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Perfluorinated alkylated substances serum concentration and breast cancer risk: Evidence from a nested case-control study in the French E3N cohort.

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Novelty & Impact Statements

- Serum concentrations of PFOS are linearly associated to increased risk of hormone-receptor-positive breast cancer tumors in nested case-control study including non-occupationally exposed postmenopausal French women.
- An increased risk of developing ER- and PR- tumors in postmenopausal women is associated to middle-low serum concentrations of PFOA and PFOS.
- Exposure to endocrine disrupting chemicals, such as PFOS and PFOA, should be considered as a potential risk factor for breast cancer, thus a serious public health issue.

Abstract

Endocrine-disrupting chemicals are proposedto increase breast cancer (BC) incidence. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), two perfluorinated alkylated substances (PFASs), are suspected to be ubiquitously present in the blood of human population worldwide. We investigated the associations between serum concentrations of these substances and BC risk.

E3N is a cohort of 98,995 French women born in 1925-1950 and followed-up since 1990. We sampled 194 BC cases and 194 controls from women with available blood samples. Serum concentrations of PFASs were measured by LC-MS/MS. Adjusted conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs). All statistical tests were two-sided.

While PFASs concentrations were not associated with BC risk overall, we found positively linear associations between PFOS concentrations and the risk of ER+ (3^{rd} quartile: OR=2.22 (CI=1.05-4.69); 4^{th} quartile: OR=2.33 (1.11-4.90); P_{trend}=0.04) and PR+ tumors (3^{rd} quartile: OR=2.47 (CI=1.07-5.65); 4^{th} quartile: OR=2.76 (CI=1.21-6.30); P_{trend}=0.02). When considering receptor-negative tumors, only the 2^{nd} quartile of PFOS was associated with risk (ER-: OR=15.40 (CI=1.84-129.19); PR-: OR=3.47 (CI=1.29-9.15)). While there was no association between PFOA and receptor-positive BC risk, the 2^{nd} quartile of PFOA was positively associated with the risk of receptor-negative tumors (ER-: OR=7.73 (CI=1.46-41.08); PR-: OR=3.44 (CI=1.30-9.10)).

Circulating levels PFASs circulating levels were differentially associated with BC risk. While PFOS concentration was linearly associated with receptor-positive tumors, only low concentrations of PFOS and PFOA were associated with receptor-negative tumors. Our findings highlight the importance of considering exposure to PFASs as a potential risk factor for BC.

Keywords

Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), breast cancer, serum levels, nested case-control study, E3N cohort

List of abbreviations

Breast cancer (BC), Endocrine-disrupting chemicals (EDCs), Perfluorinated alkylated substances (PFASs), Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA), Etude Epidémiologique auprès de femmes de l'Education Nationale (E3N), Body mass index (BMI), Phospholipids (PL), Triglycerides (TG), Total cholesterol (TC), Free cholesterol (FC), Total serum lipids (TSL), Odds ratios (ORs), 95% confidence intervals (CIs), Estrogen receptors (ER), Progesterone receptors (PR)

Background

The incidence of breast cancer (BC) has risen in the past decades among Western populations. Despite a large body of research, the etiology of BC has not been fully delineated (1, 2), as established risk factors (reproductive and lifestyle factors) cannot solely explain the unfavorable trends. There is increasing interest in understanding the contribution of environmental chemicals to the increase in BC incidence. Endocrine-disrupting chemicals (EDCs) are largely widespread in our environment and the health effects of EDC exposure in the general population are a growing concern (3). The roles of EDCs in BC pathogenesis are currently unclear, however several authors proposed that the physiological imbalance generated by EDC can alter normal patterns of tissue organization and hence interfere with stromal-epithelial interactions, altering important regulatory mechanisms and enhance the potential for neoplastic lesions. The mammary gland has been identified as an especially sensitive tissue to the action of EDCs due to its hormone-sensitivity and the complex process of differentiation, extended to the late pregnancy (4). On this regard, a list of EDCs has exhibited strong effects on the development of mammary glands, prompting the hypothesis of potential effects on BC pathogenesis (5). It has also been suggested that several EDCs lead to epigenetic changes that can in turn induce altered gene function and malignant cellular transformation (6). However, despite the biological plausibility of a strong influence of endogenous estrogens and androgens on BC, the potential involvement of EDCs in the development of BC remains to be confirmed (7).

Perfluorinated alkylated substances (PFASs) constitute a family of environmental EDCs, for which the long-term health effects are uncharacterized in terms of their relation to BC risk. PFASs include a wide group of synthetic compounds, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), that are water- and oil-repellent and are able to reduce surface tension. Due to these characteristics, PFASs have gained an important commercial value, which resulted in their use in a large number of industrial and consumer applications. The dramatic amount of PFASs produced in the last 60 years, combined with their high resistance to biodegradation, resulted in their global dissemination in the environment (8). Due to results

obtained by human biomonitoring studies revealed that PFOA and PFOS are suspected to be ubiquitously present in the blood of human population worldwide (9, 10). Given the widespread environmental distribution of these compounds, it is essential to gain deep knowledge on their long-term health effects in order to provide the necessary scientific evidence for regulating these substances.

Our objective was thus to investigate the associations between serum concentrations of PFOS and PFOA and the risk of BC in women.

Methods

The E3N study

E3N (*Etude Epidémiologique auprès de femmes de l'Education Nationale*) is a prospective cohort study involving 98,995 French women, aged 40-65 years at inclusion in 1990 and insured by a national health insurance covering workers from the French National Education System (*Mutuelle Générale de l'Education Nationale*, MGEN) (11). Participants were enrolled after completing a baseline self-administered questionnaire along with informed consent. Follow-up questionnaires were sent every 2-3 years thereafter. Detailed cancer risk factor data were collected through questionnaires at different time points during follow-up, including reproductive history, use of hormonal treatments, anthropometric characteristics, smoking habit, alcohol consumption, diet, and physical activity. The average follow-up rate per questionnaire cycle has been of 83%, and to date, the total loss to follow-up since 1990 has been <3%. The study was approved by the French National Commission for Data Protection and Privacy.

