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Risk of female breast cancer and serum concentrations of organochlorine pesticides and polychlorinated biphenyls: A case–control study in Tunisia

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HIGHLIGHTS

- We studied the association between persistent pollutants in serum and breast cancer.
- We performed a case–control study in a Tunisian population.
- Three organochlorine pesticides were individually associated with cancer risk.

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ABSTRACT

The aim of this study was to investigate the association between serum concentrations of a group of organochlorine pesticides/polychlorinated biphenyls with xenoestrogenic potential and the risk of breast cancer in a female population from Tunisia.

The relationship between serum levels of the pollutants and the risk of cancer was assessed using logistic regression analyses. In the unadjusted models, β-hexachlorocyclohexane (β-HCH), hexachlorobenzene, heptachlor, polychlorinated biphenyl congeners 138, 153, and 180, and p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE) were positively associated with breast cancer risk. However, when the models were further adjusted for the selected covariates, only β-HCH and p,p′-DDE remained statistically significant, and heptachlor was borderline significant. In addition, analyses using POP concentration tertiles corroborated a positive dose–response relationship that was significant for *p,p′*-DDE (p-trend = 0.020) and borderline significant for heptachlor (p-trend = 0.078). A similar trend was also confirmed for β-HCH, in which concentrations ≥ limit of detection were positively associated with breast cancer risk (vs. concentrations < limit of detection, OR = 3.44, $p < 0.05$). Finally, the relative influence of each chemical in the presence of the others was assessed by entering the three chemicals in a single model with all covariates, and only β-HCH remained positively associated with the risk of cancer (OR:1.18, 95%CI: 1.05–1.34).

Our findings suggest a potential association between exposure to at least one organochlorine pesticide and breast cancer risk. However, our results should be interpreted with caution, and further research is warranted to confirm these findings.

1. Introduction

Breast cancer represents 33% of female cancers in Tunisia, with approximately 1600 new cases per year. Although this disease remains less frequent in Tunisia than in European countries (Maalej et al., 2008), the crude incidence increased from 25.5 cases/100,000 women in 1995–1998 (RCNT, 1998) to 32.3 in 2004–2006 (RCNT, 2006). Breast cancer is the leading cause of cancer death among Tunisian women

Abbreviations: POPs, Persistent Organic Pollutants; PCBs, polychlorinated biphenyls; *p,p′*-DDE, *p,p′*-dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; β-HCH, hexachlorocyclohexane; LOD, limit of detection; OR, odds ratio; CI, confidence interval.

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(Lazaar-Ben et al., 2011) and is estimated to be responsible for 22% of the total cancer mortality (WHO, 2014).

This increasing incidence of breast cancer, which has been documented in most countries (Parkin et al., 2005; World Cancer Research Fund International, 2014), can only be partially explained by improvements in screening programs (Charlier et al., 2003a), and genetic factors account for a small proportion of the incidence in women (Knower et al., 2014). Hence, environmental factors are estimated to play an important role in the pathogenesis of the disease.

The development of cancer is known to be a multifactorial process, with many reported risk factors that have a hormonal component, including age at menopause, menarche, and first pregnancy, parity, accumulated lactation time, time of reproductive life, use of hormonal contraception, hormone replacement therapy, or body weight (McPherson et al., 2000). In fact, previous research has shown that breast cancer is highly influenced by long-term elevated estrogen levels (Key et al., 2002), which can be metabolized to mutagenic elements that eventually stimulate tissue growth and cause the initiation, promotion, and progression of carcinogenesis (Yager and Davidson, 2006). This led researchers to hypothesize that long-term exposure to environmental pollutants with xenoestrogenic potential might make a significant contribution to the process of carcinogenesis (Knower et al., 2014). In fact, a recent epidemiological study concluded that women occupationally exposed to chemicals with hormonal activity (e.g., agriculture, industry) had an increased risk of developing breast cancer (Brophy et al., 2012).

