI 95%)**	(Haines and Murray, (2012))
	PFOS		2880	11.13 (♂)/7.07 (♀) (6.30–12.36)	
	PFOA			2.94 (♂)/2.17 (♀) (1.99–3.15)	
USA (NHANES) (sampling 2007–2008) (serum)		12–>60		**GM (CI 95%)**	(Kato et al., (2011))
	PFOS		2100	13.2 (12.2–14.2)	
	PFOA			4.13 (4.01–4.25)	
	PFHxS			1.96 (1.76–2.17)	
	PFNA			1.49 (1.37–1.61)	

Information for PFHxS, and PFNA is available from Norway (Rylander et al., 2009), Australia (Toms et al., 2014), Korea (Cho et al., 2015) and the United States (Kato et al., 2011), with PFHxS levels also being available for Germany (Schroeter-Kermani et al., 2013). The mean values obtained were in the range 0.5–3.3 μg/L and 0.7–1.9 μg/L for PFHxS and PFNA, respectively. PFDA was only reported in Australian study (Toms et al., 2014) with a mean value of 0.3 μg/L. The Spanish values reported on our study fall within these ranges. We can therefore conclude that the Spanish population has the similar exposure patterns to PFAS than other European populations but lower PFAS levels than Australia, Canada and United States, and slightly higher than Korea.

Our study confirms findings from previous HBM surveys on PFAS, which found gender-related differences for PFOS, PFOA and PFHxS, with higher concentrations in blood-serum samples from males (Cho et al., 2015; Haines and Murray, 2012; Ingelido et al., 2010; Schroeter-Kermani et al., 2013; Toms et al., 2014; Vassiliadou et al., 2010), while the gender did not appear to have an effect for PFNA (Table 2). The geometric mean of PFOS and PFHxS concentrations increased with age in our study, whereas for PFOA, PFNA and PFDA remained constant in the range < 29–49 years old, but subsequently increasing in older age groups. Significant correlations between PFAS concentrations in human samples (blood, serum) and age have also been reported in other countries (Calafat et al., 2007b; Goralczyk et al., 2015; Haug et al., 2009; Toms et al., 2014). Differences in exposure and gender-related pharmacokinetics have been suggested for the gender differences in PFAS concentrations (Calafat et al., 2007a; Calafat et al., 2007b; Haines and Murray, 2012), although these are yet to be completely elucidated. Our data confirm that lactation could lead to a reduction in PFAS body burden in females (Fei et al., 2007; Karrman et al., 2007; Kato et al., 2011; Tao et al., 2008). In addition, menstruation has been suggested as a possible elimination route in pre-menopausal females (Harada et al., 2005; Knox et al., 2011; Taylor et al., 2014) as the gender-related difference disappears in post-menopausal women (Vassiliadou et al., 2010), and men and women of older age groups have similar accumulation patterns as judged from serum samples. Additional explanations for the differences observed between males and females could be diet and lifestyle.

Diet is one of the clearest factors influencing PFAS serum levels, with fish consumption being shown to be associated with PFHxS, PFOA, PFOS, PFNA and PFDA levels. An association between consumption of fish/shellfish and PFAS levels has also been described by other authors (Ericson et al., 2008; Falandysz et al., 2006; Haug et al., 2010; Ji et al., 2012; Rylander et al., 2010; Wu et al., 2012; Christensen et al., 2016). Similarly, the National Health and Nutrition Examination Survey (NHANES) program of United States has reported a similar relationship with fish consumption in the US population (Jain, 2014).

Although our, and the reported findings are convincing, we still have to be careful in the interpretation that fish/seafood consumption is an important determinant for the body burden PFAS. As discussed above, PFAS values in the European human population are rather uniform independent country and region, while we know from our previous studies that fish/seafood consumption differs a lot between European countries. In our Spanish material, the areas with the highest PFAS exposure are not strictly those with the highest fish/seafood consumption (Fig. 3). For comparison, human exposure to methyl-mercury is strictly correlated to consumption of marine products and we have previously shown that human exposure across Europe is clearly related to consumption of fish, both fresh or processed or canned, and seafood (Castaño et al., 2015). In addition, methyl Hg accumulation is species-, size- and age-dependent. PFAS serum levels, on the other side, only shows a weak, if any, dependency on species, size, age or type of seafood (fish, shellfish), as well as if the items are processed or canned. Therefore, although fish/seafood certainly contributes, we assume there are other sources of PFAS exposure that significantly contributes to the body burden. More studies are needed to better understand the relationship between sources and exposure to PFAS in the society.



