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Breast Cancer, Lactation History, and Serum Organochlorines

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The authors analyzed the relation between lactation history, organochlorine serum levels—in particular, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE)—and the risk of breast cancer within a subsample from a larger breast cancer case-control study conducted among women living in Mexico City, Mexico, between 1990 and 1995. From the original study, they selected a random sample of 260 subjects (1:1 case/control ratio). Analysis was restricted to 120 cases and 126 controls who had given birth to at least one child and had complete information on all key variables. Serum DDE levels were higher among cases (mean = 3.84 μg/g lipids, standard deviation = 5.98) than among controls (mean = 2.51 μg/g lipids, standard deviation = 1.97). After adjustment for age, age at menarche, duration of lactation, Quetelet index, and serum DDT levels, serum DDE levels were positively related to the risk of breast cancer (adjusted odds ratio $(OR)_{Q1\text{-}Q2}$ = 1.24, 95% confidence interval (CI): 0.50, 3.06; $OR_{Q1\text{-}Q3}$ = 2.31, 95% CI: 0.92, 5.86; $OR_{Q1\text{-}Q4}$ = 3.81, 95% CI: 1.14, 12.80; test of trend, p = 0.02). The increased risk associated with higher serum DDE levels was more apparent among postmenopausal women ($OR_{Q1\text{-}Q4}$ = 5.26, 95% CI: 0.80, 34.30; test of trend p = 0.03). A longer period of lactation was associated with a slightly decreased risk of breast cancer independently of serum DDE levels (OR = 0.91, 95% CI: 0.85, 0.99 change in risk per 10 months of lactation). Serum DDT level was not related to the risk of breast cancer. The data suggest that high levels of exposure to DDE may increase women's risk of breast cancer, particularly among postmenopausal women. *Am J Epidemiol* 2000;152:363–70.

breast neoplasms; DDE; lactation; polychlorinated biphenyls

In recent years, considerable attention has been given to the hypothesis that persistent organochlorines (particularly 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) or 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) may be associated with breast cancer. Overall, recent studies suggest that exposure to low levels of these toxins is unlikely to be associated with breast cancer risk (1, 2). However, the information available remains inconclusive, particularly for high levels of exposure (3–16). Five studies reported elevated levels of DDT or DDE (the main metabolite of DDT) among women with breast cancer (3, 7–9, 15). Seven studies found no differences in DDT or DDE levels between women with breast cancer and those without (4–6, 10–12, 14, 16), and one study (13) reported lower DDE levels in adipose tissue among women with breast cancer. The limitations of some of these studies have been discussed elsewhere (10, 17), particularly the lack of information about duration of lactation, as well as about other potential sources of estrogens, such as body mass (as a source of endogenous androgens) and replacement estrogen therapy among postmenopausal women. This is important, given that exposure to organochlorines that exert a weak estrogenic effect may increase women's risk of breast cancer (1). In some earlier studies (2–4, 6–7, 10), the lack of controlling for other sources of estrogens or potential confounders, such as lactation, may have masked a real association between organochlorine exposure and breast cancer.

In Mexico, current use of DDT is restricted to specific applications, mainly in agriculture and public health programs to control mosquitoes that transmit dengue and malaria (18). Data about levels of DDT in food and human tissue from Mexico City are scarce. However, given that foods from all regions of the country are shipped to Mexico City and that DDT accumulates in the body and has a long half-life, exposure levels to these toxins were expected to be elevated (18, 19) and most likely were higher than those reported in recent studies from the United States and Europe (12–15).

To examine further the relation between high levels of exposure to organochlorine and the risk of breast cancer, we analyzed a subsample of serum samples previously collected during a case-control study on the determinants of breast cancer among women living in Mexico City (20). For

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Abbreviations: CI, confidence interval; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; FC, free cholesterol; PCB, polychlorinated biphenyl; SD, standard deviation

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this report, we restricted the analysis to participants who breast-fed and did not use replacement hormone therapy.

MATERIALS AND METHODS

Study population

Participants included in this report were a subsample of cases and controls who were enrolled in a study to assess the relation between diet and reproductive factors and breast cancer risk among residents of Mexico City (20).