POPs are a wide group of highly lipophilic environmental pollutants that tend to accumulate and biomagnify in food chains, resulting in the considerable exposure of living organisms (UNEP, 2003). POPs include organochlorine pesticides, which have long been widely used in agriculture and public health as highly effective pest control agents (UNEP, 2003), and also polychlorinated biphenyls (PCBs), used worldwide in numerous industrial and commercial applications (La Rocca and Mantovani, 2006). Between 1980 and 1984, the Tunisian Government banned the use and import of the organochlorine pesticides included in this study (APEK, 2005). PCBs were widely used in electrical transformers in Tunisia from the 1970s until their prohibition in 1986. A situation report estimated that there were 132 dump sites containing OCPs/PCBs in the country (APEK, 2005). A previous study revealed that approximately 89% of the stocks of obsolete pesticides were stored in unsatisfactory conditions (Dasgupta et al., 2010), with the consequent risk of contaminating the environment and humans.

POPs have been detected in virtually all human populations and environmental matrices, and diet (especially fatty food) has been reported to be the main route for human exposure (Arrebola et al., 2009; Brauner et al., 2012). Once absorbed, POPs are mainly stored in adipose tissue, where they can be released or persist for long periods of time (Yu et al., 2011).

There is evidence that exposure to some POPs can cause estrogen-related effects, including an increase in uterine weight (Adami et al., 1995) or the promotion of estrogen-related tumors (Scribner and Mottet, 1981). Among the suspected mechanisms of action, in vitro studies have shown that many POPs can interact with estrogen and/or androgen receptors and exert significant effects (Andersen et al., 2002; Bonefeld-Jorgensen et al., 2001; Grunfeld and Bonefeld-Jorgensen, 2004; Soto et al., 1994), which might be a consequence of their interaction with other chemicals as well as with endogenous hormones (Arrebola et al., 2012). Additionally, there is evidence that POPs might cause cancer by other mechanisms not related to estrogen receptors, such as oxidative stress (Karami-Mohajeri and Abdollahi, 2011).

The aim of this study was to investigate the association between serum concentrations of a group of organochlorine pesticides/PCBs with (anti-)estrogenic potential and the risk of breast cancer in a female population from Tunisia. These chemicals were chosen on the basis of their suspected hormonal effects, the reported existence of obsolete stocks in the region (APEK, 2005), and the frequency of their detection in other populations.

2. Materials and methods

2.1. Study population

This case–control study is part of a wider research project designed to characterize the exposure to environmental pollutants in Tunisia and related health outcomes (Artacho-Cordon et al., in press; Belhassen et al., 2015). The study population was consecutively recruited between May and October 2012 from among patients attending the two main specialist cancer centers in the country, Salah Azaiz Hospital (Tunis state) and Ariana Hospital (Ariana state), which are both in the Grand Tunis metropolitan area (Northern Tunisia). Cases were women with breast cancer admitted to hospital for mastectomy, tumorectomy (Salah Azaiz Hospital), or chemotherapy (Cancer Center of Ariana). Out of the 96 eligible cases, 69 (72%) were finally included and provided signed consent and a blood sample. Patients were included if they were aged 18 years or over and able to give informed consent and complete a questionnaire and excluded if they had a previous history of cancer or evidenced distant metastasis at diagnosis. The control group was randomly selected from among healthy female hospital visitors, hospital staff, or blood donors who were aged 18 years and able to give informed consent and complete a questionnaire. Out of the 77 women invited to participate as controls, 56 (70%) were finally enrolled in the study. Biological samples were collected before surgery or chemotherapy. No significant differences were found between included and excluded participants in age, marital status, or occupational class (data not shown in tables).

All participants signed their informed consent to participate in the study, which was approved by the ethics committees of the hospitals.

2.2. Sample collection and extraction

Human serum samples were obtained under 12-h fasting conditions. Samples from cases were collected at the time of diagnosis and before any specific treatment. 2 mL serum was extracted using the methodology described by Turci et al. (2010) with slight modifications. Briefly, serum was spiked with *p*-chlorodibenzophenone as internal standard and further extracted with methanol and hexane/ethyl ether (1:1 v/v). The organic phase was reconstituted in 1 mL hexane and allowed to pass through a Bond Elut-PCB cartridge. The sample elution was performed with 3 ml hexane and 3 ml hexane/diethyl ether (1:1 v/v). Finally, the eluate was evaporated to dryness under a stream of nitrogen and stored at −80 °C until chemical analysis.

