



## Current exposure of Italian women of reproductive age to PFOS and PFOA: A human biomonitoring study



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### HIGHLIGHTS

- Largest human biomonitoring study in women of reproductive age in Italy.
- No differences in PFOS concentrations between urban/industrial and rural areas.
- Levels of PFOA higher in women residing in urban/industrial areas.
- A downward temporal trend in exposure is observed for both compounds.

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### ABSTRACT

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) concentrations were determined in serum samples collected in 2011–2012 from 549 nulliparous Italian women of reproductive age who resided in six different Italian Regions. Assessment of exposure to perfluorinated compounds was part of a large human biomonitoring study (Project Life Plus "Womenbiopop") that aimed at examining the exposure of women of reproductive age to priority organic pollutants.

The median concentrations of PFOS and PFOA were 2.43, and 1.55 ng g<sup>-1</sup>, respectively.

Significant differences in the concentrations of both compounds were observed among the six Regions. Women from central Italy had the highest levels of both compounds, followed by women from northern Italy, and southern Italy. No differences in the PFOS concentrations were found between women from urban/industrial areas and women from rural areas, whereas the levels of PFOA were significantly higher in women residing in urban/industrial areas than in women residing in rural areas.

Taken together, the observed concentrations confirm that the overall exposure of the Italian population is among the lowest observed in industrialized countries.

A downward temporal trend in exposure was observed for both compounds when comparing the results from the present study with those assessed in a study conducted in 2008.

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## 1. Introduction

The two perfluorinated compounds, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), represent the most widely studied members of the large family of perfluoroalkylated substances (PFAS). Because of their excellent performance as surfactants and surface protectors, PFOS and PFOA have been extensively used since the 1950s in a number of applications, including in the surface coatings of cooking pans, in making products water- and oil-repellent, in firefighting foams, and in food packaging. Humans are exposed to PFOS and PFOA principally through their diet (EFSA, 2008a), but ingesting contaminated dust and drinking water may also account for a portion of the exposure (EFSA, 2008a; Wilhelm et al., 2010; Post et al., 2012).

As a consequence of their persistence, bioaccumulation potential, toxicity, and widespread presence in environmental media, biota, and humans, the production and use of these compounds and their precursors have been restricted or banned in several countries. The EU restricted PFOS production and use from 2008 onward (Directive 2006/122/EC of the European Parliament and of the Council). In 2009, PFOS was included in Annex B of the Stockholm Convention on Persistent Organic Pollutants. The toxicity of PFOS and PFOA has been studied quite extensively, and the main toxic effects identified are hepatotoxicity, developmental toxicity, immunotoxicity, and changes in the circulating hormones and lipoproteins (EFSA, 2008b; White et al., 2011; Barouki et al., 2012; DeWitt et al., 2012). Nevertheless, the toxicological profile of these substances is far from being adequately characterized because of the marked differences in toxicokinetics that have been observed among species, which make it difficult to extrapolate the experimental data to humans (Lau, 2012). PFOA is among the substances that are scheduled for IARC evaluation on carcinogenicity this year (IARC, 2008).

PFOS and PFOA were among the persistent organic pollutants (POPs) included in the human biomonitoring project “Womenbiopop – Linking Environment and Health – A Country-based Study on Women of Reproductive Age” (April 2010–May 2013). This project, funded by the financial instrument “Life Plus” (EC, DG Environment) and co-funded by the Istituto Superiore di Sanità (Italian National Institute of Health) and by the Italian Ministry of Environment, Land and Territory, aimed to characterize the exposure of women of reproductive age to a group of priority POPs of environmental origin, including polychlorodibenzodioxins and polychlorodibenzofurans (“dioxins”), polychlorobiphenyls (PCBs), polybrominated diphenylethers (PBDEs), and some organochlorinated pesticides ( $\beta$ -hexachlorocyclohexane, *p,p'*-DDE, hexachlorobenzene), and to explore a possible correlation between exposure and women’s reproductive health.

