Serum Concentrations of Organochlorine Compounds and the Subsequent Development of Breast Cancer[1]

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Abstract

A nested case-control study was conducted to examine the association between serum concentrations of 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene (DDE), the primary metabolite of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), and polychlorinated biphenyls (PCBs) and the development of breast cancer up to 20 years later. Cases ($n = 346$) and controls ($n = 346$) were selected from cohorts of women who donated blood in 1974, 1989, or both, and were matched on age, race, menopausal status, and month and year of blood donation. Analyses were stratified by cohort participation because median DDE and PCB concentrations among the controls were 59 and 147% higher in 1974 than 1989, respectively. Median concentrations of DDE were lower among cases than controls in both time periods [11.7% lower in 1974 ($P = 0.06$) and 8.6% lower in 1989 ($P = 0.41$)]. Median concentrations of PCBs were similar among cases and controls [$P = 0.21$ for 1974 and $P = 0.37$ for 1989 (Wilcoxon signed rank test)]. The risk of developing breast cancer among women with the highest concentrations of DDE was roughly half that among women with the lowest concentrations, whether based on concentrations in 1974 [odds ratio (OR), 0.50; 95% confidence interval (CI), 0.27–0.89; $P_{trend} = 0.02$] or in 1989 (OR, 0.53; 95% CI, 0.24–1.17; $P_{trend} = 0.08$). The associations between circulating concentrations of PCBs and breast cancer were less pronounced but still in the same direction (1974: OR, 0.68; 95% CI, 0.36–12.9; $P_{trend} = 0.2$; and 1989: OR, 0.73; 95% CI, 0.37–1.46; $P_{trend} = 0.6$). Adjustment for family history of breast cancer, body mass index, age at menarche or first birth, and months of lactation did not materially alter these associations. These associations remained consistent regardless of lactation history and length of the follow-up interval, with the strongest inverse association observed among women diagnosed 16–20 years after blood drawing. Results from this prospective, community-based nested case-control study are reassuring. Even after 20 years of follow-up, exposure to relatively high concentrations of DDE or PCBs showed no evidence of contributing to an increased risk of breast cancer.

Introduction

The organochlorine compounds DDE[3] (the primary metabolite of DDT) and PCBs are suspected of having a role in breast cancer etiology because: (*a*) they are stored in adipose tissue and found in breast milk; (*b*) some have estrogen-like activity; and (*c*) some are metabolized to highly reactive compounds (1–5). Although banned in the United States in the early 1970s, DDT and PCBs are long-lived compounds that persist in the environment, with diet being the most common route of continued exposure (6–9).

Results of studies that examined the association between organochlorine compounds and breast cancer are summarized in Tables 1A and 1B (10–23). Six of the seven case-control studies based on measures of adipose tissue concentrations found similar or lower concentrations of DDE among cases than controls (10–16); four of five (11–14) observed higher adipose tissue concentrations of PCBs among cases than controls. Four case-control studies used serum organochlorine concentrations as the marker of exposure with blood drawn after the diagnosis of breast cancer (17–20). Two of these studies measured DDE and PCB concentrations (17, 20), and the other two measured only DDE concentrations (18, 19). Wolff *et al.* (17) found elevated levels of serum DDE and PCBs in newly diagnosed breast cancer cases compared with controls participating in the same screening study. DDE exhibited a dose-response relationship with the risk of breast cancer. The relationship between PCBs and breast cancer suggested a possible threshold effect. All of the serum samples of cases were obtained within 6 months of diagnosis of breast cancer. The subsequent studies have not found evidence of significantly higher levels of DDE (18–20) or PCBs (20) among women

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[3] The abbreviations used are: DDE, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene; DDT, 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane; PCB, polychlorinated biphenyl; COMT, catechol-*O*-methyltransferase; CLUE I, Campaign against Cancer and Stroke; CLUE II, Campaign against Cancer and Heart Disease; OR, odds ratio; CI, confidence interval; GT, glutathione.