2.3. Chemical analyses and lipid quantification

A group of organochlorine pesticides (*p*,*p*′-dichlorodiphenyldichloroethylene [*p*,*p*′-DDE], hexachlorobenzene [HCB], β-hexachlorocyclohexane [β-HCH], α-endosulfan, endosulfan ether, heptachlor, and oxychlordane) and PCBs (congeners 138, 153, and 180) were quantified in the extracts by gas chromatography with micro-electron capture detection, using a VARIAN CP-3800 equipped with a ^{63}Ni electron capture detector (Walnut Creek, CA, US).

Procedural blanks were extracted with the same methodology and analyzed in the gas chromatograph, always yielding a negative result. Inter- and intra-day variabilities were calculated by analyzing fortified samples within the same day (repeatability) and on different days (intermediate precision), respectively, and were always <20%. Different concentrations of laboratory-fortified matrix samples were used for the quality control. The LOD was determined as the smallest amount of the analyte that gave a signal-to-noise ratio ≥ 3 and was set at 0.05 μg/L for each POP. The recovery of POPs from serum was also studied to assess the extraction efficiency of the methods, spiking 10 serum samples with target analytes at an intermediate point on the calibration curve and processing them as described above. Recoveries ranged from 90 to 98%.

POP concentrations were calculated by using matrix-matched calibration. Concentrations below the limit of detection (LOD) were assigned a random value between zero and the LOD, which was calculated by using the random numbers function of SPSS. In addition, we repeated the multivariable analyses considering concentrations < LOD as one half of the LOD; no differences were observed with the associations observed using the random value between zero and the LOD (data not shown in tables).

Total cholesterol and triglyceride levels were enzymatically quantified in 10 μL of serum from each participant using a Covas 400 machine (Roche, Switzerland). Total serum lipids were calculated by applying the short formula of Phillips et al. (1989): TL = 2.27 TC + TG + 0.623, where TL is total lipids, TC is total cholesterol, and TG is triglycerides, all expressed in units of g/L.

2.4. Covariates

Covariates were gathered in a questionnaire administered face-to-face by a trained interviewer. It was completed by each participant and collected information on their socio-demographic characteristics, reproductive history, and lifestyle. A participant was considered a smoker (past or present) at any level of daily tobacco consumption (≥1 cig/day). Age at menarche (years), accumulated breastfeeding time (months), alcohol consumption (glasses/week), and age at last breast feeding (years) were self-reported by the participants and recorded as continuous variables. Parity was recorded as both a continuous (number of children) and dichotomous variable (nulliparous/≥1 children). The BMI, calculated as weight/height squared (kg/m^2), served as a measure of obesity.

Participants were classified according to their occupation as manual workers, non-manual workers, or homemakers and according to their residence in an urban or rural area. Self-reported information was also collected on marital status, educational level, and menopausal status. Data were also gathered from the clinical records of cases on the size of the nodule, the presence of metastasis, and any family history of breast cancer.

2.5. Statistical analysis

Descriptions of the study variables in cases and controls were performed using means, standard deviations and percentiles (quantitative variables), and frequencies (categorical variables). Bivariate analyses for the comparison between cases and controls were performed using Mann–Whitney's *U*-test for continuous variables and Fisher's exact test for categorical variables. The linear correlation between pairs of POP concentrations was assessed with Spearman's correlation test. All tests in the bivariate analyses were two-tailed, and the significance level was set at $p \leq 0.05$.

The relationship between POP serum levels and breast cancer risk was assessed by using unconditional logistic regression analyses, entering POP concentrations as continuous variables. In addition, the associations found were further explored by entering POP concentrations in the models as tertiles, with the exception of β-HCH and α-endosulfan, which were entered as dichotomous variables (<LOD/ ≥LOD) because of the low frequency of their detection. Bivariate models with individual POP concentrations as independent variable were created and then adjusted for variables whose inclusion produced changes of >10% in beta coefficients and for those described as relevant factors in the literature. Finally, a single adjusted model was created that included all covariates and POP concentrations significantly associated with breast cancer risk in the previous adjusted models, i.e., β-HCH, heptachlor, and *p,p′*-DDE. Odds ratios (ORs) for the risk of breast cancer with their corresponding 95% confidence intervals (95% CIs) were calculated, and trends were evaluated with Mantel–Haenszel's chi-square test for linear trend. In order to facilitate interpretation of the coefficients in Table 3, wet-basis concentrations were all entered as ng/mL with the exception of *p,p′*-DDE (ng/dL), while lipid-basis concentrations were all entered as mg/g lipid with the exception of *p,p′*-DDE (ng/g lipid). We considered that POP concentrations were significantly associated with the risk of breast cancer when the 95% CIs of the OR in the adjusted models did not overlap the null value (1). Data were stored and processed using SPSS Statistics 22.0 (IBM, Chicago, IL) and R statistical computing environment v3.1 (http://www.r-project.org/).