Between 1994 and 1999, E3N participants were invited to donate blood, resulting in the collection of blood samples from approximately 25,000 participants. Each sample was separated into 28 aliquots (i.e. plasma, serum, buffy-coat, leukocytes, and erythrocytes) that were stored in plastic straws in liquid nitrogen containers (−196°C) in a biobank.

BC cases were identified through self-reports in the questionnaires, from the MGEN files, or through information from death certificates. Deaths were reported by family members and by searches in the MGEN

files, and causes of death were obtained from the National Death Index. Pathology reports were obtained for 93% of incident cases. We also considered cases for which pathology reports have not been obtained, because the proportion of false-positive self-reports was low in our study population (<5%). Cases were identified up to 2013, which was therefore used as the end of the follow-up in statistical analyses.

The nested case-control study on breast cancer

Among the E3N population, we identified 281 BC cases for which at least 3 aliquots of serum were available in the biobank. From these, we excluded all cases who had not completed the dietary questionnaire in 1993 (n=27) or who were diagnosed before the blood sampling and/or before the dietary questionnaire (n=11). Cases of Paget's disease and benign breast disease were also excluded (n=3). Finally, 240 incident BC cases were available. Due to budget constraints, among those, 194 incident postmenopausal BC cases were randomly selected and included in the study.

For each case, one control was sampled from women who were free of BC at the time of diagnosis of the corresponding case (density sampling method). Controls were matched to cases by age (±2 years), menopausal status at blood collection (premenopausal or postmenopausal), body mass index (BMI) at blood collection (<25 or ≥25 kg/m^2), and year of blood collection.

The flowchart summarizing the inclusion process applied to the population of the present study is presented in figure 1.

Measurement of biomarkers of exposure

The measurement of PFOA and PFOS concentration was based on fully validated (2002/657/CE decision) and accredited methods (ISO 17025 standard). The methodology included a preliminary alkaline digestion followed by a two-stage Solid Phase Extraction purification using polymeric Oasis® HLB and graphitized carbon (ENVI-Carb®) cartridges, before liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) measurement. The quantification was achieved according to the isotopic dilution

method (i.e. using 13C labeled analogous as internal standards).

The lipid content was determined with enzymatic kits (Biolabo,Maizy, France) independently for phospholipids (PL), triglycerides (TG), total cholesterol (TC) and free cholesterol (FC). Total serum lipids (TSL) were estimated using the formula: TSL=1.677*(TC-FC)+FC+TG+PL (12).

Statistical analysis

Women were divided into quartile groups based on PFOS and PFOA serum concentrations in controls, separately for each substance. Chi-squared tests were performed to evaluate the distributions of PFOS and PFOA according to the main variables of interest. Conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of BC risk in relation to PFOS and PFOA concentration levels.

For all adjustment variables, and for each case-control pair, we considered the value collected in the last questionnaire available before the date of diagnosis in cases. For both PFOA and PFOS, we fitted an unadjusted model (Model 0) as well as three adjusted models with an increasing number of covariates. Model 1 included blood lipids (mg/mL), BMI (continuous), smoking status (smoker vs. nonsmoker), physical activity (metabolic equivalent tasks (MET)-hour/week, continuous), education level (≤14 vs. >14 years of education), personal history of benign breast disease (yes vs. no), and family history of BC (yes vs. no). Model 2 further adjusted for parity and age at first full-term pregnancy (no children, 1 or 2 children and <25 years old at delivery, ≥3 children and <25 years old at delivery, ≥ 25 years old at delivery), total breastfeeding duration (never, ≤6 or >6 months), age at menarche (continuous), age at menopause (continuous), current use of menopausal hormone therapy (yes vs.no), and use of oral contraceptives (ever vs. never). Finally, Model 3 was further adjusted for adherence scores to the Healthy and the Western dietary patterns (above the median vs. below the median), both derived using principal component analysis, as previously described (13). The selection of confounders was done a priori, based upon the known risk factors of BK available in our dataset, and potentially associated to PFAS exposure.

Using multinomial regression, we performed stratified analyses based on the BC expression of estrogen (ER– vs. ER+) and progesterone (PR– vs. PR+) receptors including all variables used in Model 3. Since multinomial regression models do not consider the matching, we additionally adjusted the multinomial models for all matching criteria: age at blood draw (continuous), BMI at blood draw (continuous), menopausal status at blood draw (premenopausal vs postmenopausal), and year of blood draw (continuous). All subjects for whom the information on hormone receptors was not available were grouped as one "missing" category and inserted in the model.

For all models, tests for linear trend were conducted by assigning the median value to each quartile group and modeling these values as continuous variables. In our study population, missing values were <5% for all variables and were imputed to the median (continuous variables) or modal value (categorical variables).

Statistical analyses were performed using SAS software (version 9.3, SAS Institute). The threshold for statistical significance was set at 5 %. All statistical tests were two-sided.

Results

PFOS and PFOA were detected in all samples and the overall median (min-max) serum concentrations in our study population were 17.51 ng/mL (5.83-85.26 ng/mL) and 6.64 ng/mL (1.29-21.39 ng/mL), respectively. In BC cases, these numbers were of 17.62 ng/mL (5.84-85.29 ng/mL) for PFOS, and of 6.39 ng/mL (2.14-21.39 ng/mL) for PFOA concentrations, while in controls they were of 17.32 ng/mL (6.61-59.12 ng/mL) and 6.78 ng/mL (1.29-17.69 ng/mL), respectively. The population's general characteristics according to the distribution of PFOS and PFOA concentrations in controls are described in **Tables 1 and 2**.

Mean age at diagnosis was 68.8 years (range 58.3-84.9 years). Information on tumor hormone receptor expression was available in 158 cases (77%) for ER, and in 155 cases (80%) for PR. In total, 132 tumors were ER+ and 98 were PR+.