Cases were recruited using a network of six hospitals that were part of the two major health providers in Mexico City, the Social Security system and the Ministry of Health. From 1990 to 1995, new (incident) cases with a histologic diagnosis of breast cancer were identified through gynecologic clinics among women attending for a biopsy of a breast lump. Only women for whom the biopsy confirmed the diagnosis of breast cancer were included in the study. A total of 537 breast cancers were identified. Of those, 94 percent ($n = 505$) agreed to participate; of these, 130 were selected at random for determination of DDE and DDT.

Controls were an age-stratified random sample of the residents of the Mexico City metropolitan area. Households were first randomly selected by using the National Household Sampling Frame. Selected households were then visited by study personnel to ascertain whether the selected unit contained a woman who matched the age group of the case and who agreed to provide a venous blood sample and to be interviewed. If so, an interview was arranged at the home of the control. If not, the procedure was repeated. Only one eligible control was included per sampling unit. Of 1,534 eligible controls, 89 percent ($n = 1,365$) agreed to participate in the study. Of these, we selected at random 130 for serum DDT/DDE measurements.

Interviewers administered a questionnaire that asked about sociodemographic variables, potential risk factors for breast cancer (including lifestyle habits), and reproductive and gynecologic history; the interview also included a food frequency questionnaire. Information on lactation history was obtained for each livebirth. Cases were interviewed at the gynecologic clinics of the recruitment hospitals before confirmatory diagnosis, while for controls interviews were conducted in the participants' home.

Laboratory analysis

For the determination of organochlorinated pesticides (21–23), 2 ml of blood plasma were spiked with polychlorinated biphenyl (PCB) 198 as an internal standard, homogenized with saturated aqueous ammonium sulfate:ethanol (1:1 v/v), and extracted with hexane (3 × 6 ml). The organic extract was concentrated to ~1 ml, and cleaned up on two deactivated Florisil columns (Fischer, Montreal, Quebec, Canada) arranged in tandem. Compounds were eluted with methylene chloride:hexane (25:75 v/v) and concentrated to 100 μl prior to analysis on an HP-5890 series II gas chromatograph equipped with dual-capillary columns (HP Ultra 1 and Ultra 2, both 50 m long, 0.2 mm internal diameter, 0.33 μm coating) and dual Ni-63 electron-capture detectors (Hewlett-Packard (Canada), Ltd., Montreal, Quebec, Canada). Peaks were identified by their relative retention times obtained on the two columns with the use of a computer program developed in house. The Ultra-1 column was used for quantitation. The limit of detection, based on three times the average standard deviation of noise, was 0.02 μg/liter for each pesticide. The average percentage recoveries ranged from 90 to 103 percent. The between-day precision ranged from 3.4 percent for DDE to 14.2 percent for other pesticides.

Internal quality control was performed by inserting a control sample (NIST SRM no. 1589, spiked with 0.4 μg/liter of each OC pesticide) after every nine samples. The between-day precision ranged from 3.4 percent (for DDE) to 14.2 percent. We verified the accuracy of our measurements by participating in the following interlaboratory comparison programs: 1) AES, QA/QC Program (Arctic Strategy Program, Environment Canada) for verification of measurements of PCBs and organochlorinated pesticides in isooctane and in extracts of adipose tissue of seal and polar bears; 2) the Canadian Association for Environmental Analytical Laboratories (CAEAL), Inc., for verification of measurements of PCBs and organochlorinated pesticides in water.; and 3) the Great Lakes Research Program's (GLRP) QA\QC project, Community Public Health Agency, Michigan, for verification of measurements of PCBs and organochlorinated pesticides in plasma. Our results were in excellent agreement with the target values in all cases. Results are presented with recovery correction. The laboratory was blinded to the case-control status of the samples.

Total serum lipid levels were estimated from measurements of total cholesterol and triglycerides. Free cholesterol (FC) levels were defined as 0.27 × total cholesterol levels as proposed by Cheek and Wease (24). Estimates of total serum lipid levels were calculated by adding the individual lipid components by the formula: total serum lipid levels = 1.677(total cholesterol – FC) + FC + triglycerides + 0.623 as proposed by Phillips et al. (25). Because variations in serum levels of organochlorine generally correlate with serum lipid variation associated with fasting and postprandial states, we expressed organochlorine levels on a lipid weight basis. This was necessary to ensure the comparability of the results, given that we were not assured that blood samples were collected from fasting individuals.

Statistical analysis

Our main goals were to identify important determinants of DDT and DDE serum levels and to assess the potential influence of these toxins in the risk of breast cancer. We expressed serum DDT and DDE levels on the basis of lipid weight and log-transformed them to normalize their distribution. We examined univariate and bivariate statistics, tabulations, and distribution plots for all variables.

