Risk and Aggressiveness of Breast Cancer in Relation to Plasma Organochlorine Concentrations[1]

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Abstract

Several organochlorines identified as "hormone mimics" were proposed as possible risk factors for breast cancer. We conducted a case-control study to assess breast cancer risk and disease aggressiveness in relation to plasma concentrations of several organochlorine compounds.

Plasma lipid concentrations of 11 chlorinated pesticides and 14 polychlorinated biphenyl congeners were measured in 315 women newly diagnosed with breast cancer, 219 hospital-based controls, and 307 population controls from the Quebec City area (Canada). Concentrations of hormonally active organochlorines or their surrogates were compared between cases and controls as well as between groups of cases defined according to tumor size and axillary-lymph-node involvement.

We found similar levels of organochlorines in cases and controls and no relationship between the relative risk of breast cancer and organochlorine exposure. However, the probability of lymph-node invasion among cases increased with exposure to 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene [*p,p′*-DDE; odds ratio, 2.54; 95% confidence interval (CI), 1.20–5.35; between the highest and the lowest tertiles]. Furthermore, *p,p′*-DDE exposure was associated with a dose-related increased relative risk of exhibiting both lymph-node involvement and a large tumor. Indeed odds ratio raised to 2.33 (95% CI, 0.94–5.77) for the second tertile relative to the first tertile and reached 3.51 (95% CI, 1.41–8.73) for the third tertile relative to the first tertile. Similar associations were noted with *β*-hexachlorocyclohexane, oxychlordane, and *trans*-nonachlor.

We conclude that exposure to persistent, hormonally active organochlorines during adulthood is not associated with breast cancer risk. The possibility that some organochlorines and especially *p,p′*-DDE may increase breast cancer aggressiveness deserves further attention.

Introduction

Exposure to environmental chemicals and particularly to organochlorines has been suggested as a possible cause of breast cancer (1). This group of persistent, lipophilic chemicals comprises industrial compounds such as PCBs[3] and agricultural pesticides, such as chlordane, DDT, and mirex, which were extensively used in the past and can be found in all ecosystems of the planet. Biological half-lives of several years have been documented in humans for the most persistent organochlorines (2, 3), resulting in their accumulation with age in body fat, including adipose tissue (4), blood lipids (5), and milk fat (6). Studies conducted 30 years ago revealed the estogenic properties of several commercial mixtures of PCBs and of *o,p′*-DDT, a minor constituent of technical DDT (7, 8). Since that time, several other organochlorines (*p,p′*-DDT, chlordane, dieldrin, endosulfan, *β*-HCH, toxaphene) were shown to elicit estrogenic responses in various *in vitro* systems (9–11). Given the suspected implication of estrogens in the pathogenesis of breast cancer (12), it has been proposed that these weakly estrogenic organochlorine compounds might be a risk factor for the disease. This hypothesis is supported by results of experimental studies in rats showing that o,p′-DDT can alter mammary gland differentiation and cell proliferation (13) and promote the growth of mammary tumors (14, 15).

Results from early human studies generally supported the existence of a relationship (16–19) or suggested a possible link (20) between breast cancer risk and organochlorine exposure, more specifically with *p,p′*-DDE, the main metabolite of DDT. In contrast, recent studies involving larger sample sizes yielded negative results (21–25). In particular, Hunter *et al.* (21) and Høyer *et al.* (25), using a nested case control study design, failed to observe a relationship between *p,p′*-DDE or PCB plasma concentrations and breast cancer risk. However, Høyer *et al.* (25) reported that high plasma concentrations of dieldrin were associated with breast cancer risk.

Previous studies have focused solely on the risk of developing a new breast cancer. However, hormonally active organochlorines might also modulate cancer growth. The present study tests the hypothesis that the risk of developing breast cancer is related to exposure to selected organochlorines that

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[3] The abbreviations used are: PCB, polychlorinated biphenyl; DDT, dichlorodiphenyltrichloroethane; *o,p′*-DDT, 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1,1-trichloroethane; *p,p′*-DDT, 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane; *p,p′*-DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; *β*-HCH, *β*-hexachlorocyclohexane; BMI, body mass index; OR, odds ratio; CI, confidence interval; ER, estrogen receptor.

either possess estrogenic properties themselves or are surrogates of past exposure to less persistent estrogenic compounds. In addition, we also investigated the possible relationship between exposure to organochlorines and two indicators of breast cancer aggressiveness and prognosis: axillary-lymph-node involvement and tumor size.