PFOS and breast cancer risk

In the unadjusted model, there was an increased risk of BC only in the 2nd quartile group of PFOS concentration when compared to the 1st quartile group (OR 1.80, 95% CI 1.01-3.21), while we observed no association between serum concentrations of PFOS and BC risk in Model 1 (**Table 3**). In Model 2, PFOS concentration was positively associated with BC risk, with ORs (95% CI) of 1.93 (1.01-3.70), and 1.96 (1.00-3.84), for the 2nd and 3rd quartile groups, respectively, while there was no association in the 4th quartile group when compared with the lowest (P_{trend}=0.26). We observed comparable results when adding dietary variables: ORs (95% CI) of 1.94 (1.00-3.78), and 2.03 (1.02-4.04), for the 2nd and 3rd quartile groups, respectively (Model 3).

When considering hormone receptor status, we found a positive and linear association between PFOS concentrations and risk of ER+ tumors (3rd quartile: OR 2.22, 95% CI 1.05-4.69; 4th quartile: OR 2.33, 95% CI 1.11-4.90; P_{trend}=0.04) (**Table 4**). In contrast, only the 2nd quartile group was associated with an increased risk of ER- tumors (OR 15.40, 95% CI 1.84-129.19). We observed similar results when stratifying according to PR expression: we found a positive and linear association between PFOS concentrations and risk of PR+ tumors, with ORs (95% CI) of 2.47 (1.07-5.65) for the 3rd quartile and of 2.76 (1.21-6.30) for the 4th quartile groups (P_{trend}=0.01), but only the 2nd quartile was positively associated with risk of PR- tumors (ORs 3.47, 95% CI 1.29-9.15).

PFOA and breast cancer risk

In the unadjusted model and in Model 1, women in the 2nd quartile group of PFOA concentrations were at increased risk of BC when compared with those in the 1st quintile group (**Table 5**). In contrast, in Model 2 and Model 3, we found no association between PFOA concentration and BC risk. Once stratified according to hormonal receptors, we observed no association between PFOA concentrations and BC risk when considering ER+ or PR+ tumors (**Table 6**). However, we found higher risks of ER- (OR 7.73, 95% CI 1.46-41.08) and PR- tumors (OR 3.44, 95% CI 1.30-9.10) in the 2nd quartile group of PFOA level only.

Discussion

The results of this nested case-control study including non-occupationally exposed postmenopausal French women suggest a linear dose-response relationship between serum concentrations of PFOS and the risk of developing hormone receptor-positive BC tumors. We also observe an association between middle-low doses of PFOA and PFOS and the risk of developing ER- and PR- tumors in postmenopausal women.

The serum concentrations measured in our study population collected between 1994 and 1999 ranged from 5.8 to 85.3 ng/mL for PFOS, and from 1.3 to 21.4 ng/mL for PFOA, which fits within the range of exposure levels in Europe: a review reported that serum and plasma sampled between 1997 and 2006 exhibited PFASs concentrations ranging from 1 to 116 ng/mL for PFOS, and from 0.5 to 40 ng/mL for PFOA in Europe (14).

Few other epidemiological studies have been conducted on the association between PFOS and PFOA and BC risk, leading to inconsistent results.

A case-control study including 31 BC cases and 115 controls, selected from an Inuit population in Greenland, found that BC risk was positively associated with PFOS serum concentrations (15), although no stratification according to hormone receptor status was performed. All cases and 31 controls from this study were then included in a larger case-control study which involved overall 97 BC cases and 93 controls from the Inuit population (16). This second study found a positive association between both PFOS and PFOA serum levels and BC risk, with no difference between ER- and ER+ cases. Although pointing towards the same results, when comparing these studies with our, some differences in the study design and in the study population have to be considered. For the Inuit studies the blood samples were collected after diagnosis and this could potentially affect PFOS and PFAS internal levels as a consequence of hormonal or other physiologic changes associated with the disease. Moreover, it has been indicated that susceptibility to environmental contaminants differs between different ethnic groups, and indeed the Inuit population is known

to present some different genetic polymorphisms in comparison to European populations. These differences could be responsible of a specific susceptibility of the Inuit population to PFAS exposure (17).

No association was found between PFOS or PFOA serum concentrations and BC risk neither in a case-control study nested in the Danish National Birth Cohort, nor in the case-control study nested in the California Teachers Study (18, 19). Concerning the case-control study nested in the Danish National Birth Cohort (based on 250 cases and 233 controls), it should be noted that there are two main differences between this study and ours: first, the Danish study was conducted in a younger population for whom the average age at BC diagnosis was 40.8 years, while in our study the average age at diagnosis was 68.4 years. Although tumor receptor status was not reported in the Danish study, a younger age at BC diagnosis is associated with a lower prevalence of ER+ tumors and a higher proportion of ER–/PR– tumors (20). Thus, it is probable that within the Danish National Birth Cohort there was a higher proportion of ER–/PR– tumors. Second, higher serum concentrations were reported for PFOS (mean: 30.6 ng/mL) in the Danish study than in our population. These two differences may explain the inconsistency with our findings. Indeed, our results highlighted a positive association between high concentrations of PFOS and BC risk only when the analyses were restricted to ER+/PR+ tumors. On the opposite, the PFOS was associated to an increased risk of ER-/PR- tumors only for low concentrations (13.6-17.3 ng/mL), which were lower than those measured in the group used as reference in the Danish study.

With regard to the case-control study nested in the California Teachers Study (based on 902 cases and 858 controls), the main difference compared to our study, is that cases' blood sampling was performed post-diagnosis and pot-treatment. As for the Inuit studies, the reverse causation bias cannot be fully ruled out due to the fact that that PFOS and PFAS internal levels could have be affected both by the onset and treatment of breast cancer (19).

Few animal and *in vitro* studies are available on the effects of PFOS and PFOA on the mammary gland. In order to assess the potential chronic toxicity and tumorigenicity of PFOA, Butenhoff et al. (2007)

conducted a 2-year dietary study on rats demonstrating that gestational exposure to this compound was associated with altered mammary gland development in dams and female offspring, and to a reduced mammary differentiation in dams (21). Moreover, it has been observed that PFOA stimulates mammary gland development in mice by promoting steroid hormone production and increasing growth factor levels (22). The biological plausibility is supported by limited yet consistent cell-based mechanistic studies. For instance, the exposure of PFOS at 1-10 μM stimulated in a dose-dependent manner the proliferation, migration and invasiveness of immortalized BC cells MCF-10A, through the up-regulation of CDK4 and down-regulation of p27, p21, and p53 (23, 24).The authors did not find any alteration of ERα and ERβ levels, nonetheless the cell proliferation was partially blocked when cells were co-exposed to an ER receptor inhibitor. On this regard, the authors suggested other alternative mechanisms contributed to explain the effects triggered by PFOS such as the pathway involving the growth factor receptor EGFR/HER2. Overall the effects of PFOS and PFOA on the ER pathway remain inconclusive.