We first studied the distribution DDT/DDE in relation to age groups, using indicator variables for the 5-year age categories. We also modeled the age effect using linear and quadratic terms to account for the nonlinear relation that was

observed between age and DDT/DDE levels (figure 1). We then included all other potential predictors of DDT/DDE levels. For these analyses, we used linear regression models (26).

We assessed the relation between serum DDT and DDE levels and risk of breast cancer using multivariate logistic regression analyses (27) that accounted for established risk factors for breast cancer. These included age (in 5-year groups), socioeconomic status (low, medium, or high), age at menarche (≤11, 12–15, or ≥16 years), age at full-term pregnancy (≤19, 20–29, or ≥30 years), parity (0, 1–2, 3–4, or ≥5), menopausal status (yes/no), and family history of breast cancer (yes/no). We evaluated DDT and DDE serum levels as continuous and as quartile categories, with cut-points based on the distribution in the control series (DDT: Q_1 = 0.023–0.070 μg/g lipids, Q_2 = 0.071–0.10 μg/g lipids, Q_3= 0.11–0.18 μg/g lipids, and Q_4 = 0.19–5.41 μg/g lipids; DDE: Q_1 = 0.20–1.16 μg/g lipids, Q_2 = 1.17–1.96 μg/g lipids, Q_3 = 1.97–3.48 μg/g lipids, Q_4 = 3.49–14.84 μg/g lipids). In our models, we also adjusted serum DDE levels by serum DDT levels. Given that serum DDT and DDE levels were highly correlated (Pearson correlation = 0.71), we adjusted DDE levels by DDT using linear regression analysis of log-transformed DDE and DDT levels. We then used the DDE-adjusted values (residuals) in the logistic regression models. We tested for linear trend in logit risks with increasing exposure by using the likelihood ratio test under the assumption of a linear relation (27). We performed all statistical analyses with Stata Software (Stata Statistical Software, Release 5.0, Stata Corporation College Station, Texas).

From the 260 cases and controls selected, the analysis was restricted to 126 controls and 120 cases. Eight cases were excluded because they were never pregnant. Four controls and two cases were excluded because the serum samples were not large enough to determine lipid levels.

RESULTS

The baseline characteristics of women who participated in the initial case-control study and those who were selected for the DDT/DDE analysis are described in table 1. Although we did not detect meaningful differences between groups, the effect of some risk factors became more apparent in the subsample selected for the DDE/DDT analysis (table 1). After age adjustment, parity and lifetime accumulated months of lactation were inversely associated with the risk of developing breast cancer. Cases had a higher body mass index than did controls. Early age at menarche and late age at first full-term pregnancy posed an increased risk of breast cancer. A higher proportion of cases reported a family history of breast cancer; however, this difference was not significant.

The mean ages of the 126 cases and 120 controls were 48 years (standard deviation (SD) = 13) and 52 years (standard deviation = 14), respectively. Serum DDE levels were significantly related to age. DDE levels increased sharply among women aged 30–40 years and then continued to increase linearly, but at a slower pace (figure 1). This relation was best fitted by a power parameterization of age (table 2) and was similar for cases and controls. After adjustment for age, we observed a significant decrease in the age-adjusted DDE concentrations with increasing time of lactation (= –0.0027 per month, figure 2). When we examined variables simultaneously in a multivariate regression model, we observed that the major predictors of serum DDE levels were serum DDT levels, age, duration of lactation, parity, and socioeconomic level (table 2). Serum DDT levels were also strongly related to age; however, after adjustment for age, we found that only body mass index remained positively associated with DDT serum levels (table 2).

Overall, the mean DDE level was significantly higher among cases (mean = 3.84 μg/g lipids, median = 2.62 μg/g