PFOS and PFOA were included in the group of POPs that were of interest for the project because of the increasing burden of toxicology and epidemiology studies that have shown a possible impact of these substances on female reproductive health. In particular, a number of animal toxicology studies have shown that exposure to PFOS and PFOA can alter ovarian function (Zhai et al., 2012) and affect the development of mammary gland tissue (White et al., 2011). Epidemiologic research, which exists primarily as cross-sectional studies, on the possible effects on female reproductive health has so far produced inconsistent results. Fei et al. (2009) observed an association between high serum levels of PFOS and PFOA and a longer time to pregnancy, but no such association was found in other studies (Vestergaard et al., 2012; Whitworth et al., 2012). PFOS and PFOA pass through the placenta (Needham et al., 2011) and are excreted in milk, although less so than other POPs (Haug et al., 2011; Mannetje et al., 2012). Effects of *in utero* exposure to PFAS on the female reproductive functions have also been reported. In a recent study (Kristensen et al., 2013)

daughters who were exposed *in utero* to levels of PFOA that were higher than the reference group showed a later age of menarche.

In the Project “Womenbiopop”, 549 nulliparous women were enrolled. These women resided in six different Regions that were representative of northern, central, and southern/insular Italy and in areas that are characterized by different levels of anthropogenic activity. The present paper reports the determinations of PFOS and PFOA that were conducted on women’s serum samples.

## 2. Methods

### 2.1. Study design and samples

The regions included in the study were Trentino-Alto Adige and Piemonte (northern Italy); Umbria and Lazio (central Italy); Puglia (southern Italy); and Sicilia (insular Italy).

Areas that had presumed different exposure to POPs were included in all Regions, such as areas with background exposure (rural or mountainous areas with no major industrial settlements) and areas with possible incremental exposure (urban areas and/or areas with industrial settlements). Women were enrolled in the following areas (Fig. 1):

In Trentino Alto-Adige, the town of Trento and two mountainous areas in the Province of Trento (Val di Non and Valsugana, where a steel plant has been operating for the last thirty years).

In Piemonte, the town of Torino and two mountainous areas in the Province of Torino (Val Chisone and Val di Susa, where a steel plant has been operating for decades).

In Umbria, the town of Terni (where a large steel plant has been operating since the end of the 19th century) and country areas in the Province of Terni.

In Lazio, the town of Rome, the town of Latina and country areas in the Province of Latina.

In Puglia, the town of Taranto (where one of the largest steel plants in Europe has been operating since 1960) and country areas in the Province of Taranto.

In Sicilia, the town of Palermo and country areas in the Province of Palermo.

Enrolment was done in the years 2011–2012. Thirty to 50 women were enrolled in each area, for a total of 549 women.

Only nulliparous women (or women who never breastfed) were enrolled, considering that breastfeeding may alter women’s body burden.

The women’s age was in the range 20–40 years. All women had resided in the area for at least 10 years.

The Project was approved by local Ethics Committees. Prior to blood drawing, signed informed consent was acquired from each woman, and a questionnaire was administered by medical doctors or trained nurses.

### 2.2. Questionnaires

The questionnaires contained questions on anthropometric and socio-demographic variables (age, weight, height, educational level, employment status) and questions aimed at characterizing sources of exposure to POPs (dietary habits, and lifestyle factors). A section of the questionnaire focused on woman’s reproductive health and contained specific questions on a range of diseases/dysfunctions (e.g., infertility, repeated miscarriages, endometriosis, thyroid dysfunctions) for which exposure to POPs has been hypothesized to play a role in their etiology.

The dietary variables investigated were the intake of meat (poultry, beef, pork, lamb, ham and salami), fish and seafood, milk and dairy products (milk, yogurt, cheese), eggs, and vegetables (raw and cooked). Because approximately one half of women



Fig. 1. Sampling areas: Trentino-Alto Adige, Piemonte (northern Italy), Umbria, Lazio (central Italy), Puglia (southern Italy) and Sicilia (insular Italy).

resided in rural or mountainous areas, where the consumption of food of either local or their own production could have accounted for a major fraction of the intake of some food categories, the women were asked to specify the source of purchase (gross distribution or retail vs. local or their own production) for the food categories considered.