With a combination of short-term *in vitro* and *in vivo* zebrafish assays, Du et al. (2012, 2013) reported that PFOS and PFOA have estrogenic activity and anti-thyroid hormone activity, altering steroid hormone synthesis and the expression of the major steroidogenic genes (25, 26). On the other hand, another *in vitro* study, which analyzed PFOA and PFOS estrogenic effects on T47D hormone-dependent BC cells, found that while these compounds did not have estrogenic activity, they enhanced the effects of estradiol on estrogen-responsive gene expression, ERK1/2 activation, and the growth of the hormone-deprived T47D cells (27).

Strengths/limitations

Several limitations should be considered when interpreting our findings. First, despite that the overall sample size of our study was sufficient to detect moderate to strong associations, we had limited power when stratifying on hormone receptor status (due to the low prevalence of ER-/PR- tumors in our population, and to the relative high number of cases for which this information was missing). This led to extremely wide CIs, although it should be noticed that the trend was consistent for both kind of hormone-receptors considered and

across substances. In addition, only a single measurement of PFOS and PFOA concentrations was available. However, it can be assumed that the degree of exposure did not drastically change across time for two reasons: first, PFASs have a long half-life in the human body (28); second, the environmental contamination levels change extremely slowly due to the resistance to biodegradation of PFASs (29), and consequently the general population is exposed to a constant level of PFASs.

Despite these limitations, our study presents several strengths. Thanks to the long follow-up of the E3N cohort, we were able to investigate prospectively the long-term health effects of PFOS and PFOA. Moreover, extensive information concerning the main BC risk factors, such as reproductive history, use of hormonal treatments, anthropometric characteristics, smoking habits, alcohol consumption, diet, and physical activity, was collected prospectively in the cohort and thus at the same time for cases and controls. Finally, we collected information on BC expression of estrogen and progesterone receptors, allowing us to better characterize the tumors and to perform stratified analyses.

Conclusion

This study provides some evidence of an association between PFASs serum concentrations and BC risk. In particular, for PFOS associated positive linear dose-response relationship was found with the risk of developing ER+/PR+ tumors, starting from internal values of 17.3 ng/mL, while only a low-dose effect for both PFOS and PFOA was associated with ER-/PR- tumors, although this should be confirmed in larger studies.

This study reflects exposure of non-occupationally exposed postmenopausal women in France. Our findings support the hypothesis previously suggested by other authors (30, 31) of a differential effect of exposure to PFASs depending on the dose, highlighting the importance of considering also on low-dose effects when studying EDCs, although further studies are warranted to explain the biological mechanism underlying the relationship between PFAS and BC. Exposure to EDCs, and in particular to PFAS, should be considered as a potential risk factor for BC, thus as a serious public health issue.

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Declaration of competing financial interests

The authors declare they have no actual or potential competing financial interests.

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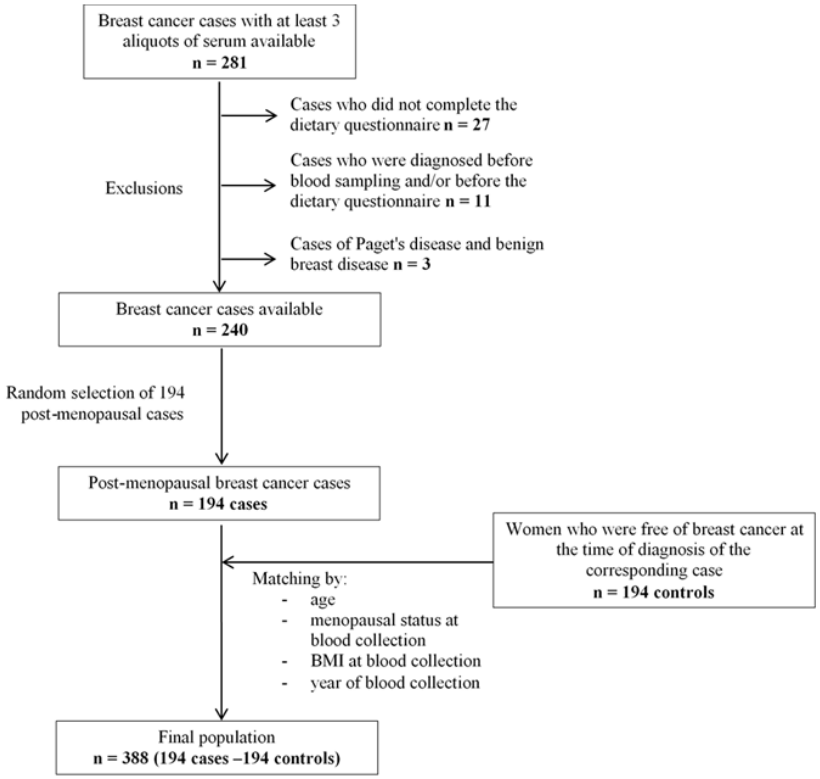

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Table 1 Distribution of serum levels of PFOS according to categorized variables of interest and p-value of chi² tests performed to evaluate the dependence of distributions.