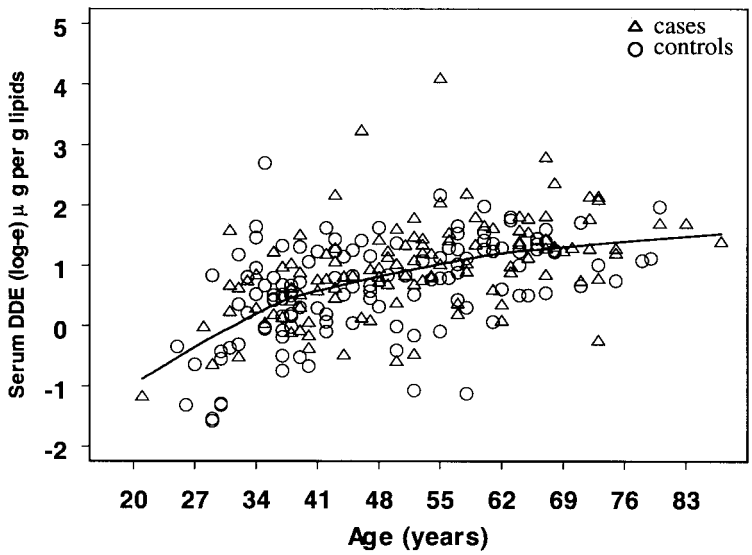

FIGURE 1. Relation between age and serum 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (DDE) levels (μg/g lipids) (lowess smoother), Mexico City, Mexico, 1990–1995.

TABLE 1. Distribution of selected characteristics among women selected for the DDT/DDE* study, those who were not selected, and observed associations between risk factors and breast cancer in the subsample that was included in the DDT/DDE study, Mexico City, Mexico, 1990–1995

Variable	Women not selected for the DDT/DDE analysis (mean (SD*))		Subsample in DDT/DDE analysis (mean (SD))			
	Cases (n = 385)	Controls (n = 1,239)	Cases (n = 120)	Controls (n = 126)	OR*,†	95% CI*
Age (years)	52 (12.9)	49 (13.4)	52 (14)	48 (13)	1.02	1.00, 1.04
Lifetime accumulated months of lactation (months)	34 (46)	48 (54)	32 (35)	45 (44)	0.98	0.97, 0.99
Parity	3.6 (3.2)	4.5 (3.3)	4.2 (2.8)	4.7 (2.7)	0.90	0.82, 0.99
Body mass index (k/m^2)	27.3 (4.7)	27.3 (4.7)	28.0 (4.4)	27.1 (4.5)	1.05	0.99, 1.10
Age at menarche (years)	13.0 (1.56)	13.1 (1.62)	12.9 (1.4)	13.4 (1.6)	0.76	0.62, 0.92
First livebirth over age 30 years (% yes)	10.6	6.5	10.8	5.6	2.0	0.75, 5.54
Menopause (% yes)	62.7	51.3	53.3	49.2	0.27	0.08, 0.85
Family history of breast cancer (% yes)	8.4	4.1	3.1	0.8	3.5	0.03, 41.4
SES* (%)						
Low	19.8	28.6	20.8	25.4	1.0	
Medium	36.0	45.8	39.2	41.3	1.07	0.55, 2.09
High	44.2	25.6	40.0	33.3	1.26	0.62, 2.51

* DDT/DDE, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane/1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; SD, standard deviation; OR, odds ratio; CI, confidence interval; SES, socioeconomic status.

† Odds ratios age-adjusted using age in 5-year intervals categories.

TABLE 2. Predictors of serum DDE* and DDT* levels, Mexico City, Mexico, 1990–1995

Variables	Beta	SE*	p
Serum DDE†			
Age	0.060	0.016	0.000
Age^2	–0.004	0.0001	0.012
SES*			
Low	Reference		
Medium	0.248	0.079	0.002
High	0.341	0.081	0.000
Lactation	–0.0027	0.0009	0.004
Parity	–0.044	0.013	0.001
Serum DDT	0.606	0.038	0.000
Serum DDT‡			
Age	0.082	0.025	0.002
Age^2	–0.0005	0.000	0.016
Body mass index	0.029	0.010	0.008

* DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; SE, standard error; SES, socioeconomic status.

† Log-transformed. All variables are simultaneously entered in the model to predict the log-transformed serum DDE levels expressed on a lipid basis. Lactation is expressed as the number of months of lactation and parity as the number of children (R^2 = 0.68).

‡ All variables are simultaneously entered in the model to predict serum DDT levels. Quetelet index is expressed as weight/height^2, R^2 = 0.16.

lipids, SD = 5.98) than among controls (mean = 2.51μg/g lipids, median = 2.0 μg/g lipids, SD = 1.97; $\log_e$ transformed and age-adjusted t test: $p = 0.02$). The observed differences were more apparent for postmenopausal women (mean difference = 1.94 μg/g lipids; $\log_e$-transformed age-adjusted t test: $p = 0.032$) and were only marginally significant among premenopausal women (table 3).