Materials and Methods

Subjects. From October 1994 to March 1997, 315 women with histologically confirmed infiltrating primary breast cancer and 219 controls were recruited in four hospitals of the Quebec City area (Quebec, Canada). A second control group included 307 women randomly selected from the general population files of the Régie de l'Assurance maladie du Québec. Cases and controls were matched for age (5-year age groups) and region of residence (rural/urban). Cases were excluded if they had a previous history of breast cancer or any other cancer (except cervical intraepithelial neoplasm or basocellular skin cancer) or if they showed distant metastasis at diagnosis. All participants had to reside in the Quebec City area and be aged between 30 and 70 years. Hospital controls had, in addition, to be free of gynecological illnesses; they were admitted for digestive surgery (50%), orthopedic surgery (25%), vascular surgery (14%), or other surgeries (11%). Participation rates were 91% for cases, 89% for hospital controls, and 47% for population controls.

Blood samples were obtained from cases and hospital controls after surgery and for cases before the initiation of chemotherapy or radiotherapy. A research nurse visited population controls at their residence, and blood sampling was performed during this visit. Information on demographic, anthropometric characteristics, life style habits, and reproductive history were obtained from cases and controls by telephone interview.

Assay Methods. Blood samples (20 ml) were collected in vials containing EDTA and were centrifuged (10 min, 5000 rpm), and the plasma was transferred in glass vials prewashed with hexane. Plasma samples were stored frozen at −20°C until the time of analysis. A 2-ml aliquot was extracted with hexane, and the lipid extract was cleaned up on Florisil columns. Fourteen PCB congeners (International Union of Pure and Applied Chemistry numbers 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187) and 11 chlorinated pesticides or their metabolites (aldrin, α-chlordane, γ-chlordane, *p,p′*-DDT, *p,p′*-DDE, hexachlorobenzene, β-HCH, mirex, *cis*-nonachlor, *trans*-nonachlor, oxychlordane) were quantified in the eluate using a HP-5890 series II gas chromatograph equipped with dual capillary columns and dual Ni-63 electron-capture detectors. Peaks were identified by their relative retention times obtained on the two columns using a computer program developed in-house. Quantification was mainly performed on the Ultra-1 column. The limit of detection, based on three times the average SD of noise, was 0.02 μg/liter for PCB congeners and chlorinated pesticides, with the exception of *p,p′*-DDT and β-HCH (0.03 μg/liter). The average percentage recoveries were >95% for PCB congeners and ranged from 90 to 103% for chlorinated pesticides. The between-day precision ranged from 3.3 to 7.0% for PCB congeners and from 5.5 to 14.2% for chlorinated pesticides.

Because organochlorines distribute mainly in body fat, and blood was collected from nonfasting participants, results are reported in μg/kg of plasma lipids to take into account the postprandial rise in blood lipids (26). Total and free cholesterol, phospholipids, and triglycerides were quantified by enzymatic assays.

Statistical Analysis. Characteristics of cases were compared to those of control groups using Student's *t* tests for continuous variables or χ^2 tests for categorical variables. Variance analysis was used to compare the mean concentrations of organochlorines between cases and controls. Organochlorine concentrations in plasma lipids displayed log-normal distributions and therefore, these statistical analyses were performed using the natural logarithm of organochlorine concentrations. A concentration equal to half the detection limit was assumed for samples with organochlorine levels below the detection limit.

Point and interval estimates of relative risk were based on unconditional logistic regression analysis. Quintile and tertile limits of plasma organochlorine concentrations were based on the distribution observed among controls. Risks were calculated relative to the lowest category. Age (30–40<, 40–50<, 50–60<, ≥60 years) and region of residence (rural/urban) were included in all multivariate models. The other variables tested for confounding were BMI (kg/m^2), total energy consumed, alcohol consumption, age at first cigarette, number of fertile years, age at first child, total breast feeding duration, use of oral contraceptive, use of hormone therapy, first-degree family history of breast cancer, history of benign breast disease, and time separating blood sampling from surgery. A variable was considered as a confounder when its inclusion in the model modified OR (adjusted for age and region of residence) by >10%. All statistical analyses were performed using the SAS software (SAS Institute Inc., Cary, NC).