Table 1 Published studies of the association between organochlorine compounds and breast cancer

Study[a]	Year (place)	No. of cases/ controls	Mean concentrations (SD)		OR by categories					P_{trend}
			Cases	Controls	1 (lowest)	2	3	4	5 (highest)	
A. Published studies of the association between DDT compounds and breast cancer										
Case-Control										
Adipose Tissue										
Davies (10)	1975	29/29	5.4 ppm	7.1 ppm						
Wasserman (11)	1976 (Brazil)	9/5	2.7 ppm	6.7 ppm						
Unger (12)	1982 (Denmark)	14/21	1.2 (0.63) ppm	1.2 (0.8) ppm						
Mussalo-Rauhamaa (13)	1985–86 (Finland)	41/33	0.96 (0.6) mg/kg	0.98 (0.9) mg/kg						
Falck (14)	1987 (United States)	20/20	1877 (1283) ng/g[c]	1174 (630) ng/g[c]						
van't Veer (15)	1991–92 (Europe)	265/341	1.35 μg/g	1.51 μg/g	1.0	1.14	0.71	0.48		0.02
Dewailly (16)	1991–92 (Canada)	20/17	ER−[b] 0.6 (0.3) μg/g ER+ 2.1 (2.0) μg/g	0.7 (0.5) μg/g						
Serum										
Wolff (17)	1985–91 (United States)	58/171	11.0 (9.1) ppb	7.7 (6.8) ppb	1.0	1.67	4.37	2.31	3.68	0.04
Schechter (18)	1994 (Vietnam)	21/21	12.2 (2.4) ng/ml	16.8 (4.1) ng/ml	1.0	0.45	1.14			
Lopez-Carillo (19)	1994–96	141/141	562.5 (676.2) ng/g[c]	505.5 (567.2) ng/g[c]	1.0	0.60	0.76			
Moysich (20)	1986–91	154/192	11.5 (10.5) ng/g[c]	10.8 (10.6) ng/g	1.0	1.01	1.34			0.25
Prospective studies, nested case-control										
Serum										
Krieger (21)	1964–71 (United States)	150/150	43.3 (25.9) ppb	43.1 (23.7) ppb	1.0	1.31	1.26			0.43
Hunter (22)	1989–90 (United States)	240/240	6.01 (4.56) ppb[c]	6.97 (5.99) ppb[c]	1.0	0.88	0.56	0.73	0.66	0.22
Hoyer (23)	1976 (Denmark)	240/477	NA		1.0	0.79	0.92	0.84		0.65
B. Published studies of the association between PCBs and breast cancer										
Case-Control										
Adipose Tissue										
Wasserman (11)	1976 (Brazil)	9/5	9.1 ppm	3.0 ppm						
Unger (12)	1982 (Denmark)	14/21	3.9 (1.0) ppm	3.9 (1.3) ppm						
		18/35	6.47 ± 2.35	5.1 (2.4) ppm						
Mussalo-Rauhamaa (13)	1985–86 (Finland)	20/20	1.1 (0.6) mg/kg	1.3 (0.8) mg/kg						
Falck (14)	1987 (United States)	41/33	1669 (894) ng/g	1105 (424) ng/g						
Dewailly (16)	1991–92 (Canada)	20/17	ER− 0.3 (0.7) μg/g ER+ 0.4 (0.1) μg/g	04 (0.2) μg/g						
Serum										
Wolff (17)	1985–91 (United States)	58/171	8.0 ± 4.1 ppb	6.7 ± 2.9 ppb	1.0	5.18	7.02	4.10	4.35	0.16
Moysich (20)	1986–91	154/192	4.3 (2.4) mg/g[c]	4.1 (2.2) mg/g	1.0	0.70	1.14			0.51
Prospective studies, nested case-control										
Serum										
Krieger (21)	1964–71 (United States)	150/150	4.4 (1.8) ppb	4.8 (2.5) ppb	1.0	1.17	0.94	0.55	0.59	0.9
Hunter (22)	1989–90 (United States)	240/240	5.1 (2.5) ppb[c]	5.2 (2.3) ppb	1.0	0.57	0.54			0.26
Hoyer (23)	1976 (Denmark)	240/477	NA		1.0	0.92	0.78	1.11		0.77

[a] Number in parentheses cites reference.
[b] ER−, estrogen receptor negative; ER+, estrogen receptor positive; NA, not available.
[c] Lipid-adjusted.

with breast cancer compared with control women, with the exception of one study that found an association among parous women who never breast-fed (20). Two of these studies were conducted in Mexico and Vietnam where DDT is actively used (18, 19).