~~Fig. 3.~~Fig. 3

PFAS serum levels by area of residence expressed as geometric mean in µg/L. In the maps values have been distributed in four intervals. A.1 Galicia, A.2 Asturias–Cantabria, A.3 Basque Country, A.4 Navarre–La Rioja–Aragon, A.5 Catalonia, A.6 Castile and Leon, A.7 Madrid, A.8 Castile la Mancha–Extremadura, A.9 Valencia–Balearic Islands, A.10 Andalusia–Ceuta, A.11 Murcia, A.12 Canary Islands. For detailed information see Tables A1–A6 in the Supplementary information.

alt-text: Fig. 3

~~5.~~5 Conclusion

We present values for serum PFAS levels in a national cross-section of the Spanish population in active employment, and these values are the best proxy for the baseline values for Spanish adults in the age range 18–65 years available until this moment. The mean PFAS concentrations reported here are in accordance with the levels described for populations living in neighbouring countries. They confirm the importance of age and gender with respect to PFAS levels, with men having higher levels than women, and with PFAS levels increasing with age, particularly in men. In women lactation has a significant influence in reducing PFAS levels. We found a geographic variation with the highest levels in the north and east of Spain and the lowest in the Canary Islands. Other PFAS exposure factors were the number of years living in Spain, the water used to cook, and to some extent foods items (mainly fish, beer, wine). Our results and the comparison to values reported from other countries, that there are significant sources of PFAS exposure not covered by this study. Therefore it is important to continue with HBM surveys combined with appropriately designed questionnaires to identify additional exposure sources and on basis of this develop risk-management actions.

Detailed information concerning the descriptive results of serum PFAS levels in the study population and statistical tables for the univariate and multivariate models applied in this study. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2017.06.031.

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E-Extra

PFAS serum levels by area of residence expressed as geometric mean in µg/L. In the maps values have been distributed in four intervals. A.1 Galicia, A.2 Asturias–Cantabria, A.3 Basque Country, A.4 Navarre–La Rioja–Aragon, A.5 Catalonia, A.6 Castile and Leon, A.7 Madrid, A.8 Castile la Mancha–Extremadura, A.9 Valencia–Balearic Islands, A.10 Andalusia–Ceuta, A.11 Murcia, A.12 Canary Islands. For detailed information see Tables A1-A6 in the Supplementary information.

Results of multiple regression analysis of PFAS for different exposure factors related to lifestyle. Values have been adjusted for gender, age and sampling trimester. For detailed information see Supplementary material

Tables A7–A11.

PFOA, PFOS and PFNA were detected in 100%, 99.7% and 99.9% of the samples respectively, while PFHxS and PFDA were detected in 84.8% and 86.4% of samples. Only 3.3% of the samples contained *N*-MeFOSAA. The arithmetic (AM) and geometric means (GM), and selected percentiles of PFAS at national level are summarized in Table 1, whereas the other descriptive variables, age, sex, occupational sector and geographical information are available in Tables A1 to A6 as Appendix A.

~~Table 1.~~ Table 1 Arithmetic (AM) and geometric means (GM) for PFAS, and selected percentiles for all samples.

alt-text: Table 1

PFAS	N	AM (µg/L) IC 95%	GM (µg/L) IC 95%	p10	p25	p50	p75	p90	p95	< LQ (%)
PFOS	755	9.16 8.33–9.98	7.67 7.17–8.19	3.76	5.30	7.55	11.10	16.05	19.33	0.3
PFOA	755	2.35 2.19–2.51	1.99 1.89–2.09	1.09	1.40	2.03	2.99	4.37	5.48	0.0
PFHxS	755	1.18 0.97–1.39	0.91 0.84–0.99	< LQ	0.52	0.82	1.27	2.27	2.84	15.2
PFNA	755	1.11 1.01–1.21	0.96 0.89–1.03	0.51	0.65	0.92	1.34	1.89	2.44	0.1
PFDA	755	0.49 0.43–0.55	0.42 0.39–0.46	< LQ	0.25	0.36	0.55	0.78	0.99	13.6
N-MeFOSAA	719			< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	96.7

The region of residence has an impact on PFAS serum levels (for detailed information see Tables A1–A5 in Supplementary information). Subjects residing in the northeast of Spain (Catalonia) and in the northwest (Galicia) had higher geometric means than in other areas. Catalonia inhabitants are the most exposed for PFOA (2.72 µg/L, $p < 0.001$), PFHxS (1.26 µg/L, $p < 0.001$) and PFNA (1.28 µg/L, $p = 0.003$), whereas subjects from Galicia had slightly higher levels of PFOS (10.46 µg/L, $p = 0.041$) and PFDA (0.50 µg/L, $p = 0.012$) than those from Catalonia. It proved impossible to make any comparisons for *N*-MeFOSAA since only a few samples had levels above the limit of quantification, with this substance only being detected in a few individuals from Catalonia, the Basque country, Aragon and Madrid. The lowest levels for all PFAS were found in residents of the Canary Islands. As the model includes the quarter of the year as covariate, these regional results are not affected by seasonal influences because of the design and timing of the survey.