The following organochlorines were detected in <70% of plasma samples: aldrin, α-chlordane, γ-chlordane, *cis*-nonachlor, mirex, and PCB congeners 28, 52, 101, 105, and 128. Regardless of their biological activity, these compounds were excluded from statistical analyses because the precision of measurement decreases when values approach the limit of detection. Out of those remaining, the following compounds were selected that either display estrogenic properties or are indicators of past exposure to less persistent xenoestrogens: *p,p′*-DDT, *p,p′*-DDE, β-HCH, *trans*-nonachlor, and oxychlordane. 2,2′,4,4′,5,5′-Hexachlorobiphenyl (PCB-153) was selected as a surrogate for all PCB congeners because this persistent congener is the most abundant in plasma samples and exhibits a strong correlation ($r \geq 0.72$; $P < 0.0001$; Pearson correlation coefficient) with other highly prevalent congeners (99, 118, 138, 156, 170, 180, 183, 187).

Results

Cases and population controls did not differ with regard to most characteristics presented in Table 1, with the exception of family history of breast cancer and history of benign breast disease, which were reported more frequently by cases than controls ($P = 0.001$). Cases were older ($P = 0.01$), had more fertile years ($P = 0.0001$), gave birth to their first child at a later age ($P = 0.002$), showed a smaller BMI ($P = 0.0003$), and were more likely to report a personal history of benign breast disease ($P = 0.001$) than hospital controls but not population controls. Therefore, hospital and populations controls were considered separately in further statistical analyses.

Mean concentrations of chlorinated pesticides and metabolites in women who were diagnosed with breast cancer were not statistically different from those of hospital or population controls (Table 2). The mean plasma PCB-153 level in cases was also essentially the same as in hospital or population controls. Moreover, high concentrations of organochlorines

Table 1 Characteristics of cases and controls

	Cases (n = 315)	Controls	
		Hospital (n = 219)	Population (n = 307)
Age	53 ± 9[a]	51 ± 11	53 ± 10
Age at menarche	13 ± 5	13 ± 2	13 ± 2
No. of fertile years	32 ± 7	29 ± 7	31 ± 7
Age at first birth[b]	25 ± 4	24 ± 5	25 ± 4
No. of deliveries	2.2 ± 2	2.4 ± 2	2.2 ± 2
BMI (kg/m^2)	25 ± 4	26 ± 5	24 ± 4
Breast fed >6 mo[b]	11%	8%	11%
Ever use of hormonal contraceptives	68%	65%	62%
Ever use of hormonal replacement therapy	42%	35%	36%
History of breast cancer in first-degree relatives[c]	22%	16%	12%
History of benign breast disease[d]	35%	20%	17%

[a] Mean ± SD.
[b] Among parous women only.
[c] Data for this variable were available for 310 cases, 214 hospital controls, and 304 population controls.
[d] Cyst puncture or benign breast illness investigated with surgery.

were not related to breast cancer risk, whatever the control group used in OR calculations (Table 3). For instance, the adjusted OR was 1.00 (95% CI, 0.60–1.67) for the highest quintile of plasma *p,p′*-DDE relative to the lowest quintile (comparing cases to population controls). Breast cancer risk increased only slightly with PCB-153 plasma concentrations. The adjusted OR with reference to population controls calculated for the highest quintile of plasma PCB-153 as compared to the lowest quintile was 1.28 (95% CI, 0.74–2.19). Similar results were obtained when using the sum of the 14 PCB congeners to calculate ORs (OR, 1.45; 95% CI, 0.82–2.58; for the highest relative to the lowest quintiles).

Additional statistical analyses were restricted to cases and sought to investigate the possible relation of the aggressiveness of breast cancer to plasma organochlorine concentrations. Among the 299 cases with available information on tumor diameter at pathology, 142 (47%) had a large tumor (largest diameter ≥ 2 cm). In the crude analysis, mean organochlorine plasma levels in this group of patients were not different from those measured in patients with smaller tumors, and the OR did not increase with plasma organochlorine concentrations (data not shown). After adjusting for age, region of residence, BMI, breast feeding duration, number of fertile years, and time separating blood sampling from surgery, the relative risk of having a large tumor increased with plasma concentrations of most organochlorines, although statistically-significant ORs were observed only for β-HCH (OR, 2.25; 95% CI, 1.12–4.51) and *trans*-nonachlor (OR, 2.27; 95% CI, 1.11–4.65), comparing the highest to the lowest tertiles (Table 4).