Three prospective studies using nested case-control study designs have examined the association between DDE and PCBs and subsequent development of breast cancer (21–23). The study by Krieger *et al.* (21) in California examined concentrations in blood samples drawn between 1964 and 1971 from 150 women who went on to develop breast cancer and 150 matched control women. The development of breast cancer was not associated with serum concentrations of DDE or PCBs regardless of race, year of diagnosis, length of follow-up, or estrogen receptor status (21). Hunter *et al.* (22) examined concentrations of organochlorine compounds in blood samples drawn in 1989 or 1990 among 240 women who developed breast cancer by 1992 and matched control women. The risk of breast cancer tended to be lower among women with higher serum concentrations of DDE and PCBs but the trends were not statistically significant. The exposure assessment in this study preceded the breast cancer but only by a maximum period of 3 years. Høyer *et al.* measured concentrations of organochlorine compounds in blood samples obtained in 1976 from 240 women who developed breast cancer by 1995 and 477 matched control women (23). The development of cancer was not associated with DDE, total DDT, or total PCB concentrations.

We used the resources of two specimen banks established in Washington County, Maryland in 1974 and 1989 to prospectively examine the association between exposure to DDE and PCBs and the development of breast cancer through 1994. The 1974 cohort provides a long follow-up period, and the serum

concentrations were likely to be maximal in 1974, close to the time organochlorine compounds were banned in the United States. The 1989 cohort was used to examine the association between more current organochlorine concentrations and breast cancer.

The susceptibility to exogenous exposures such as organochlorine compounds may vary by levels of detoxification enzymes such as glutathione transferases. Glutathione-μ, encoded by *GSTM1* genotype, is known to catalyze the conjugation of glutathione with oxidative intermediates of a variety of carcinogenic compounds. Studies in rodents indicate that oxidative intermediates of organochlorines are excreted in urine and feces as conjugates (24–26), and other organochlorine compounds—3,4-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene—are known to be a substrate for GT-μ and other GT family enzymes (27). We recently reported an association between putative high-risk genotypes for *GSTM1*, *GSTP1*, and *GSTT1* and the development of breast cancer (28). There is also a suggestion of an interaction between *COMT* activity and *GSTM1* null genotype and the risk of breast cancer (29). The present study examines the association between organochlorine compounds and the risk of breast cancer, stratifying by genotype among women who participated in the 1989 cohort.

Materials and Methods

From August through November 1974, the Campaign against Cancer and Stroke was conducted in Washington County, MD. Referred to as CLUE I (from the slogan, "Give us a Clue to Cancer and Stroke"), it was designed to collect blood samples from as many adults as possible to provide specimens for a serum bank. A total of 25,802 persons donated blood, of whom 20,305 were county residents. Linkage of the records from this program to those of a private census in the summer of 1975 indicated that almost one-third of the adult population of the county had participated. Participation was best in the age group 35–65 years and was slightly better among females, the better-educated, and nonsmokers. A brief history form was completed at the time of blood collection. Blood was drawn into 15-ml Vacutainers (Becton Dickinson), and serum aliquots were stored at −70°C.

The Campaign against Cancer and Heart Disease (CLUE II) was similar to CLUE I and was conducted from May through October 1989. Brief histories and blood pressures were taken, and 20 ml of blood were drawn into heparinized Vacutainers (Becton Dickinson). Plasma, buffy coat, and 1.8 ml of packed red cells were stored at −70°C. A total of 32,892 persons participated, of whom 25,080 were Washington County residents. A total of 8395 residents participated in both CLUE I and CLUE II campaigns. Comparisons with published figures from the 1990 Census indicated that again approximately 30% of adult residents had participated. As before, women and the better educated had higher than average participation rates, as did the age group 45–70 years.