3. Results

The main characteristics of cases and controls are summarized in Table 1. In comparison to controls, cases were older (median: 49 vs. 44 yrs, respectively) and had given birth to more children (median of 3 vs. 2 children, respectively). Among the cases, there was a larger proportion (vs. controls) of homemakers (71.0% vs. 27.8%, respectively), women with only primary schooling or less (76.8% vs. 35.2%, respectively), post-menopausal women (50.7% vs. 25.9%), and residents in rural areas (42.0% vs. 11.1%, respectively). No member of the control group reported a family history of breast cancer, while 17 (24.6%) cases declared at least one case of breast cancer in their family.

Table 2 exhibits the serum POP concentrations and detection frequencies in cases and controls. Concentrations of β-HCH, heptachlor, PCB 138, PCB 180, and *p,p′*-DDE were significantly or borderline significantly higher in cases than in controls. Due to the low number of positive samples, endosulfan-ether and oxychlordane were not further considered in the statistical models. Spearman correlation tests among individual POP concentrations are shown as Supplementary Material.

Table 3 exhibits the results of the logistic regression analyses for both wet- and lipid-basis POP concentrations, which showed very similar associations. In the unadjusted models, β-HCH, HCB, heptachlor, PCB 138, PCB 153, PCB 180, and *p,p′*-DDE were positively associated with the risk of breast cancer, i.e., the risk increased with higher exposure levels. However, when the models were further adjusted for the selected covariates, only β-HCH and *p,p′*-DDE remained statistically significant, and heptachlor was borderline significant. In addition, analyses using POP concentration tertiles corroborated a positive dose–response relationship that was significant for *p,p′*-DDE (p-trend = 0.020) and borderline significant for heptachlor (p-trend = 0.078). A similar trend was also confirmed for β-HCH, in which concentrations ≥ LOD were positively associated with breast cancer risk (vs. concentrations < LOD, OR = 3.44, $p < 0.05$). Finally, the relative influence of each chemical in the presence of the others was assessed by entering the three chemicals as continuous variables in a single model with all covariates, and only β-HCH remained positively associated with the risk of cancer (OR:1.18, 95%CI: 1.05–1.34, wet-basis concentrations, data not shown in tables). In an attempt to assess the potential effect modification of the associations found, we searched for interactions between POP concentrations in the final model by entering the following interaction terms: β-HCH × heptachlor, β-HCH × *p,p′*-DDE, and heptachlor × *p,p′*-DDE; however, no statistically significant interaction was found (data not shown in tables).

4. Discussion

In our study, high serum concentrations of β-HCH, *p,p′*-DDE, and heptachlor were associated with a greater risk of breast cancer in the adjusted models, and only β-HCH remained positively associated when the three chemicals were entered in a single model with all covariates. Many OCPs and PCBs have proven to interact with estrogen and/or androgen receptors (Bonefeld Jorgensen et al., 1997; Bonefeld-Jorgensen et al., 2001; Grunfeld and Bonefeld-Jorgensen, 2004; Sonnenschein and Soto, 1998; Soto et al., 1998), and epidemiological efforts have traditionally focused on hormone-dependent cancers, e.g., breast and prostate tumors (Xu et al., 2010). In this regard, the endocrine disrupting properties of these chemicals have been demonstrated in previous studies. β-HCH has been reported to promote

Table 1
Description of the study population.