Among the 273 cases who had axillary surgery, 118 (43%) showed axillary-lymph-node involvement. Again, cases with and without axillary-lymph-node involvement displayed similar mean organochlorine concentrations, and the OR did not increase with organochlorine concentrations in the crude analysis (data not shown). However, after adjusting for the confounding factors, the relative risk of axillary-lymph-node involvement increased with tertiles of most organochlorines and reached statistical significance for *p,p′*-DDE (OR, 2.91; 95% CI, 1.43–5.91; third tertile *versus* first), oxychlordane (OR, 2.34; 95% CI, 1.10–4.97; third tertile *versus* first), and PCB-153 (OR, 2.12; 95% CI, 1.05–4.30; third tertile *versus* first; Table 4).

The relation of breast cancer aggressiveness to plasma concentration of *p,p′*-DDE was further explored by considering both tumor size and lymph-node involvement in the same model (Table 5). ORs of having involved nodes after adjustment for tumor size and confounding factors increased with tertiles of *p,p′*-DDE, reaching 2.54 (95% CI, 1.20–5.35) for the highest tertile as compared to the lowest. In contrast, ORs of having a large tumor after adjustment for lymph-node involvement and confounding factors did not increase with *p,p′*-DDE plasma concentrations (OR, 1.18; 95% CI, 0.56–2.21; for the highest tertile as compared to the lowest). However, using cases with a small tumor and without lymph-node involvement as the referent group, the multivariate relative risk of breast cancer characterized by both a large tumor and lymph-node involvement increased in a dose-related manner with tertiles of *p,p′*-DDE. The OR comparing the second tertile to the first was 2.33 (95% CI, 0.94–5.77) and 3.51 (95% CI, 1.41–8.73) for the third tertile relative to the first. Similar associations were noted with β-HCH (OR, 3.91; 95% CI, 1.47–10.35; third tertile *versus* first), oxychlordane (OR, 3.22; 95% CI, 1.18–8.80; third tertile *versus* first), and *trans*-nonachlor (OR, 3.92; 95% CI, 1.42–10.82; third tertile *versus* first).

Information on the hormonal status of the tumor was available for 292 cases, and 204 (70%) of them had ER-positive tumors. Women with ER-negative tumors and those with ER-positive tumors had similar organochlorine plasma levels (data not shown). There was no interaction between organochlorine exposure and the hormonal status of the tumor with regard to either axillary-lymph-node involvement or tumor size.

Discussion

Our data do not support the hypothesis that exposure to hormonally active organochlorines is related to the risk of developing breast cancer. Although organochlorine plasma levels tended to be higher in cases than controls, differences did not reach statistical significance, and there was no evidence of an increased relative risk associated with organochlorine exposure. Two early prospective studies provided some evidence of a relation between *p,p′*-DDE and breast cancer risk. Wolff *et al.* (19) conducted a nested case-control study involving 58 cases and 171 matched controls from a cohort in New York City of 14,290 women. The relative risk of breast cancer increased with *p,p′*-DDE plasma concentration; the adjusted relative risk in the highest quintile compared to the lowest was 3.7 (95% CI, 1.0–13.5). Krieger *et al.* (20) compared serum levels of PCBs and *p,p′*-DDE in 150 cases (50 white, 50 black, and 50 Asian) and 150 matched controls from a cohort of 57,040 women of the San Francisco Bay area. When all ethnic groups were combined, breast cancer risk was not associated with PCB or *p,p′*-DDE exposure. Among black women, however, an adjusted relative risk of 3.9 (95% CI, 0.9–16.1) was obtained when comparing the third tertile of *p,p′*-DDE to the first. Several recent studies did not observe associations of organochlorines with breast cancer risk (21–25). Our results further confirm that there is little or no relation between breast cancer risk and PCB or *p,p′*-DDE exposure and extend this conclusion to other hormonally active organochlorines. Taken together, results from six large epidemiological studies reported during the last 2 years, including our own, provide little indication that organochlorine exposure is a risk factor for the disease.