			Quartile of serum level of PFOS				
		All (N=388)	**Q1 : 5.8-13.6 ng/mL (N=80)**	**Q2 : 13.6-17.3 ng/mL (N=109)**	**Q3 : 17.3-22.5 ng/mL (N=99)**	**Q4 : 22.5-85.3 ng/mL (N=100)**	**Global p-value**
Case/control status	Control	194 (50.0)	48 (60.0)	49 (45.0)	48 (48.5)	49 (49.0)	0.21
	Case	194 (50.0)	32 (40.0)	60 (55.0)	51 (51.5)	51 (51.0)	
Estrogen receptor status of the breast cancer	**Control**	**194 (50.0)**	**48 (59.9)**	**49 (45.0)**	**48 (48.5)**	**49 (49.0)**	**0.01**
	ER+	**132 (34.0)**	**20 (24.9)**	**37 (33.9)**	**35 (35.4)**	**40 (40.0)**	
	ER-	**26 (6.7)**	**1 (1.3)**	**15 (13.8)**	**4 (4.0)**	**6 (6.0)**	
	Missing	**36 (9.3)**	**11 (13.8)**	**8 (7.3)**	**12 (12.1)**	**5 (5.0)**	
Progesterone receptor status of the breast cancer	Control	194 (49.9)	48 (59.9)	49 (45.0)	48 (48.5)	49 (49.0)	0.06
	PR+	98 (25.3)	14 (17.4)	26 (23.9)	27 (27.3)	31 (31.0)	
	PR-	57 (14.7)	7 (8.8)	25 (22.8)	12 (12.1)	13 (13.0)	
	Missing	39 (10.1)	11 (13.8)	9 (8.3)	12 (12.1)	7 (7.0)	
Age at blood draw	≤ 55.5 years old	191 (49.2)	45 (56.2)	60 (55.0)	45 (45.5)	41 (41.0)	0.09
	> 55.5 years old	197 (50.8)	35 (43.7)	49 (45.0)	54 (54.5)	59 (59.0)	
Total serum lipid level (mg/mL)	**≤ 6.5**	**193 (49.7)**	**50 (62.5)**	**60 (55.0)**	**43 (43.4)**	**40 (40.0)**	**0.01**
	> 6.5	**195 (50.3)**	**30 (37.5)**	**49 (45.0)**	**56 (56.6)**	**60 (60.0)**	
BMI (kg/m²) at blood draw	< 25	293 (75.5)	64 (80.0)	83 (76.1)	72 (72.7)	74 (74.0)	0.69
	≥ 25	95 (24.5)	16 (20.0)	26 (23.9)	27 (27.3)	26 (26.0)	
Smoking status at blood draw	Never	191 (49.2)	35 (43.7)	56 (51.4)	43 (43.4)	57 (57.0)	0.17
	Ever	197 (50.8)	45 (56.2)	53 (48.6)	56 (56.6)	43 (43.0)	
Physical activity (METs.h/ week) at blood draw	**≤ 34**	**193 (49.7)**	**52 (65.0)**	**50 (45.9)**	**46 (46.5)**	**45 (45.0)**	**0.02**
	> 34	**195 (50.3)**	**28 (35.0)**	**59 (54.1)**	**53 (53.5)**	**55 (55.0)**	
Education level (years)	≤ 14	230 (59.3)	47 (58.7)	60 (55.0)	68 (68.7)	55 (55.0)	0.15
	> 14	158 (40.7)	33 (41.2)	49 (45.0)	31 (31.3)	45 (45.0)	
Personal history of benign breast disease	No	248 (63.9)	48 (60.0)	72 (66.1)	60 (60.6)	68 (68.0)	0.58
	Yes	140 (36.1)	32 (40.0)	37 (33.9)	39 (39.4)	32 (32.0)	
Family history of breast cancer	No	280 (72.2)	58 (72.5)	77 (70.6)	70 (70.7)	75 (75.0)	0.88
	Yes	108 (27.8)	22 (27.5)	32 (29.4)	29 (29.3)	25 (25.0)	
Parity	Nulliparous	57 (14.7)	8 (9.9)	17 (15.6)	12 (12.1)	20 (20.0)	0.59
	1 child	52 (13.4)	11 (13.8)	11 (10.1)	15 (15.2)	15 (15.0)	
	2 children	173 (44.6)	34 (42.4)	53 (48.6)	45 (45.5)	41 (41.0)	
	≥ 3 children	106 (27.3)	27 (33.8)	28 (25.7)	27 (27.3)	24 (24.0)	
Age at first full-term pregnancy	Nulliparous	57 (14.7)	8 (10.0)	17 (15.6)	12 (12.1)	20 (20.0)	0.27
	< 25 years old	174 (44.8)	32 (40.0)	51 (46.8)	50 (50.5)	41 (41.0)	
	≥ 25 years old	157 (40.5)	40 (50.0)	41 (37.6)	37 (37.4)	39 (39.0)	
Total breastfeeding duration (months)	Never	152 (39.2)	30 (37.4)	40 (36.7)	39 (39.4)	43 (43.0)	0.94
	≤ 6	160 (41.2)	33 (41.3)	47 (43.1)	43 (43.4)	37 (37.0)	
	> 6	76 (19.6)	17 (21.3)	22 (20.2)	17 (17.2)	20 (20.0)	
Age at menarche	**< 13**	**174 (44.8)**	**44 (55.0)**	**41 (37.6)**	**39 (39.4)**	**50 (50.0)**	**0.05**
	≥ 13	**214 (55.2)**	**36 (45.0)**	**68 (62.4)**	**60 (60.6)**	**50 (50.0)**	
Age at menopause	< 51	190 (49.0)	38 (47.5)	50 (45.9)	52 (52.5)	50 (50.0)	0.79
	≥ 51	198 (51.0)	42 (52.5)	59 (54.1)	47 (47.5)	50 (50.0)	
Menopausal status at blood draw	Premenopausal	100 (25.8)	28 (35.0)	30 (27.5)	24 (24.2)	18 (18.0)	0.07
	Postmenopausal	288 (74.2)	52 (65.0)	79 (72.5)	75 (75.8)	82 (82.0)	
Oral contraceptive use at blood draw	**Never**	**158 (40.7)**	**28 (35.0)**	**37 (33.9)**	**41 (41.4)**	**52 (52.0)**	**0.04**
	Ever	**230 (59.3)**	**52 (65.0)**	**72 (66.1)**	**58 (58.6)**	**48 (48.0)**	
Menopausal hormone	No	230 (59.3)	54 (67.5)	59 (54.1)	57 (57.6)	60 (60.0)	0.31

therapy use at blood draw	Yes	158 (40.7)	26 (32.5)	50 (45.9)	42 (42.4)	40 (40.0)	
Score of adherence to the Mediterranean diet	< 0.19	191 (49.2)	42 (52.5)	58 (53.2)	37 (37.4)	54 (54.0)	0.06
	≥ 0.19	197 (50.8)	38 (47.5)	51 (46.8)	62 (62.6)	46 (46.0)	
Score of adherence to the Western diet	< 0.12	193 (49.7)	37 (46.2)	60 (55.0)	54 (54.5)	42 (42.0)	0.17
	≥ 0.12	195 (50.3)	43 (53.7)	49 (45.0)	45 (45.5)	58 (58.0)	

Table 2 Distribution of serum levels of PFOA according to categorized variables of interest and p-value of chi² tests performed to evaluate the dependence of distributions.