	Cases (n = 69)					Controls (n = 54)					p-Value
	Mean	SD[a]	Percentiles			Mean	SD[a]	Percentiles			
			25th	50th	75th			25th	50th	75th	
Age (years)	49.9	11.0	42.0	49.0	57.5	43.9	8.7	38.0	43.5	50.0	0.001
Body mass index (kg/m^2)	27.1	5.5	22.9	26.2	30.5	26.7	4.4	23.8	26.1	28.6	0.967
Age at menarche (years)	13.0	1.6	12.0	13.0	14.0	12.9	1.5	12.0	13.0	13.3	0.881
Number of children	2.8	2.0	1.5	3.0	4.0	2.2	1.4	1.0	2.0	3.0	0.056
Accumulated breastfeeding time (months)	31.4	38.5	2.0	18.0	48.0	23.3	28.3	5.3	12.0	36.0	0.663
Age at last breastfeeding (years)	27.1	14.8	27.0	32.0	38.0	28.5	13.0	28.3	32.5	36.8	0.901
Size of first nodule (cm)	2.3	2.6	0.3	1.8	3.5	–	–	–	–	–	
		n	%				n	%			
Marital status											0.999
Single		10	14.5				8	14.8			
Married/divorced		59	85.5				46	85.2			
Occupational class											<0.001
Homemaker		49	71.0				15	27.8			
Manual worker		5	7.3				8	14.8			
Non-manual worker		15	21.7				31	57.4			
Education											<0.001
Up to primary		53	76.8				19	35.2			
Secondary		4	5.8				22	40.7			
University		12	17.4				13	24.1			
Residence											<0.001
Urban		40	58.0				48	88.9			
Rural		29	42.0				6	11.1			
Menopausal status											0.006
Pre menopausal		34	49.3				40	74.1			
Post menopausal		35	50.7				14	25.9			
Parity											0.133
Nulliparous		14	20.3				8	14.8			
≥1 children		55	79.7				46	85.2			
Family history of breast cancer		17	24.6				0	0.0			–
Smoker		2	2.9				2	3.7			0.999
Alcohol consumption		0	0.0				0	0.0			–

[a] Standard deviation.

tumors in mice (Wong and Matsumura, 2007), as well as the transformation and invasiveness of human MCF-7 breast cancer cells (Zou and Matsumura, 2003). In addition, heptachlor has been shown to induce estrogenic effects in various in vitro assays (Chow et al., 2013) or human breast preneoplastic and cancerous cell lines (Shekhar et al., 1997), and to modulate estrogenic function in rainbow trout hepatocytes (Okoumassoun et al., 2002). However, Kim et al. (2011) found no evidence of estrogenic activity induced by heptachlor in a stably

Table 2
POP concentrations in cases and controls.

		Cases (n = 69)						Controls (n = 54)						p-Value
		% ≥ LOD[a]	Mean	SD[b]	Percentiles			% ≥ LOD[a]	Mean	SD[b]	Percentiles			
					25th	50th	75th				25th	50th	75th	
β-HCH	ng/mL	55.1	0.14	0.32	<LOD	0.05	0.11	33.3	<LOD	–	<LOD	<LOD	0.05	0.005
	ng/g lipid		25.17	57.74	<LOD	9.29	18.06		<LOD	–	<LOD	<LOD	9.51	0.003
α-Endosulfan	ng/mL	26.1	<LOD	–	<LOD	<LOD	0.05	33.3	<LOD	–	<LOD	<LOD	0.05	0.508
	ng/g lipid		–	–	<LOD	<LOD	7.76		<LOD	–	<LOD	<LOD	7.92	0.531
Endosulfan-ether	ng/mL	0.0	<LOD	–	<LOD	<LOD	<LOD	0.0	<LOD	–	<LOD	<LOD	<LOD	–
	ng/g lipid		<LOD	–	<LOD	<LOD	<LOD		<LOD	–	<LOD	<LOD	<LOD	–
HCB	ng/mL	94.2	0.19	0.16	0.09	0.13	0.22	100	0.14	0.06	0.09	0.12	0.16	0.151
	ng/g lipid		33.48	29.23	17.09	21.60	40.24		23.91	10.02	18.25	19.98	28.22	0.201
Heptachlor	ng/mL	89.9	0.13	0.09	0.07	0.12	0.15	79.6	0.09	0.06	0.05	0.08	0.11	0.001
	ng/g lipid		22.49	15.12	13.05	19.90	26.57		14.88	14.86	6.36	12.39	16.33	0.001
Oxychlordane	ng/mL	7.2	<LOD	–	<LOD	<LOD	<LOD	3.7	<LOD	–	<LOD	<LOD	<LOD	0.740
	ng/g lipid		<LOD	–	<LOD	<LOD	<LOD		<LOD	–	<LOD	<LOD	<LOD	0.582
PCB 138	ng/mL	98.6	0.21	0.10	0.14	0.18	0.23	100	0.17	0.08	0.13	0.15	0.18	0.003
	ng/g lipid		37.74	19.37	25.68	31.45	42.60		28.59	16.64	22.26	26.08	31.33	0.002
PCB 153	ng/mL	100	0.72	0.27	0.53	0.68	0.83	100	0.68	0.21	0.54	0.62	0.76	0.463
	ng/g lipid		131.54	51.58	94.13	118.95	149.07		119.07	35.96	88.90	111.99	149.57	0.412
PCB 180	ng/mL	98.6	0.23	0.12	0.15	0.19	0.27	98.1	0.18	0.06	0.14	0.17	0.23	0.087
	ng/g lipid		41.35	24.57	26.05	34.78	49.01		31.74	11.00	22.76	29.84	40.44	0.095
p,p′-DDE	ng/mL	100	2.10	3.05	0.60	1.07	2.08	98.1	1.07	1.18	0.38	0.60	1.53	0.009
	ng/g lipid		381.97	562.49	106.18	196.49	362.86		215.05	214.72	66.02	127.59	276.93	0.008