Because half-lives of the most persistent organochlorines are in the order of 10–20 years, our measure of exposure is

Table 2 Plasma organochlorine concentrations in cases and controls

Organochlorines	Cases (n = 314)		Hospital controls (n = 218)			Population controls (n = 305)		
	Mean ± SD[a]	Median	Mean ± SD	Median	P^b	Mean ± SD	Median	P^c
β-HCH	21.1 ± 40.5	15.5	19.4 ± 37.1	15.3	0.54	17.5 ± 11.4	15.1	0.86
p,p′-DDE	508.9 ± 491.1	386.0	462.7 ± 447.7	337.0	0.39	480.4 ± 408.1	351.7	0.88
p,p′-DDT	12.7 ± 17.1	9.1	12.5 ± 11.8	8.8	0.70	11.0 ± 6.8	9.0	0.87
Oxychlordane	12.9 ± 5.3	11.9	13.0 ± 6.7	11.8	0.08	12.2 ± 4.5	11.5	0.27
trans-Nonachlor	16.6 ± 7.4	15.2	16.7 ± 8.5	14.7	0.10	16.0 ± 6.3	14.9	0.68
PCB-153	58.7 ± 27.5	55.0	53.3 ± 20.9	50.4	0.85	55.6 ± 23.4	51.1	0.53

[a] Arithmetic mean ± SD; μg/kg, lipid basis.
[b] *P* from ANOVA analysis adjusting for age, region of residence, BMI, breast feeding duration, age at first child, number of fertile years, family history of breast cancer, and history of benign breast disease.
[c] *P* from ANOVA analysis adjusting for age and region of residence.

Table 3 Relative risk of breast cancer according to plasma organochlorine concentrations

Organochlorines	Quintile limits[a]	No. of women[b]	Controls used in OR calculations	
			Hospital OR (95% CI)[c]	Population OR (95% CI)[d]
β-HCH	10.2<	69/49/55	1.00	1.00
	10.2–13.6<	51/42/63	0.71 (0.38–1.33)	0.60 (0.35–1.01)
	13.6–17.2<	60/34/70	0.85 (0.44–1.62)	0.62 (0.37–1.04)
	17.2–22.6<	65/51/54	0.71 (0.38–1.32)	0.86 (0.50–1.49)
	≥22.6	69/42/63	0.83 (0.43–1.61)	0.80 (0.47–1.35)
p,p′-DDE	184.4<	67/44/61	1.00	1.00
	184.4–282.5<	52/41/63	0.85 (0.45–1.59)	0.75 (0.45–1.25)
	282.5–427.8<	56/57/47	0.66 (0.37–1.19)	1.06 (0.62–1.79)
	427.8–680.0<	67/36/69	1.54 (0.81–2.95)	0.86 (0.52–1.42)
	≥680.0	72/40/65	1.36 (0.71–2.63)	1.00 (0.60–1.67)
p,p′-DDT	6.0<	79/49/55	1.00	1.00
	6.0–7.9<	52/44/62	0.85 (0.47–1.54)	0.57 (0.34–0.95)
	7.9–10.6<	50/36/68	1.06 (0.57–1.98)	0.50 (0.30–0.84)
	10.6–15.0<	63/44/60	1.07 (0.59–1.94)	0.71 (0.43–1.19)
	≥15.0	70/45/60	1.37 (0.73–2.56)	0.81 (0.48–1.37)
Oxychlordane	8.4<	53/43/61	1.00	1.00
	8.4–10.6<	62/42/63	1.10 (0.58–2.09)	1.09 (0.65–1.82)
	10.6–12.6<	60/39/66	0.96 (0.49–1.88)	1.00 (0.59–1.69)
	12.6–16.3<	70/44/61	0.81 (0.41–1.61)	1.26 (0.74–2.16)
	≥16.3	69/50/54	0.55 (0.27–1.13)	1.47 (0.83–2.62)
trans-Nonachlor	10.6<	52/48/56	1.00	1.00
	<10.6–13.5	56/36/69	1.25 (0.64–2.42)	0.82 (0.49–1.40)
	13.5–16.9<	93/44/60	1.46 (0.77–2.76)	1.53 (0.91–2.59)
	16.9–20.7<	46/41/65	0.59 (0.29–1.20)	0.69 (0.39–1.23)
	≥20.7	67/49/55	0.74 (0.38–1.47)	1.20 (0.68–2.13)
PCB-153	36.3<	51/47/57	1.00	1.00
	36.3–46.6<	66/43/63	1.02 (0.54–1.94)	1.12 (0.66–1.88)
	46.6–57.1<	56/39/64	0.99 (0.50–1.93)	0.94 (0.55–1.62)
	57.1–69.3<	61/51/54	0.64 (0.33–1.23)	1.18 (0.68–2.05)
	≥69.3	80/38/67	1.07 (0.54–2.12)	1.28 (0.74–2.19)