### 2.3. Analysis

To analyze all of the POPs considered in the study, approximately 50 mL of blood was drawn from each woman and centrifuged to obtain serum. An aliquot of approximately 250  $\mu\text{L}$  was taken from each serum sample to analyze PFOS and PFOA. The aliquot was fortified with  $^{13}\text{C}_4$ -labelled PFOS and PFOA (Wellington Laboratories Inc., Guelph, Ontario, Canada, purity grade >99%, 1.150  $\mu\text{g ml}^{-1}$  of each compound), and allowed to rest overnight at 4  $^\circ\text{C}$ . Extraction with acetonitrile (Sigma–Aldrich Corp, Saint Louis, MO, USA) was performed in a centrifuge tube. After centrifugation and separation of the two phases, the volume of acetonitrile was reduced and transferred to an autosampler vial to undergo instrumental analysis (Inoue et al., 2004; Ingelido et al., 2010). Instrumental analysis was carried out by HPLC (Waters Alliance 2695, Waters Corporation, Milford, MA, USA) interfaced with a triple quadrupole mass spectrometer (Micromass QuattroMicroTM

API, Waters Corporation, Milford, MA, USA) operated in the electrospray negative mode. The working conditions were as follows: Desolvation gas flow, 600  $\text{L h}^{-1}$ ; Cone gas flow, 25  $\text{L h}^{-1}$ ; Source temperature, 120  $^\circ\text{C}$ ; Desolvation temperature, 450  $^\circ\text{C}$ ; Capillary voltage, 1 kV. The direct injection volume was 30  $\mu\text{L}$ . Data were acquired using multiple reaction monitoring (MRM). Mass transitions for  $^{12}\text{C}$  PFOA were set at 412 > 369 and 412 > 169, and those for  $^{13}\text{C}$  PFOA were set at 417 > 372. Mass transitions for  $^{12}\text{C}$  PFOS were set at 499 > 99 and 499 > 80, and those for  $^{13}\text{C}$  PFOS were set at 503 > 99. The isotope dilution technique was applied throughout the analyses. The recovery ranges were 80–110% for the  $^{13}\text{C}$ -labelled internal standards. The limits of quantification for PFOS and PFOA were 0.05  $\text{ng g}^{-1}$  and 0.1  $\text{ng g}^{-1}$ , respectively. The concentrations of PFOS were above the limit of quantitation (LOQ) in all samples, and those of PFOA were above LOQ in 98% of samples.

All analyses were conducted by the laboratory of Toxicological Chemistry. The laboratory is accredited for the analysis of POPs according to ISO/IEC 17025. Since 2011, it has participated twice a year in the intercomparison exercise “AMAP Ring Test for Persistent Organic Pollutants in Human Serum,” which is organized by the Institut National de Santé Publique du Québec, Centre de Toxicologie du Québec (Canada), and has always met the performance acceptability criteria for PFOS and PFOA.

## 2.4. Statistical analysis

Non-parametric Kruskal–Wallis and Mann–Whitney U tests and the Spearman correlation were used to investigate the statistical significance of the differences in the PFOS and PFOA serum concentrations between the groups and the possible correlations between PFOS and PFOA concentrations and variables from the questionnaires.

Information from questionnaires was incomplete for 70 women. As a consequence, the statistical analysis was performed on 479 of the 549 women enrolled.

## 3. Results

Anthropometric variables and socio-demographic characteristics of women enrolled, including the age distribution, body mass index (BMI), educational level, employment status, and residence, are shown in Table 1. The mean age was 27.7 years. Most women (64%) were less than 30 years old, which was a consequence of the difficulty in finding women who had never breastfed in the upper age class (30–41 years). Most subjects (71%) had a normal BMI, with almost the same percentage (11 and 13, respectively) of women being under or over weight, and a small percent (4) were obese. Participants were well distributed according to their place of residence (56% from urban and industrial areas and 44% from rural areas). Most women (93%) had a medium to high educational level and were employed (66%).