[a] Limit of detection.
[b] Standard deviation.

Table 3
Serum POP concentrations and risk of breast cancer. Logistic regression analyses.

	Wet-basis model						Lipid-basis model					
	Unadjusted model			Adjusted model[a]			Unadjusted model			Adjusted model[a]		
	OR	95% CI[b]		OR	95% CI[b]		OR	95% CI[b]		OR	95% CI[b]	
		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
β-HCH (ng/dL)	1.09**	1.02	1.19	1.16**	1.05	1.30	1.05**	1.01	1.11	1.10**	1.03	1.18
<LOD	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
>LOD	2.45**	1.18	5.21	3.44**	1.30	9.72	2.45**	1.18	5.21	3.44**	1.30	9.72
α-Endosulfan (ng/dL)	0.99	0.92	1.07	0.96	0.87	1.06	1.00	0.95	1.04	0.98	0.92	1.03
<LOD	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
>LOD	0.71	0.32	1.54	0.72	0.25	1.98	0.71	0.32	1.54	0.72	0.25	1.98
HCB (ng/dL)	1.04**	1.01	1.09	1.04	0.98	1.12	1.03**	1.00	1.06	1.02	0.99	1.07
T1	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
T2	0.91	0.38	2.16	1.95	0.58	6.99	0.55	0.23	1.32	1.21	0.36	4.15
T3	2.05	0.84	5.13	2.73	0.62	13.01	1.89	0.77	4.80	2.94	0.68	13.80
Heptachlor (ng/dL)	1.09**	1.03	1.17	1.06*	1.00	1.15	1.05**	1.01	1.09	1.03*	1.00	1.08
T1	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
T2	1.34	0.56	3.24	1.31	0.42	4.14	1.34	0.56	3.24	1.27	0.40	4.07
T3	5.02**	1.97	13.74	3.24*	0.92	12.18	5.02**	1.97	13.74	2.87*	0.93	10.63
PCB 138 (ng/dL)	1.06**	1.01	1.12	1.03	0.97	1.10	1.04**	1.01	1.07	1.02	0.99	1.06
T1	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
T2	1.48	0.62	3.58	2.06	0.58	7.77	1.22	0.51	2.92	2.31	0.67	8.54
T3	4.38**	1.74	11.67	4.40	0.90	18.88	3.96**	1.58	10.52	2.99	0.75	13.42
PCB 153 (ng/dL)	1.01*	0.99	1.02	1.01	0.98	1.03	1.01*	1.00	1.02	1.01	0.99	1.02
T1	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
T2	1.01	0.42	2.39	1.08	0.30	3.91	0.91	0.38	2.16	0.63	0.17	2.16
T3	1.35	0.56	3.28	1.23	0.29	5.31	1.50	0.62	3.66	1.25	0.31	5.11
PCB 180 (ng/dL)	1.05**	1.01	1.10	1.03	0.96	1.10	1.03**	1.01	1.05	1.01	0.98	1.06
T1	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
T2	1.34	0.56	3.23	1.28	0.36	4.67	1.48	0.62	3.58	1.33	0.40	4.45
T3	1.82	0.76	4.46	1.62	0.37	7.32	1.64	0.69	3.99	1.11	0.26	4.64
p,p′-DDE (ng/mL)	1.33**	1.06	1.83	1.72**	1.11	3.13	1.18**	1.04	1.42	1.37**	1.07	1.94
T1	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
T2	3.73**	1.52	9.57	7.79**	2.04	35.31	2.71**	1.12	6.75	6.26**	1.62	28.33
T3	3.00**	1.24	7.54	7.08**	1.15	38.47	3.01**	1.24	7.58	9.65**	1.81	63.33