			Quartile of serum level of PFOA				
		All (N=388)	**Q1 : 1.3-4.8 ng/mL (N=85)**	**Q2 : 4.8-6.8 ng/mL (N=118)**	**Q3 : 6.8-8.8 ng/mL (N=91)**	**Q4 : 8.8-21.4 ng/mL (N=94)**	**Global p-value**
Case/control status	Control	194 (50.0)	48 (56.5)	49 (41.5)	48 (52.7)	49 (52.1)	0.15
	Case	194 (50.0)	37 (43.5)	69 (58.5)	43 (47.3)	45 (47.9)	
Estrogen receptor status of the breast cancer	Control	194 (50.0)	48 (56.5)	49 (41.5)	48 (52.7)	49 (52.1)	0.28
	ER+	132 (34.0)	25 (29.4)	43 (36.4)	31 (34.1)	33 (35.1)	
	ER-	26 (6.7)	2 (2.4)	12 (10.2)	6 (6.6)	6 (6.4)	
	Missing	36 (9.3)	10 (11.8)	14 (11.9)	6 (6.6)	6 (6.4)	
Progesterone receptor status of the breast cancer	Control	194 (49.9)	48 (56.5)	49 (41.5)	48 (52.7)	49 (52.1)	0.17
	PR+	98 (25.3)	20 (23.5)	28 (23.7)	23 (25.3)	27 (28.7)	
	PR-	57 (14.7)	7 (8.2)	25 (21.2)	13 (14.3)	12 (12.8)	
	Missing	39 (10.1)	10 (11.8)	16 (13.6)	7 (7.7)	6 (6.4)	
Age at blood draw	**≤ 55.5 years old**	**191 (49.2)**	**51 (60.0)**	**67 (56.8)**	**36 (39.6)**	**37 (39.4)**	**0.003**
	> 55.5 years old	**197 (50.8)**	**34 (40.0)**	**51 (43.2)**	**55 (60.4)**	**57 (60.6)**	
Total serum lipid level (mg/mL)	**≤ 6.5**	**193 (49.7)**	**53 (62.4)**	**57 (48.3)**	**45 (49.5)**	**38 (40.4)**	**0.03**
	> 6.5	**195 (50.3)**	**32 (37.6)**	**61 (51.7)**	**46 (50.5)**	**56 (59.6)**	
BMI (kg/m²) at blood draw	< 25	293 (75.5)	65 (76.5)	96 (81.4)	69 (75.8)	63 (67.0)	0.11
	≥ 25	95 (24.5)	20 (23.5)	22 (18.6)	22 (24.2)	31 (33.0)	
Smoking status at blood draw	Never	191 (49.2)	39 (45.9)	61 (51.7)	50 (54.9)	41 (43.6)	0.38
	Ever	197 (50.8)	46 (54.1)	57 (48.3)	41 (45.1)	53 (56.4)	
Physical activity (METs.h/ week) at blood draw	≤ 34	193 (49.7)	52 (61.2)	51 (43.2)	41 (45.1)	49 (52.1)	0.05
	> 34	195 (50.3)	33 (38.8)	67 (56.8)	50 (54.9)	45 (47.9)	
Education level (years)	≤ 14	230 (59.3)	52 (61.2)	76 (64.4)	52 (57.1)	50 (53.2)	0.38
	> 14	158 (40.7)	33 (38.8)	42 (35.6)	39 (42.9)	44 (46.8)	
Personal history of benign breast disease	No	248 (63.9)	54 (63.5)	69 (58.5)	58 (63.7)	67 (71.3)	0.29
	Yes	140 (36.1)	31 (36.5)	49 (41.5)	33 (36.3)	27 (28.7)	
Family history of breast cancer	No	280 (72.2)	61 (71.8)	87 (73.7)	67 (73.6)	65 (69.1)	0.87
	Yes	108 (27.8)	24 (28.2)	31 (26.3)	24 (26.4)	29 (30.9)	
Parity	**Nulliparous**	**57 (14.7)**	**6 (7.1)**	**16 (13.6)**	**15 (16.5)**	**20 (21.3)**	**0.003**
	1 child	**52 (13.4)**	**10 (11.8)**	**19 (16.1)**	**13 (14.3)**	**10 (10.6)**	
	2 children	**173 (44.6)**	**43 (50.6)**	**65 (55.1)**	**30 (33.0)**	**35 (37.2)**	
	≥ 3 children	**106 (27.3)**	**26 (30.6)**	**18 (15.3)**	**33 (36.3)**	**29 (30.9)**	
Age at first full-term pregnancy	Nulliparous	57 (14.7)	6 (7.1)	16 (13.6)	15 (16.5)	20 (21.3)	0.25
	< 25 years old	174 (44.8)	43 (50.6)	53 (44.9)	39 (42.9)	39 (41.5)	
	≥ 25 years old	157 (40.5)	36 (42.4)	49 (41.5)	37 (40.7)	35 (37.2)	
Total breastfeeding duration (months)	Never	152 (39.2)	27 (31.8)	46 (38.9)	44 (48.4)	35 (37.3)	0.16
	≤ 6	160 (41.2)	40 (47.1)	54 (45.8)	31 (34.1)	35 (37.3)	
	> 6	76 (19.6)	18 (21.1)	18 (15.3)	16 (17.6)	24 (25.5)	
Age at menarche	< 13	174 (44.8)	31 (36.5)	57 (48.3)	41 (45.1)	45 (47.9)	0.34
	≥ 13	214 (55.2)	54 (63.5)	61 (51.7)	50 (54.9)	49 (52.1)	
Age at menopause	< 51	190 (49.0)	42 (49.4)	62 (52.5)	40 (44.0)	46 (48.9)	0.67
	≥ 51	198 (51.0)	43 (50.6)	56 (47.5)	51 (56.0)	48 (51.1)	
Menopausal status at blood draw	Premenopausal	100 (25.8)	28 (32.9)	32 (27.1)	21 (23.1)	19 (20.2)	0.2348
	Postmenopausal	288 (74.2)	57 (67.1)	86 (72.9)	70 (76.9)	75 (79.8)	
Oral contraceptive use at blood draw	**Never**	**158 (40.7)**	**28 (32.9)**	**42 (35.6)**	**40 (44.0)**	**48 (51.1)**	**0.05**
	Ever	**230 (59.3)**	**57 (67.1)**	**76 (64.4)**	**51 (56.0)**	**46 (48.9)**	
Menopausal hormone	No	230 (59.3)	49 (57.6)	73 (61.9)	53 (58.2)	55 (58.5)	0.92