Table 2 summarizes the PFOS and PFOA concentrations stratified by age and by region. The data distributions were not normal and were skewed to the right for both compounds. The PFOS and PFOA median concentrations were 2.43 and 1.55 ng g<sup>-1</sup>, respectively. The P95 and maximum values were, respectively, 6.68 and 58.9 ng g<sup>-1</sup> for PFOS and 3.46 and 9.99 ng g<sup>-1</sup> for PFOA. The median serum concentrations of both compounds were lowest in Puglia and highest in Umbria.

The women who had the highest concentrations of PFOS resided in Piemonte (58.9 and 25.4 ng g<sup>-1</sup>) and in Umbria (14.9 ng g<sup>-1</sup>). The highest PFOA values were observed in two women residing in Umbria (9.99 and 7.99 ng g<sup>-1</sup>) and one in Piemonte (7.36 ng g<sup>-1</sup>). Significant differences in concentration were observed between regions for PFOS and PFOA. In particular, women

**Table 1**  
Anthropometric and socio-demographic characteristics of study participants.

	Mean (std dev)	N (%)
Age (years)	27.7 (5.6)	
20–29		307 (64)
30–41		172 (36)
Body mass index (kg m <sup>-2</sup> )	22.2 (3.5)	
Underweight (BMI < 18.5)		55 (11)
Normal (18.5 < BMI < 25)		342 (71)
Overweight (25 < BMI < 30)		64 (13)
Obese (BMI ≥ 30)		18 (4)
Residence		
Urban or Industrial		267 (56)
Rural		212 (44)
Educational level		
Primary school		1 (0)
Secondary school (1st stage)		34 (7)
Secondary school (2nd stage)		248 (52)
University		195 (41)
Employment status		
Employed		311 (66)
Housewife		3 (1)
Student		134 (28)
Unemployed		26 (5)

from Umbria had significantly higher concentration of PFOS than did women from Trentino Alto Adige, Piemonte, Puglia, and Sicilia (Kruskall–Wallis Test,  $p \ll 0.0001$ ). Women from Umbria had a significantly higher serum concentration of PFOA than did women from Piemonte, Lazio, Puglia, and Sicilia ( $p \ll 0.0001$ ).

Though the PFOS concentrations did not significantly differ among areas of different typology, the PFOA concentrations were significantly higher in women residing in urban and industrial areas than in women from rural areas ( $p = 0.017$ ). The concentrations of the two compounds were strongly correlated ( $p \ll 0.0001$ ).

A statistically significant ( $p < 0.05$ ) correlation was found between the concentrations of PFOS and PFOA and some of the variables from the questionnaires. In particular, a direct correlation with age was observed for PFOS ( $p \ll 0.0001$ ), but not for PFOA.

Additionally, women who had the highest educational level (university degree) showed significantly higher levels of PFOA than did women in the other education levels ( $p < 0.05$ ). The same tendency was also observed for PFOS.

The contaminant concentrations did not differ significantly among the four categories of employment status, but students and employed subjects had higher serum levels of both compounds than did housewives and unemployed women; this difference was only marginally significant ( $p < 0.1$ ) for PFOS. However, this result might have been affected by the low number of housewives and unemployed subjects ( $N = 29$ ).

For each participant, the daily intake of specific food items was calculated based on the reported food consumption frequencies. The consumption figures derived from the questionnaires were in good agreement with figures reported by European Food Safety Authority (EFSA) for Italy for the same population group for the food categories considered (EFSA, 2008b). The results of the correlation analysis between the PFOS and PFOA serum concentrations and the consumption of selected food items are reported in Table 3, together with the results of the Mann Whitney U test applied to PFOS and PFOA concentrations in consumers of locally produced food vs. consumers of food obtained from gross distribution or retail. The PFOS levels were significantly correlated with the consumption of beef ( $p \ll 0.01$ ), pork ( $p \ll 0.01$ ), fish ( $p = 0.05$ ), and vegetables ( $p \ll 0.01$ ). As for PFOA, no statistically significant correlation was found with any of the food categories considered, except for a weak inverse correlation with chicken and ham consumption. The concentrations of both compounds were significantly higher ( $p < 0.05$ ) in women who consumed beef, pork, lamb, ham and salami, fish and seafood from food retailers, whereas those of PFOA were higher ( $p \leq 0.04$ ) in women who consumed cheese, eggs, liver and vegetables from food retailers.