In order to facilitate interpretation of the coefficients, wet-basis concentrations were all entered in units of ng/mL, with the exception of *p,p′-DDE* (ng/dL), while lipid-basis concentrations were all entered in units of mg/g lipid, with the exception of *p,p′-DDE* (ng/g lipid).
NS: Not statistically significant. T: Tertiles of POP concentrations.
[a] Adjusted for age, body mass index, occupational class, residence, education, accumulated lactation time, parity, menopausal status, family history of breast cancer, total serum lipids.
[b] Confidence interval.
* $p < 0.100$.
** $p < 0.050$.

transfected human estrogen receptor-α transcriptional activation assay. Furthermore, researchers using MCF-7 breast cancer cells observed that several constituents of the pesticide DDT (i.e., *o,p′*-DDT, *o,p′*-DDE, *p,p′*-DDT, and *p,p′*-DDE) induced cell proliferation (Andersen et al., 1999; Shekhar et al., 1997; Soto et al., 1995).

In addition, there is a growing evidence that these chemicals might also cause cancer via other action mechanisms, including: disruption of the epigenomic landscape in cancers (reviewed by: Knower et al., 2014), the induction of enzymes that produce genotoxic intermediates and DNA adduct (Yanez et al., 2004), and an increase in reactive oxygen and nitrogen species through the induction of cytochrome P450 or mitochondrial alterations (Karami-Mohajeri and Abdollahi, 2011). This issue becomes even more complex if we take into account evidence of potential gene–environment interactions affecting the putative relationship between POP exposure and breast cancer, which include a potential modifying effect of cytochrome P4501A1 (CYP1A1) and the p53 gene on the effect of PCBs on cancer (Hoyer et al., 2002; Laden et al., 2002; Moysich et al., 1999; Zhang et al., 2004).

At an epidemiological level, a number of studies have investigated the association between human exposure to POPs and the risk of breast cancer, but their conclusions have been controversial, with some authors reporting positive associations but many others finding no evidence to support a causal association (Cassidy et al., 2005; Charlier et al., 2003a,2003b; Demers et al., 2000, 2002; Gatto et al., 2007; Hoyer et al., 2001; Itoh et al., 2009; Laden et al., 2001; Lopez-Carrillo et al., 1997, 2002; Lopez-Cervantes et al., 2004; Olaya-Contreras et al., 1998; Recio-Vega et al., 2011; Snedeker, 2001; Ward et al., 2000; Wolff et al., 2000a,2000b; Zheng et al., 1999a,1999b). Hoyer et al. (2001) found no association between organochlorine pesticides and breast cancer but observed that they might contribute to a worse prognosis. These discrepancies may be attributable to various factors, including differences in study designs, in biological matrices used to estimate exposure, and in target populations, with highly varied historical and current exposure levels to POPs and distinct ethnicities, age groups and/or dietary patterns. Epidemiological studies reporting positive associations also differ in the chemicals responsible for the observed effect, including POPs such as DDT and HCB (Charlier et al., 2003a), *p,p′*-DDE (Olaya-Contreras et al., 1998), or PCBs (Recio-Vega et al., 2011).