therapy use at blood draw	Yes	158 (40.7)	36 (42.4)	45 (38.1)	38 (41.8)	39 (41.5)	
Score of adherence to the Mediterranean diet	< 0.19	191 (49.2)	40 (47.1)	65 (55.1)	44 (48.4)	42 (44.7)	0.46
	≥ 0.19	197 (50.8)	45 (52.9)	53 (44.9)	47 (51.6)	52 (55.3)	
Score of adherence to the Western diet	< 0.12	193 (49.7)	39 (45.9)	57 (48.3)	52 (57.1)	45 (47.9)	0.43
	≥ 0.12	195 (50.3)	46 (54.1)	61 (51.7)	39 (42.9)	49 (52.1)	

Table 3 Conditional logistic regression models to estimate the association between quartiles of serum levels of PFOS and breast cancer risk in postmenopausal women: adjusted odds ratios (ORs) and 95% confidence intervals (CIs) (N=388).

	Quartile of serum level of PFOS				
Model	**Q1 : 5.8-13.6 ng/mL (n=80)**	**Q2 : 13.6-17.3 ng/ mL (N=109)**	**Q3 : 17.3-22.5 ng/ mL (N=99)**	**Q4 : 22.5-85.3 ng/ mL (N=100)**	**p-trend**
0	Ref.	**1.80 (1.01; 3.21)**	1.59 (0.88; 2.90)	1.53 (0.85; 2.74)	0.38
1	Ref.	1.80 (0.98; 3.28)	1.78 (0.95; 3.34)	1.67 (0.90; 3.10)	0.23
2	Ref.	**1.93 (1.01; 3.70)**	**1.96 (1.00; 3.84)**	1.70 (0.88; 3.28)	0.26
3	Ref.	**1.94 (1.00; 3.78)**	**2.03 (1.02; 4.04)**	1.72 (0.88; 3.36)	0.25

Model 0: univariable analysis;
Model 1: adjusted for total serum lipids (unit), BMI (kg/m²), smoking status (non-smoker vs. smoker), physical activity (MET-h/week), education level (≤14 vs. >14 years), personal history of benign breast disease (yes vs. no), family history of breast cancer (yes vs. no);
Model 2: model 1+ parity/age at first full-term pregnancy (nulliparous, 1 or 2 children and <25 years old at delivery, ≥3 children and <25 years old at delivery, ≥25 years old at delivery), total breastfeeding duration (never, ≤6 months, >6 months), age at menarche, age at menopause, use of oral contraceptives (ever vs. never), current use of menopausal hormone therapy (yes vs.no);
Model 3: model 2 + score of adherence to the Western diet (high vs. low) and to the Mediterranean diet (high vs. low).

Table 4 Multinomial regression models to estimate the association between quartiles of PFOS serum level and breast cancer risk stratified according to hormone receptor status in postmenopausal women: adjusted odds ratios (ORs) and 95% confidence intervals (CIs) (N=388).

	Quartile of serum level of PFOS				
Estrogen receptor status	**Q1 : 5.8-13.6 ng/mL (n=80)**	**Q2 : 13.6-17.3 ng/ mL (N=109)**	**Q3 : 17.3-22.5 ng/ mL (N=99)**	**Q4 : 22.5-85.3 ng/ mL (N=100)**	**p-trend**
Control (n=194)	Ref	Ref	Ref	Ref	Ref
Case ER+ (n=132)	Ref	1.85 (0.90-3.82)	**2.22 (1.05-4.69)**	**2.33 (1.11-4.90)**	**0.04**
Case ER- (n=26)	Ref	**15.40 (1.84-129.19)**	4.74 (0.45-49.62)	7.07 (0.73-68.03)	0.72
Case with missing ER status (n=36)	Ref	0.67 (0.23-1.97)	1.25 (0.45-3.43)	0.41 (0.12-1.44)	0.27
Progesterone receptor status	**Q1 : 5.8-13.6 ng/mL (n=80)**	**Q2 : 13.6-17.3 ng/ mL (N=109)**	**Q3 : 17.3-22.5 ng/ mL (N=99)**	**Q4 : 22.5-85.3 ng/ mL (N=100)**	**P trend**
Control (n=194)	Ref	Ref	Ref	Ref	Ref
Case PR+ (n=98)	Ref	1.84 (0.82-4.14)	**2.47 (1.07-5.65)**	**2.76 (1.21-6.30)**	**0.02**
Case PR- (n=57)	Ref	**3.47 (1.29-9.15)**	1.82 (0.61-5.45)	1.71 (0.57-5.10)	0.93
Case with missing PR status (n=39)	Ref	0.78 (0.27-2.21)	1.30 (0.47-3.56)	0.64 (0.20-2.01)	0.58

Model 3: adjusted for total serum lipids (unit), BMI (kg/m²), smoking status (non-smoker vs. smoker), physical activity (MET-h/week), education level (≤14 vs. >14 years), personal history of benign breast disease (yes vs. no), family history of breast cancer (yes vs. no), parity*age at first full-term pregnancy (nulliparous, 1 or 2 children and <25 years old at delivery, ≥3 children and <25

years old at delivery, ≥25 years old at delivery), total breastfeeding duration (never, ≤6 months, >6 months), age at menarche, age at menopause, use of oral contraceptives (ever vs. never), current use of menopausal hormone therapy (yes vs.no), score of adherence to the Western diet and to the Mediterranean diet, age (years) at blood draw, BMI (kg/m²) at blood draw, menopausal status (premenopausal vs. postmenopausal) at blood draw, and year of blood draw.