[a] Organochlorine concentrations are divided in quintiles according to the distribution among the 526 controls (μg/kg, lipid basis).
[b] Cases/hospital controls/population controls.
[c] OR adjusted for age, region of residence, BMI, breast feeding duration, age at first child, number of fertile years, family history of breast cancer, and history of benign breast disease.
[d] OR adjusted for age and region of residence.

probably a good estimate of the body burden that prevailed in women during the decade before diagnosis. Hence, our conclusion and those of other case-control studies conducted on this subject only apply to organochlorine exposure occurring relatively late during adulthood, not to prenatal exposure or exposure during puberty.

Women diagnosed with breast cancer who had higher plasma concentrations of *p,p′*-DDE, β-HCH, oxychlordane, or *trans*-nonachlor were more likely to show both a large tumor (diameter ≥ 2 cm) and axillary-lymph-node involvement. These results suggest that organochlorine exposure may influence the growth or aggressiveness of the disease rather than initiate breast cancer, at least in the range of organochlorine concentrations found in women from the Quebec City area. With regard to breast cancer aggressiveness, only Hunter *et al.* (21) stratified cases according to axillary-lymph-node involvement at diagnosis and reported similar levels of *p,p′*-DDE and PCBs in both groups of women. It is unclear whether these authors made an adjustment for confounding factors. In the present study, crude comparisons of organochlorine concentra-

Table 4 Relative risk of having a large tumor (≥2 cm) or axillary-lymph-node involvement among cases, according to plasma organochlorine concentrations

Pesticides	Tertile limits[a]	Tumor size		Lymph-node involvement	
		≥2 cm/<2 cm	OR[b] (95% CI)	+/−	OR[b] (95% CI)
β-HCH	12.7<	49/46	1.00	42/49	1.00
	12.7–18.9<	33/62	0.65 (0.34–1.25)	27/54	0.89 (0.44–1.79)
	≥18.9	60/49	2.25 (1.12–4.51)	49/52	2.03 (0.98–4.19)
p,p′-DDE	250.0<	42/55	1.00	32/57	1.00
	250.0–495.3<	45/43	1.39 (0.74–2.62)	38/43	2.06 (1.02–4.15)
	≥495.3	55/59	1.64 (0.87–3.08)	48/55	2.91 (1.43–5.91)
p,p′-DDT	7.2<	46/58	1.00	40/57	1.00
	7.2–11.8<	44/44	1.43 (0.77–2.64)	36/43	1.31 (0.68–2.53)
	≥11.8	52/55	1.59 (0.84–3.03)	42/55	1.51 (0.77–2.95)
Oxychlordane	10.0<	43/39	1.00	37/46	1.00
	10.0–13.7<	46/62	0.99 (0.52–1.89)	35/57	1.12 (0.56–2.24)
	≥13.7	53/56	1.67 (0.81–3.44)	46/52	2.34 (1.10–4.97)
trans-Nonachlor	12.6<	37/44	1.00	34/41	1.00
	12.6–17.9<	54/64	1.52 (0.80–2.89)	43/65	1.32 (0.66–2.63)
	≥17.9	51/49	2.27 (1.11–4.65)	41/49	1.95 (0.92–4.15)
PCB-153	43.0<	38/45	1.00	32/43	1.00
	43.0–61.7<	51/52	1.39 (0.73–2.64)	38/59	1.22 (0.61–2.43)
	≥61.7	53/60	1.49 (0.77–2.86)	48/53	2.12 (1.05–4.30)

[a] Organochlorine concentrations are divided in tertiles according to the distribution among the 526 controls (μg/kg, lipid basis).
[b] ORs are adjusted for age, region of residence, BMI, time separating blood sampling from surgery, breast feeding duration, and number of fertile years.