Serum DDE levels were significantly related to breast cancer risk (age-adjusted OR = 1.59 per $\log_e$ unit of lipid-adjusted DDE in serum, 95 percent CI: 1.09, 2.32 per $\log_e$ unit of lipid adjusted DDE in serum), but serum DDT levels were not (age-adjusted OR = 1.03 per $\log_e$ unit of lipid-adjusted DDT in serum, 95 percent CI: 0.74, 1.43). When we examined serum DDE levels as quartiles, after adjustment for age, age at menarche, duration of breastfeeding, Quetelet index, and menopausal status, we observed a positive trend in the risk of breast cancer with increasing levels of serum DDE that was marginally significant ($OR_{Q1\text{-}Q4}$ = 2.16, 95 percent CI: 0.85, 5.50; test for trend $p = 0.06$) (table 3). This increased risk became more apparent after adjustment for serum DDT levels ($OR_{Q1\text{-}Q2}$ = 1.24, 95 percent CI: 0.50, 3.06; $OR_{Q1\text{-}Q3}$ = 2.31, 95 percent CI: 0.92, 5.86; $OR_{Q1\text{-}Q4}$ = 3.81, 95 percent CI: 1.14, 12.80; test for trend $p = 0.020$).

We next evaluated the relation between DDE and DDT levels and menopause status. Among those women who had experienced menopause, the relation between serum levels of DDE and the risk of breast cancer was stronger than that observed for the total sample ($OR_{Q1\text{-}Q4}$ = 5.26, 95 percent CI: 0.80, 34.30), and we observed a significant positive trend between DDE levels and the risk of breast cancer (table 4). In contrast, the relation between DDE levels and risk of breast cancer was weaker among premenopausal women, and we did not observe a significant trend in the risk of breast cancer in relation to DDE levels.

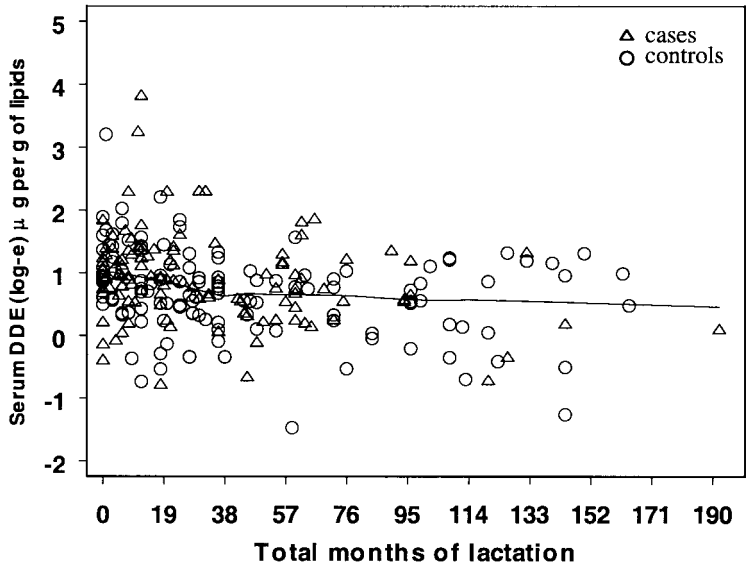

FIGURE 2. Relation between lifetime accumulated months of lactation and serum 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) levels (μg/g lipids) (lowess smoother), Mexico City, Mexico, 1990–1995.

TABLE 3. Serum concentrations of DDT* and DDE* in breast cancer patients and population-based controls, Mexico City, Mexico, 1990–1995

Variables	Cases		Controls	
	Mean†	10–90 percentile	Mean	10–90 percentile
All women	*n* = 120		*n* = 126	
p,p′-DDT (μg/liter)	1.05	0.25, 2.04	1.41	0.28, 2.21
p,p′-DDT (μg/g lipids)	0.15	0.038, 0.31	0.23	0.04, 0.33
p,p′-DDE (μg/liter)‡	24.2	4.42, 32.50	17.5	5.54, 42.58
p,p′-DDE (μg/g lipids)‡	3.84	0.60, 4.77	2.51	0.97, 6.05
Premenopausal women	*n* = 56		*n* = 64	
p,p′-DDT (μg/liter)	1.19	0.21, 1.49	0.82	0.25, 1.21
p,p′-DDT (μg/g lipids)	0.22	0.033, 0.25	0.13	0.035, 0.21
p,p′-DDE (μg/liter)‡	14.01	3.67, 22.08	12.6	2.96, 22.86
p,p′-DDE (μg/g lipids)‡	2.40	0.61, 3.84	1.93	0.49, 3.73
Postmenopausal women	*n* = 64		*n* = 62	
p,p′-DDT (μg/liter)	1.60	0.33, 2.42	1.19	0.31, 2.03
p,p′-DDT (μg/g lipids)	0.25	0.05, 0.32	0.18	0.046, 0.028
p,p′-DDE (μg/liter)‡	33.29	16.22, 55.41	14.01	3.16, 30.57
p,p′-DDE (μg/g lipids)‡	5.10	1.60, 8.41	3.12	0.47, 4.35

* DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene.
† Arithmetic mean.
‡ Mean difference statistically significant (*t* test, $p < 0.05$). *t* tests were performed in $\log_e$-transformed variables.

DISCUSSION

We observed a modest association between elevated serum DDE levels and risk of breast cancer. Women whose serum DDE levels were in the top quartile (Q_4, 3.49–14.84 μg of DDE per g lipids) were twice as likely to have a breast cancer diagnosis as were women with DDE levels in the bottom quartile (Q_1, 0.20–1.16 μg/g of DDE per g lipids). We also observed a statistically significant positive trend in the risk of breast cancer and increasing serum DDE levels. The observed associations were attenuated when we adjusted for potential confounders, such as duration of lactation and body mass index. Following the hypothesis that serum DDT levels may reflect recent exposure to organochlorines and that adjusting by this variable will allow us to account for long-term exposure to DDE, we reexpressed DDE levels by adjusting for serum DDT concentrations. When we included DDT-adjusted levels of DDE in the models, the relation between DDE levels and breast cancer risk became more apparent. We do not have a direct explanation for this observed phenomenon, but it is likely that adjusting for DDT increased our ability to evaluate long-term exposures with a single measurement of DDE. We did not observe any evidence of effect modification by DDT. Our results should be interpreted with caution, and testing of the observed association in other data sets will be needed before concluding any causal association.

As reported in other studies (28, 29), age was positively related and lactation was negatively related to serum DDE levels, mainly because lactation is the chief route by which women excrete organochlorines. Duration of lactation has been associated with decreased risk of breast cancer in various studies (20, 30). Lactation may reduce a woman's risk of breast cancer by "flushing out" carcinogens from the breast tissue. Support for this hypothesis is derived from studies that compared women who had breastfed unilaterally with those who had breastfed from both breasts and documented a significant increased risk for cancer in the unsuckled breast (31). Our findings that duration of lactation correlated with a decreased risk of breast cancer independently of serum DDE levels suggest that the effect of lactation may also be related to other factors, such as a decrease in cumulative exposure to estrogens. It is important to note that DDT and DDE are lipophilic compounds that accumulate in body fat. These compounds have an estrogenic activ-

TABLE 4. Odds ratios for breast cancer according to serum DDE* levels among women in Mexico City, Mexico, 1990–1995

DDE levels (quartiles)	Cases	Controls	Model 1†		Model 2‡		Model 3§	
			OR*	95% CI*	OR	95% CI	OR	95% CI
All women								
1¶	18	31	1.00		1.00		1.00	
2	20	32	1.08	0.47, 2.45	1.06	0.44, 2.55	1.24	0.50, 3.06
3	38	32	1.79	0.82, 3.93	1.75	0.76, 4.09	2.31	0.92, 5.86
4	44	31	2.20	0.96, 5.07	2.16	0.85, 5.50	3.81	1.14, 12.80
			Test for trend, $p = 0.03$		Test for trend, $p = 0.06$		Test for trend, $p = 0.02$	
Premenopausal women								
1	15	24	1.00		1.00		1.00	
2	15	20	1.09	0.42, 2.97	1.47	0.50, 4.28	1.38	0.46, 4.17
3	19	13	2.09	0.76, 5.69	2.39	0.82, 6.97	2.53	0.75, 8.49
4	7	7	1.46	0.41, 5.16	2.16	0.54, 8.60	2.41	0.37, 15.81
			Test for trend, $p = 0.24$		Test for trend, $p = 0.11$		Test for trend, $p = 0.16$	
Postmenopausal women								
1	3	7	1.00		1.00		1.00	
2	5	12	1.03	0.18, 5.87	0.97	0.14, 6.47	1.06	0.15, 7.27
3	19	19	2.19	0.48, 9.99	1.79	0.35, 9.06	2.40	0.44, 12.98
4	37	24	3.46	0.78, 15.37	2.41	0.47, 12.30	5.26	0.80, 34.30
			Test for trend, $p = 0.02$		Test for trend, $p = 0.12$		Test for trend, $p = 0.03$	

* DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; OR, odds ratio; CI, confidence interval.
† Model 1, adjusted for age (5-year age categories).
‡ Model 2, adjusted for age (5-year age categories), age at menarche (with dummy variables for ages 14 and ≥15 years), duration of lactation (months), and Quetelet index (kg/m^2).
§ Model 3, adjusted for age (5-year age categories), age at menarche (with dummy variables for ages 14 and ≥15 years), duration of lactation (months), Quetelet index (kg/m^2), and serum dichlorodiphenyltrichloroethane (DDT) levels (μg/g lipids).
¶ Quartiles of serum DDE levels: Q_1 = 0.20–1.16 μg/g lipids; Q_2 = 1.17–1.96 μg/g lipids; Q_3 = 1.97–3.48 μg/g lipids; and Q_4 = 3.49–14.84 μg/g lipids.

ity and have been classified as potential carcinogens in humans.

Although the estrogenic activity of DDE is rather weak compared with that of 17-beta estradiol (32), because of the long half-life of organochlorine compounds, ductal and other breast cells are exposed to these compounds over several decades. Therefore, the long-term exposure may counterbalance the low estrogenic potency of this organochlorine (12). In addition, a high organochlorine body burden has been found in women with estrogen receptor-positive breast cancer (33), which is concordant with the association we observed in our study. Postmenopausal women are more likely to have hormone-dependent tumors (34) and higher DDE levels that may interact with estrogen-binding protein and promote the growth of estrogenic tumors (35–37).

Several studies have examined the relation between DDT/DDE and breast cancer. Results of these studies have been discussed recently (1, 2, 32). However, a direct comparison of reported results remains difficult because of the different sensitivity and specificity of laboratory assays used, the different biomarkers of exposure measured, and the differences in expressed units.

Early case-control studies were based on small numbers of cases and compared DDE levels in mammary adipose tissue from women with breast cancer with levels in tissue from women with nonmalignant breast disease (2–4, 6, 7); they did not account for major confounding factors such as lactation history and parity. Two nested case-control studies among large cohorts of women (8, 10) yielded conflicting results. Wolff et al. (8) reported higher DDE levels among women with breast cancer than among controls (11.0 vs. 7.7 ppb) and a significant increasing trend in the risk of breast cancer with increasing levels of DDE. The adjusted relative risk among women with DDE levels in the top quintile (11.9–44.3 ppb) compared with that among women with DDE levels in the bottom quintile (0.5–3.2 ppb) was 3.68 (95 percent CI: 1.10, 13.50). Krieger et al. (10) examined 150 breast cancer cases selected from a cohort of 57,040 San Francisco Bay-area women. In that study, mean DDE levels were similar for cases and controls (43.3 vs. 43.1 ppb). However, the data suggested a threefold increase in the risk of breast cancer among a subgroup of Black women whose DDE levels were in the highest tercile (47.7–149.5 ppb) compared with those in the lower tercile (5.3–29.6 ppb).

In contrast to the results from these earlier studies, recent findings do not support the hypothesis that elevated levels of DDE increase a woman's risk of breast cancer. A large European case-control study, which included 264 women with breast cancer, reported a significant inverse trend between levels of DDE in adipose tissue and risk of breast cancer (13). This unexpected finding and the varying response rates among controls in participating centers (22–91 percent) raise the issue of selection bias. The authors did not provide a meaningful explanation of why higher