## 4. Discussion

This study represents the widest human biomonitoring study on PFOS and PFOA that has been carried out in Italy so far and, to the best of our knowledge, one of the widest carried out on women of reproductive age in the EU countries. To characterize exposure as a function of different geographical latitudes and environmental situations, the study design was based on a bifocal/trifocal approach. Consequently, in each of the six Regions considered, women residing in rural and/or mountainous areas and women residing in areas at a higher level of anthropic activity (urban and/or industrial areas) were included in the study.

The results show that a relevant exposure variability to both compounds exists among the Regions. Women from the Regions in southern Italy had the lowest ( $p \ll 0.0001$ ) levels of both PFOS and PFOA, followed by women from northern and central Italy. The Umbria Region seemed to be particularly affected by these contaminants. Although the reasons for the observed differences

**Table 2**Descriptive statistics of PFOS and PFOA concentrations (ng g<sup>-1</sup>) in serum samples stratified by age and Region of residence.

	N	Minimum	P5	P25	Median	Mean	P75	P95	Maximum
<i>PFOS</i>									
All data	479	0.34	0.95	1.73	2.43	3.06	3.45	6.68	58.9
Age 20–29	307	0.34	0.904	1.70	2.31	2.92	3.11	6.04	58.9
Age 30–40	172	0.61	1.02	1.77	2.72	3.31	4.02	6.73	25.4
Lazio	61	0.97	1.49	1.94	2.56	3.23	3.49	7.78 <sup>a</sup>	14.1
Piemonte	104	0.56	0.81	1.54	2.42	3.68	3.62	7.09 <sup>a</sup>	58.9
Puglia	85	0.34	0.74	1.34	1.71	1.96	2.56	3.45 <sup>a</sup>	5.50
Sicilia	39	0.53	0.57	1.72	2.36	2.95	3.77	5.95 <sup>a</sup>	12.3
Trentino	90	1.03	1.30	1.78	2.18	2.41	2.75	4.42 <sup>a</sup>	7.06
Umbria	100	0.51	1.40	2.59	3.43	3.87	4.69	6.99 <sup>a</sup>	14.9
<i>PFOA</i>									
All data	470	<0.1	0.300	1.04	1.55	1.70	2.04	3.46	9.99
Age 20–29	300	<0.1	0.310	1.06	1.54	1.66	2.01	3.20	9.99
Age 30–40	170	0.12	0.28	0.97	1.59	1.76	2.14	4.00	7.36
Lazio	61	0.20	0.23	0.41	1.52	1.47	2.17	3.23 <sup>a</sup>	3.91
Piemonte	95	<0.1	0.16	1.03	1.44	1.57	1.78	3.40 <sup>a</sup>	7.36
Puglia	85	0.21	0.37	0.75	1.05	1.23	1.60	2.42 <sup>a</sup>	3.72
Sicilia	39	0.45	0.46	0.88	1.32	1.49	1.84	3.17 <sup>a</sup>	5.22
Trentino	90	0.59	1.08	1.37	1.71	1.73	1.94	2.82 <sup>a</sup>	4.05
Umbria	100	0.33	0.54	1.49	2.04	2.41	2.81	5.57 <sup>a</sup>	9.99

<sup>a</sup> P95 calculated on a number of subjects  $N < 160$  only provide a rough indication of high levels of serum concentrations EFSA (2008b).

**Table 3**Correlation between PFOS and PFOA serum concentrations and consumption of selected food items and differences between concentrations in commercial vs. local food consumers. The values highlighted in bold are statistically significant ( $p < 0.05$ ).