Multiple regression analysis including gender, age, occupational sector, geographic area and time of the year confirmed the non-significance of the occupational sector as an exposure source for all PFAS (Table 2). In a multiple regression analysis in which gender, age, occupational sector and geographic area were held constant, additional exposure factors for different PFAS could be identified (Table 3 and Supplementary information in Tables A7 to A11). The serum concentrations of all the studied PFAS increased with the number of years of living in Spain, suggesting that current levels have been acquired as a result of exposure in Spain.

Lifestyle-related environmental factors were not common for all the substances studied. The type of water used in the household appeared to be important with respect to PFAS exposure: when municipal tap water is taken as a reference, consumers of water from private wells had significant higher levels of PFOA and PFDA, but lower levels of PFHxS (Table 3) which follows the general trend of PFHxS exposure being lower in rural than in urban areas (Table A9).

Data from the food frequency questionnaire allowed us to assess the possible influence of fish, beer and wine consumption on PFAS exposure (Tables 3 and A7–A11 in the ~~suplemnent~~Supplement). Fish consumption (including canned fish) showed a positive association with PFHxS, PFOA, PFOS, PFNA and PFDA levels, and the strength of the association increased with consumption frequency irrespective of fish species. Beer and wine consumption also influence PFAS levels. Therefore, regular beer drinkers (1 to 6/week) showed an association with PFOA and PFOS, but not significant with PFHxS ($p = 0.223$). On the other hand, consumers of wine also had a positive association with the levels of PFOA, PFOS, PFNA and PFDA. However, regular red wine consumers (1 to 6 times/week) did not show a significant association with PFNA

(p = 0.135) and PFDA (p = 0.374).

E-component

The following are the supplementary data related to this article.

Multimedia Component 1

Table A1 Descriptive results of serum levels of PFOS for the studied population. Geometric mean and selected percentiles for the whole samples and in groups categorized according to personal and socio-demographic characteristics.

alt-text: Table A1

Multimedia Component 2

Table A2 Descriptive results of serum levels of PFOA for the studied population. Geometric mean and selected percentiles for the whole samples and in groups categorized according to personal and socio-demographic characteristics.

alt-text: Table A2

Multimedia Component 3

Table A3 Descriptive results of serum levels of PFHxS for the studied population. Geometric mean and selected percentiles for the whole samples and in groups categorized according to personal and socio-demographic characteristics.

alt-text: Table A3

Multimedia Component 4

Table A4 Descriptive results of serum levels of PFNA for the studied population. Geometric mean and selected percentiles for the whole samples and in groups categorized according to personal and socio-demographic characteristics.

alt-text: Table A4

Multimedia Component 5

Table A5 Descriptive results of serum levels of PFDA for the studied population. Geometric mean and selected percentiles for the whole samples and in groups categorized according to personal and socio-demographic characteristics.

alt-text: Table A5

Multimedia Component 6

Table A6 Descriptive results of serum levels of *N*-MeFOSAA for the studied population. Geometric mean and selected percentiles for the whole samples and in groups categorized regarding personal and socio-demographic characteristics.

alt-text: Table A6

Multimedia Component 7

Table A7 Results of multiple regression analysis for PFOS to identify additional exposure factors related to lifestyle (adjusted for gender, age and sampling trimester).

alt-text: Table A7

Multimedia Component 8

Table A8 Results of multiple regression analysis for PFOA to identify additional exposure factors related to lifestyle (adjusted for gender, age and sampling trimester).

alt-text: Table A8

Multimedia Component 9

Table A9 Results of multiple regression analysis for PFHxS to identify additional exposure factors related to lifestyle (adjusted for gender, age and sampling trimester).

alt-text: Table A9

Multimedia Component 10

Table A10 Results of multiple regression analysis for PFNA to identify additional exposure factors related to lifestyle (adjusted for gender, age and sampling trimester).

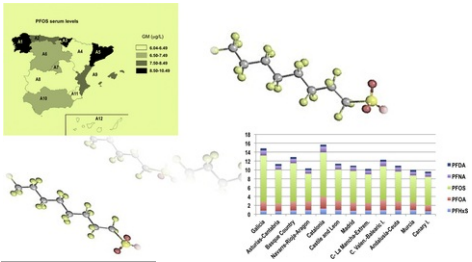
alt-text: Table A10

Multimedia Component 11

Table A11 Results of multiple regression analysis for PFDA to identify additional exposure factors related to lifestyle (adjusted for gender, age and sampling trimester).

alt-text: Table A11

Graphical abstract



alt-text: Image 1

Highlights

- A new information has been reported for PFAS serum levels of Spanish adults.
- Exposure to PFAS was related with sex, age, diet and geographic area.
- Breast feeding decreases PFAS serum levels in women.
- Northeast Spain was the area with the highest exposure to PFAS levels.
- Spanish population has similar exposure levels than their European neighbours.

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