DDE levels may decrease a woman's risk of breast cancer. Plasma DDE levels were measured among 240 cases and controls selected from a large cohort of 32,826 nurses participating in the Nurses' Health Study (12). No significant difference in serum DDE levels was observed between cases and controls (median, 4.71 vs. 5.35 ppb). The multivariate relative risk of breast cancer among women in the highest quintile of exposure compared with those in the lowest quintile was 0.72 (95 percent CI: 0.37, 1.40). A population-based case-control study that included 154 menopausal women with breast cancer and 192 menopausal community controls reported no association between serum DDE levels and breast cancer risk (mean serum DDE levels, 11.47 among cases and 10.77 ppb among controls). However, among parous women who had never lactated, there was some indication of a modest increased risk associated with DDE levels ($OR_{Q1\text{-}Q3}$ = 1.83, 95 percent CI: 0.63, 5.33) (14). In a recent nested case-control study, researchers from Denmark reported that breast cancer risk was twice as high among women with dieldrin serum levels in the fourth quartile as among those with levels in the first quartile; they also found that levels of PCB, DDT, and *p,p′*-DDE were not associated with risk of breast cancer (38). However, the levels of DDE that they observed were only one third of those observed in our study, and their data were not adjusted for lactation. This may have affected the results.

In a recent hospital-based case-control study conducted among women living in Mexico City (141 cases and 141 age-matched controls), Lopez-Carrillo et al. (11) did not report any association between DDE levels and risk of breast cancer. However, the women in that study had surprisingly low DDE and DDT. The serum DDE levels among the women in our study were considerably higher (arithmetic mean among breast cancer patients, 24.2 vs. 4.75 μg/liter; arithmetic mean among controls, 17.5 vs. 4.07 μg/liter). Cases from both studies came from similar hospitals, and there were no apparent differences between case and control selection that may explain these large differences. Therefore, it is difficult to explain the differences in DDE levels observed in the two studies by causes other than laboratory procedures or result reporting. Because DDT is steadily metabolized in the human body into DDE and other metabolites, the DDE/DDT ratio provides information regarding the timing of exposure to DDT. In the study by Lopez-Carrillo et al. (19), the DDE/DDT ratio was close to seven, whereas in our study, the ratio was close to 23. Low DDE/DDT ratios suggest recent exposure to DDT, which is unlikely for women living in Mexico City because current use of DDT in Mexico is limited and occurs only in remote areas where malaria is endemic (19). To our knowledge, there are no other studies published for Mexico City regarding serum DDT/DDE levels. However, in a small pilot study that we conducted among women living in Mexico City, we found levels comparable with those observed in the study reported here (Romieu et al., unpublished data).

In our study, age, lactation, parity, and socioeconomic status were strong predictors of serum DDE levels and of breast cancer risk, which is consistent with previous reports (28–30). Other variables associated with breast cancer risk (age at menarche, menopause, and body mass index) were also identified.

Two major concerns in our study and in those reported by others are the use of a single measurement of organochlorine levels to assess exposure and the lack of information on the relevant period of exposure. DDT and DDE accumulate in human adipose tissue (37), and serum DDE levels have been shown to correlate very well with DDE levels in adipose tissue (33, 39, 40). In addition, repeated blood sampling among nonoccupationally exposed women has also shown a high reproducibility (41, 42). Furthermore, there is no evidence that breast cancer cells modify the metabolism of DDE stored in breast adipose tissue. Blood DDE levels (lipid adjusted) before and after breast cancer treatment have been reported to be similar (mean difference, 0.05 μg/g lipids) (43). This provides evidence that blood levels are stable markers of exposure to DDE. In our study, blood samples were collected from women attending a gynecologic clinic for biopsy of a breast lump before the results of the biopsy were known. In fact, 40 percent of these potential cases were found to have a benign breast disease. We believe that the serum DDE levels reported in our study provide a good estimate of participants' cumulative exposure to DDE and that disease status is unlikely to have modified the serum DDE levels of participants. It is also unlikely that serum DDE or DDT levels may have conditioned study participation.

Our results support the hypothesis that long-term exposure to high DDE levels may be associated with a small increase in the risk of breast cancer among postmenopausal women. We postulated that exposure to organochlorines such as DDT or DDE may increase a woman's risk of breast cancer through their weak estrogenic properties and promote the late stages of carcinogenesis among postmenopausal women. This hypothesis is supported by several studies in which high body mass index and high plasma levels of estradiol among postmenopausal women with no previous use of hormone replacement therapy were shown to be risk factors of breast cancer (44–46). In our study, none of the participants had been exposed to replacement estrogen therapy; consequently, exogenous sources of estrogen were small. Therefore, the increased risk of breast cancer attributable to DDE may have been more apparent even though DDE has a weak estrogenic effect. The interaction between endogenous and exogenous sources of estrogen is complex. There is a need for further studies to determine the impact of high exposure to organochlorine among subgroups of postmenopausal women not exposed to other sources of exogenous estrogens.

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