	Number of consumers <i>N</i>	Percentage of consumers of local food (%)	Spearman correlation		Mann–Whitney test <sup>a</sup>	
			PFOS	PFOA	PFOS	PFOA
			<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>
Milk and dairy products	447	19	0.78	0.74	0.49	<b>0.01</b>
Milk	372	3	0.37	0.33	– <sup>b</sup>	–
Yogurt	317	1	0.19	0.16	–	–
Cheese	355	22	0.13	0.25	0.27	<b>0.01</b>
Eggs	396	37	0.59	0.20	0.30	<b>0.01</b>
Meat and meat products	454	27	<b>&lt;&lt;0.01<sup>c</sup></b>	0.84	0.99	0.12
Chicken	423	24	0.71	<b>0.04<sup>d</sup></b>	0.74	0.18
Beef	365	19	<b>&lt;&lt;0.01<sup>c</sup></b>	0.24	<b>&lt;0.01</b>	<b>&lt;&lt;0.01</b>
Liver	68	19	0.71	0.18	0.21	<b>0.04</b>
Pork	317	19	<b>&lt;&lt;0.01<sup>c</sup></b>	0.42	<b>0.02</b>	<b>0.03</b>
Lamb	84	38	0.27	0.47	<b>&lt;&lt;0.01</b>	<b>&lt;&lt;0.01</b>
Ham, salami	390	12	0.40	<b>0.04<sup>d</sup></b>	<b>0.04</b>	<b>0.03</b>
Fish and seafood	422	19	0.15	0.86	<b>&lt;&lt;0.01</b>	<b>&lt;&lt;0.01</b>
Fish	415	19	<b>0.05<sup>c</sup></b>	0.70	<b>&lt;&lt;0.01</b>	<b>&lt;&lt;0.01</b>
Seafood	188	24	0.74	0.35	<b>&lt;&lt;0.01</b>	<b>0.02</b>
Raw and/or cooked vegetables	462	46	<b>&lt;&lt;0.01<sup>c</sup></b>	0.34	0.13	<b>&lt;&lt;0.01</b>
Cooked vegetables	445	41	<b>&lt;&lt;0.01<sup>c</sup></b>	0.48	0.08	<b>&lt;&lt;0.01</b>
Raw vegetables	449	47	<b>0.01<sup>c</sup></b>	0.25	0.14	<b>&lt;&lt;0.01</b>

<sup>a</sup> For all significant ( $p < 0.05$ ) differences serum concentrations are higher in commercial food consumers.

<sup>b</sup> Mann–Whitney test was not applied when the percentage of consumers of local food was <10%.

<sup>c</sup> Direct correlation.

<sup>d</sup> Inverse correlation.

are difficult to identify, they are probably partly associated with differences in dietary habits and lifestyle.

Diet could play a particular role in PFOS exposure, as suggested by the significant correlation with meat and fish consumption. People from northern and central Italy traditionally have a diet that is richer in meat and meat products than do people from the south (Piccinelli et al., 2011), which was also confirmed by the questionnaires. Additionally, women residing in the northern Regions might be exposed to indoor dust containing higher levels of PFAS because of the greater use of treated carpets and textiles in colder regions (Kubwabo et al., 2005; Gewurtz et al., 2009).

Women residing in urban areas appear to be exposed to higher levels of PFOA. A comparatively higher exposure to PFOA was also observed for women with a high degree of education, who were more represented in urban areas in this study. Such an association between the higher levels of PFOA and a higher educational level has also been observed in other studies (Calafat et al., 2007; Melzer et al., 2010). These findings are not imputable to known variables, but lifestyle factors might play a role; particularly different dietary habits (i.e., a higher rate of consumption of food obtained from the retail level and of packaged fast-food) and more prolonged exposure to indoor environments, which are known to

represent an important exposure pathway to PFAS, particularly to PFOA (Björklund et al., 2009; Haug et al., 2011).