We cannot rule out that the associations found with single chemicals are a surrogate of exposure to other unmeasured pollutants with similar physicochemical properties or even to mixtures of pollutants that exert a combined effect. In fact, heptachlor epoxide levels (an oxidation product and one of the most important metabolites of heptachlor) were previously found to be positively associated with the prevalence of breast cancer in biopsies and to contribute to the initiation, promotion, and progression of cancer (Cassidy et al., 2005). Likewise, the risk of breast cancer was found to increase with higher adipose tissue concentrations of the pesticide lindane (in which β-HCH is commonly present) in a case–control study of a female population recruited in Southern Spain (Ibarluzea et al., 2004). The concentrations of many POPs are often positively correlated, which poses methodological problems in the statistical modeling (Holford et al., 2000). In this regard, our population

showed positive correlations between β-HCH and HCB levels (Spearman coefficient = 0.27, $p < 0.05$, Supplementary Material) and between heptachlor and the concentrations of the three PCB congeners (Spearman coefficients = 0.2–0.3, $p < 0.05$, Supplementary Material). In our study, no statistically significant interaction was found between POP concentrations in the final model. However, there is a need to developing biomarkers of combined effects in order to improve our understanding of potential interactions among chemicals. Assessment of the effects of human exposure to environmental chemicals is a highly complicated issue, given that most individuals are exposed to complex mixtures of chemicals that can exert synergic and/or antagonistic effects (Aube et al., 2011) and can interact with endogenous hormones (Bonefeld-Jorgensen et al., 2014; Sonnenschein and Soto, 1998; Soto et al., 1998). In a study of a population from the Canary Islands (Spain), Boada et al. (2012) concluded that a combination of aldrin, *p,p′*-DDE and dichlorodiphenyldichloroethane may represent a risk factor for breast cancer.

The development and treatment of cancer usually implies modifications in metabolism and adipose tissue mobilization, which might alter serum POP concentrations (Boada et al., 2012). Hence, it is theoretically possible that the higher concentrations observed in cases are a consequence of the disease rather than the other way round. However, as argued by Charlier et al. (2003b), this seems unlikely given that samples were taken at the time of diagnosis in individuals with no history of cancer and before the start of chemotherapy or radiotherapy.

The use of serum POP concentrations has some limitations in comparison to adipose tissue. It has been reported that serum POP levels are good estimators of ongoing exposure but not always of long-term exposure, because they can be affected by lipid mobilization and current exposure levels (Archibeque-Engle et al., 1997; Crinnion, 2009). However, in the present study, all women were sampled under 12-h fasting conditions and adjustment for total serum lipids was performed in the multivariable models, which are known methods for minimizing this bias, at least in part (Charlier et al., 2003b; Schisterman et al., 2005).

No adjustment for food intake was performed in the present study. Diet, especially of animal origin, has been acknowledged as an important risk factor for cancer (Vieira et al., 2011) but is also responsible for most of the POP exposure in the general population (Agudo et al., 2009). Our hypothesis is that previously observed positive associations between the consumption of certain fatty foods and cancer risk (Jordan et al., 2013; Khodarahmi and Azadbakht, 2014) might be partially caused by the POP present in these food items. Therefore, we consider that adjustment for diet would imply the inclusion of covariates that are in the same causal pathway (diet ➔ POPs ➔ cancer).

In the present study, serum concentrations of organochlorine pesticides were comparable to those reported previously in some populations in Tunisia (Ben Hassine et al., 2014; Ennaceur and Driss, 2010) and other Mediterranean countries, such as Egypt (Ahmed et al., 2002), Italy (Bergonzi et al., 2009), Spain (Llop et al., 2010), or Greece (Vafeiadi et al., 2014). However, PCB concentrations were in general higher than those reported in other Tunisian populations and were comparable to those reported in more industrialized Mediterranean countries such as Italy (Bergonzi et al., 2009) and Spain (Llop et al., 2010), which may be attributable to the recent rapid technological development of Tunisia.

Although the sample size of our study was relatively limited, it yielded significant results that were consistent in both bivariate and multivariable models. The findings of the present study suggest a potential association between baseline serum concentrations of at least one organochlorine pesticide and breast cancer, underlining the need for measures to reduce current exposure levels. However, our results should be interpreted with caution and need to be verified in further studies.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.03.045.

Conflict of interest

The authors declare no conflict of interest.

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