Table 5 Relative risk of aggressive breast cancer among cases in relation to plasma *p,p′*-DDE concentrations

	Tertile 1[a] (<250.0)	Tertile 2 (250.0–495.3<)	Tertile 3 (≥495.3)
Tumor size			
% tumors ≥2 cm	45	51	49
Total no. of cases	90	86	111
OR (95% CI)[b,c]	1.00	1.11 (0.52–2.04)	1.18 (0.56–2.21)
Lymph-node involvement			
% with invaded nodes	34	47	47
Total no. of cases	79	79	99
OR (95% CI)[b,c]	1.00	1.89 (0.91–3.93)	2.54 (1.20–5.35)
Lymph-node involvement and tumor size			
% lymph node positive and tumors ≥2 cm	20	33	34
Total no. of cases	79	79	99
OR (95% CI)[b,d]	1.00	2.33 (0.94–5.77)	3.51 (1.41–8.73)

[a] *p,p′*-DDE concentrations are divided in tertiles according to the distribution among the 526 controls (μg/kg, lipid basis).
[b] ORs are adjusted for age, region of residence, BMI, time separating blood sampling from surgery, breast feeding duration, and number of fertile years.
[c] ORs for lymph-node involvement were adjusted for tumor size, and ORs for tumor size were adjusted for lymph-node involvement.
[d] OR of having positive nodes and a tumor ≥2 cm *versus* having negative nodes and a tumor <2 cm. Women with a tumor <2 cm and negative lymph-node status represent respectively 35, 31, and 34% of women in tertiles 1, 2, and 3.

tions and OR calculations did not reveal any association either with axillary-lymph-node involvement or tumor size. Statistically significant associations were revealed only after adjustment for confounding factors. Age was the most important confounding factor because it was strongly associated with both organochlorine plasma levels and lymph-node involvement. Log values of *p,p′*-DDE concentration increased with age ($r = 0.36$; $P = 0.0001$; Pearson correlation coefficient), and women <50 years of age were more likely to exhibit axillary-lymph-node involvement than older women (55% *versus* 37%; $\chi^2 = 8.2$; $P = 0.004$).

Alternatively, the toxicokinetics of persistent lipophilic organochlorines may be different in women developing a more aggressive disease, leading to higher plasma concentrations and artifactual associations. In particular, it might be argued that because blood samples in the present study were collected near the time of diagnosis, the disease process might have modulated organochlorine concentrations in plasma lipids. Indeed, severe weight loss can lead to an augmentation in organochlorine plasma lipid levels (27, 28). However, this is unlikely to have affected our results because severe weight loss is not expected in early stages of breast cancer. Few cases in our study reported losing weight during the year before diagnosis (4.5% of cases with positive lymph-node status and 4.3% of those with negative lymph-node status).

The mechanism by which organochlorines might influence the growth or aggressiveness of breast cancer is not clear but may involve the capacity of some of them to mimic or antagonize the effects of endogenous sex hormones. There are no data on the endocrine disrupting potential of oxychlordane and *trans*-nonachlor. Both compounds are indicators of past exposure to chlordane, which displayed weak estrogenic activity in a yeast-based ER assay (11). β-HCH was shown to stimulate the proliferation of MCF-7 and T47D breast cancer cell lines (10). *p,p′*-DDT, the main component of technical DDT, also elicited weak estrogenic effects in several *in vitro* systems (9,

29, 30). In addition to being an indicator of past DDT exposure, *p,p′*-DDE has been identified as a potent antiandrogen agent (31). Androgens have been shown to inhibit the proliferation of hormone-responsive breast cancer cell lines (32) and the growth of mammary carcinoma in the rat (33). This inhibitory effect on tumor growth was alleviated by blocking androgen receptors with flutamide (33). The antiandrogen *p,p′*-DDE could in a similar way counteract cancer growth inhibition normally exerted by androgens and possibly accelerate breast cancer progression.

In conclusion, we observed no association between exposure during adulthood to various persistent, hormonally active organochlorines and breast cancer risk. However, this exposure was linked to an increased risk of having a large tumor and axillary-lymph-node involvement among cases. Replication of these results and additional studies examining other prognostic factors as well as survival are needed to determine whether exposure to organochlorines, especially to *p,p′*-DDE, can lead to more aggressive breast tumors and a less favorable clinical course.

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