Regarding the role of food consumption patterns, a significant correlation was detected between the PFOS concentrations and the intake of some meat products, fish and vegetables. These findings were, overall, consistent with the EFSA opinion (EFSA, 2012), which identified fish and meat as major contributors to dietary exposure to PFOS. Analysis of the association between concentrations of PFOS and PFOA and consumption of foodstuffs from the retail level or from local or their own production showed that retail food contributes to exposure more consistently than does locally produced food for most of the categories considered.

As already observed in a study conducted in 2007–2009 (Ingelido et al., 2010), the PFOS and PFOA serum concentrations in Italy are among the lowest recently observed in Europe (Kärman et al., 2013). The reasons for this are unknown. Dietary intake is unanimously recognized as the major exposure pathway for PFAS. However, other factors, mainly dust ingestion, also contribute to overall exposure and account for a fraction that may be highly variable; they range from a few percentage units to approximately 40% in the worst case (Björklund et al., 2009). We hypothesize that the generally limited use of carpets in Italy might play a role, as suggested by some studies that showed a positive correlation between the percentage of home carpeting and PFAS internal dose (Kubwabo et al., 2005; Gewurtz et al., 2009), which was also confirmed by studies carried out in other Mediterranean

countries (Ericson Jogsten et al., 2012), which, like Italy, have limited or no use of fitted carpets.

The serum concentrations assessed in the present study were compared with the concentrations observed in a study carried out in 2007–2009 (Ingelido et al., 2010) in 230 subjects (males and females, ages 20–65) residing in Rome and Brescia. The PFOS and PFOA levels appear to have significantly declined (Mann–Whitney  $U$  test,  $p \ll 0.001$ ), and the median values decreased from 6.31 to 2.43  $\text{ng g}^{-1}$  for PFOS and from 3.59 to 1.55  $\text{ng g}^{-1}$  for PFOA. For a better comparison that which was unaffected by differences in the subjects' age and sex, we selected two subgroups of subjects from the 2010 database. The first subgroup included men and women of the same age range as the present study (61 subject, 20–41 years), and the second included only a group of 24 women, whose characteristics were perfectly comparable to those of the women enrolled in the present study with respect to nulliparity and age. A comparison of the observed median concentrations in this study with those assessed for such subgroups showed a decline in the PFOS and PFOA concentrations of approximately 50% (Fig. 2).

A decline in PFOS serum concentrations over the last decade has been observed in other countries, including Germany (Schröter-Kermani et al., 2013), the U.S. (Olsen et al., 2012), Norway (Haug et al., 2009) and Sweden (Glynn et al., 2012). Further, a decrease in exposure to PFOA has been observed, although such a decrease is, on average, less marked with respect to PFOS (Glynn et al., 2012).