Table 5 Conditional logistic regression models to estimate the association between quartiles of serum levels of PFOA and breast cancer risk in postmenopausal women: adjusted odds ratios (ORs) and 95% confidence intervals (CIs) (N=388).

	Quartile of serum level of PFOA				
Model	**Q1 : 1.3-4.8 ng/mL (N=85)**	**Q2 : 4.8-6.8 ng/ mL (N=118)**	**Q3 : 6.8-8.8 ng/ mL (N=91)**	**Q4 : 8.8-21.4 ng/ mL (N=94)**	**p-trend**
0	Ref.	**1.75 (1.00; 3.08)**	1.15 (0.61; 2.15)	1.12 (0.58; 2.16)	0.72
1	Ref.	**1.86 (1.03; 3.36)**	1.08 (0.56; 2.09)	1.18 (0.59; 2.37)	0.85
2	Ref.	1.79 (0.94; 3.40)	0.95 (0.47; 1.92)	0.98 (0.46; 2.08)	0.49
3	Ref.	1.69 (0.89; 3.21)	0.88 (0.43; 1.80)	0.92 (0.43; 1.98)	0.43

Model 0: univariable analysis;
Model 1: adjusted for total serum lipids (unit), BMI (kg/m²), smoking status (non-smoker vs. smoker), physical activity (MET-h/week), education level (≤14 vs. >14 years), personal history of benign breast disease (yes vs. no), family history of breast cancer (yes vs. no);
Model 2: model 1+ parity/age at first full-term pregnancy (nulliparous, 1 or 2 children and <25 years old at delivery, ≥3 children and <25 years old at delivery, ≥25 years old at delivery), total breastfeeding duration (never, ≤6 months, >6 months), age at menarche, age at menopause, use of oral contraceptives (ever vs. never), current use of menopausal hormone therapy (yes vs.no);
Model 3: model 2 + score of adherence to the Western diet (high vs. low) and to the Mediterranean diet (high vs. low).

Table 6 Multinomial regression models to estimate the association between quartiles of PFOA serum level and breast cancer risk stratified according to hormone receptor status in postmenopausal women: adjusted odds ratios (ORs) and 95% confidence intervals (CIs) (N=388).

	Quartile of serum level of PFOA				
Estrogens receptor status	**Q1 : 1.3-4.8 ng/mL (N=85)**	**Q2 : 4.8-6.8 ng/ mL (N=118)**	**Q3 : 6.8-8.8 ng/ mL (N=91)**	**Q4 : 8.8-21.4 ng/ mL (N=94)**	**p-trend**
Control (n=194)	Ref	Ref	Ref	Ref	Ref
Case ER+ (n=132)	Ref	1.72 (0.88-3.36)	1.34 (0.66-2.73)	1.42 (0.68-2.95)	0.64
Case ER- (n=26)	Ref	**7.73 (1.46-41.08)**	3.18 (0.55-18.47)	3.98 (0.67-23.52)	0.59
Case with missing ER status (n=36)	Ref	1.46 (0.55-3.85)	0.48 (0.15-1.59)	0.41 (0.12-1.40)	0.06
Progesterone receptor status	**Q1 : 1.3-4.8 ng/mL (N=85)**	**Q2 : 4.8-6.8 ng/ mL (N=118)**	**Q3 : 6.8-8.8 ng/ mL (N=91)**	**Q4 : 8.8-21.4 ng/ mL (N=94)**	**P trend**
Control (n=194)	Ref	Ref	Ref	Ref	Ref
Case PR+ (n=98)	Ref	1.40 (0.67-2.93)	1.28 (0.59-2.77)	1.54 (0.70-3.69)	0.37

Case PR- (n=57)	Ref	**3.44 (1.30-9.10)**	1.80 (0.62-5.19)	1.69 (0.56-3.12)	0.90
Case with missing PR status (n=39)	Ref	1.68 (0.65-4.36)	0.58 (0.18-1.83)	0.43 (0.13-1.43)	0.06

Model 3: adjusted for total serum lipids (unit), BMI (kg/m²), smoking status (non-smoker vs. smoker), physical activity (MET-h/week), education level (≤14 vs. >14 years), personal history of benign breast disease (yes vs. no), family history of breast cancer (yes vs. no), parity*age at first full-term pregnancy (nulliparous, 1 or 2 children and <25 years old at delivery, ≥3 children and <25 years old at delivery, ≥25 years old at delivery), total breastfeeding duration (never, ≤6 months, >6 months), age at menarche, age at menopause, use of oral contraceptives (ever vs. never), current use of menopausal hormone therapy (yes vs.no), score of adherence to the Western diet and to the Mediterranean diet, age (years) at blood draw, BMI (kg/m²) at blood draw, menopausal status (premenopausal vs. postmenopausal) at blood draw, and year of blood draw.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are two environmental endocrine-disrupting chemicals suspected to be ubiquitously present in the blood of the human population. This nested case-control study including non-occupationally exposed postmenopausal French women suggests a linear dose-response relationship between PFOS serum concentrations and the risk of developing hormone receptor-positive breast cancer. Furthermore, an increased risk of developing ER- and PR- tumors is associated to middle-low serum concentrations of PFOA and PFOS. Exposure to endocrine-disrupting chemicals should be considered as a potential risk factor for breast cancer, thus a serious public health issue.

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