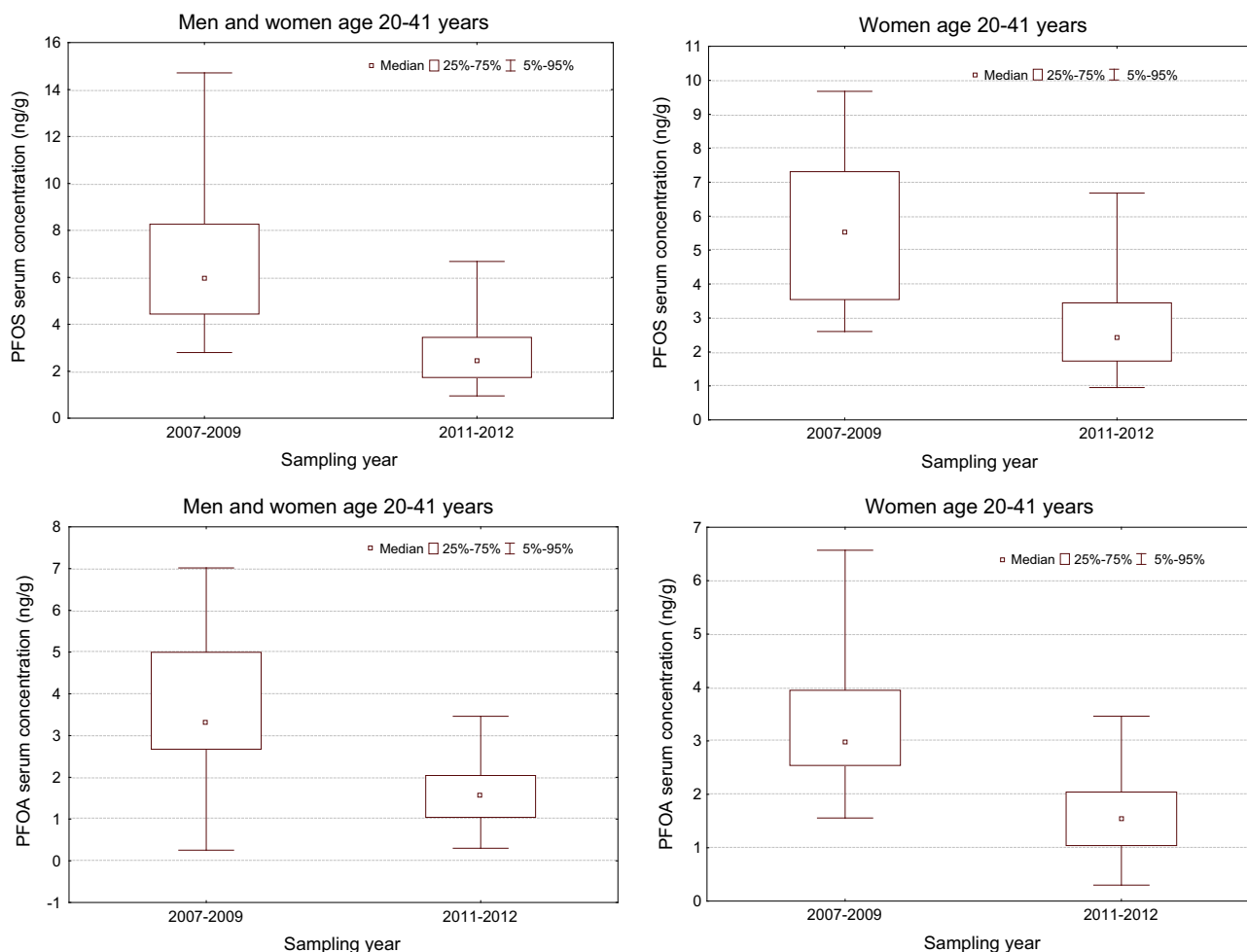


Fig. 2. Time trend of PFOS and PFOA serum concentrations ( $\text{ng g}^{-1}$ ) in men and women (left) and women only (right).

Though the decline observed for PFOS is attributable to the production of this compound ceasing in Europe and North America, the less pronounced decline in exposure to PFOA could be due to its ongoing production and the use of PFOA and its precursors (Glynn et al., 2012).

It is worth noting that the decrease in serum concentrations observed for Italy is significantly sharper than generally observed for both compounds in other studies, most notably for PFOA.

The distributions of the observed PFOS and PFOA concentrations were markedly skewed to the right; the highest values were 10–25-fold higher than the median for PFOS, and 5–6-fold higher than the median for PFOA. Such exposure variability, together with evidence of placental transfer (approximately 30–80% for PFOS, and 70–150% for PFOA, Fromme et al., 2010; Needham et al., 2011) as well as the observation that in a population residing in a polluted area (Mondal et al., 2012) both compounds were shown to accumulate in children via their mothers, indicates the need to continue monitoring exposure to PFAS in women of reproductive age.

This holds particularly true in the light of some recent toxicological findings (Grandjean and Budtz-Jørgensen, 2013) that showed that immunotoxicity in children might be associated with below average serum concentrations of PFOS and PFOA in women who were of reproductive age in recent population studies (Brede et al., 2010; Kärrman et al., 2013; Axmon et al., 2014; Berg et al., 2014) and below the median concentration values assessed in the present study.

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