Cases were drawn from participants who were residents of Washington County, Maryland who donated blood for the serum bank in 1974 only (CLUE I), in 1989 only (CLUE II), or in both programs (CLUE I and II). The cases were women who were first diagnosed as having breast cancer (ICD9, 174) after having donated blood for one of the two CLUE programs. Cases were identified by linkage of the cohort participants to the Washington County Cancer Registry. Registry cases were identified from discharge records of the Washington County Hospital, the only hospital in the county, and from death certificates. Through June 1994, 346 women were identified who had developed breast cancer after donating blood, had no other invasive cancers, and had serum available. One hundred ninety-nine women had local disease; of these, 23 were *in situ* ductal carcinoma, 109 had regional disease, and 26 were of unknown stage. The majority of women (295) had participated in CLUE I; 115 women had participated in CLUE II, and 64 of these had also donated blood to CLUE I. Participants who had donated to both CLUE programs developed their breast cancer after the CLUE II program (1989) and were included in each program-specific analysis. Completeness of ascertainment was estimated by comparing the number of breast cancer cases reported to the Maryland Cancer Registry (in operation since 1992) to the number of cases obtained through the Washington County Registry for 1993 (30). In that year, 90 cases of breast cancer were recorded in the Washington County Cancer Registry and 81 of these were reported to the Maryland Cancer Registry.

Controls were selected from participants who were residents of Washington County when they donated blood and were not diagnosed with an invasive cancer (with the possible exception of basal or squamous cell cancer of the skin) at the time when cases were diagnosed. Each case was matched to one control by sex (all were women), race (all were Caucasian), age (within one year), menopausal status, date of blood donation, and the CLUE programs in which they participated. Among premenopausal women, cases and controls were also matched by the day of the menstrual cycle at the time of blood donation.

One case-control set from CLUE II was eliminated because the chosen control had been diagnosed with breast cancer; 26 sets were excluded from CLUE I because of inadequate quantity of sera for assays; and 34 sets from CLUE I and 10 sets from CLUE II were eliminated because either the case or the control sera failed to meet quality control standards for added marker compounds during the assay. Thus, data on organochlorine compounds were available for 235 matched sets from CLUE I and 105 matched sets from CLUE II.

Questionnaire Data

At the time of blood donation, participants completed a brief questionnaire that ascertained smoking status, height, weight, and medication use in the previous 48 h. Women with breast cancer and matched controls (or their next of kin, if deceased) were sent a self-administered questionnaire in 1995 to obtain more detailed information about breast cancer risk factors. Eighty-nine percent of cases and 76% of control women returned the questionnaires. Of the returned questionnaires, 31 (10.6%) were answered by surrogates of cases and 14 (5.3%) were answered by surrogates of controls. Exposure to factors such as the use of hormone replacement therapy and alcohol intake was truncated at the date of diagnosis of the case for both cases and controls.

Laboratory Assays

DDE and PCBs. Blood was collected in red-topped Vacutainer tubes in 1974 and in heparinized green-topped Vacutainer tubes in 1989. Serum or plasma was prepared from blood samples within 24 h of collection and usually within 6 h (note the term serum will be used generally to denote serum from CLUE I and plasma from CLUE II specimens.) In 1989, plasma, buffy coat, and RBCs were separated and stored at −70°C within 24 h of collection. In that year, 90 cases of breast cancer were recorded in the Washington County Cancer Registry, and 81 of these were reported to the Maryland Cancer Registry.

DDE and PCBs in sera were assayed using solid-phase extraction followed by gas chromatography with electron capture detection as described previously by Brock *et al.* (31).

Plasma samples were assayed using liquid/liquid extraction and adsorption chromatography. The method was similar to that described by Burse *et al.* (32) with the following exceptions: (*a*) only one elution fraction was collected from Florisil [6% ethyl ether/petroleum ether that contained pesticides (except Dieldrin and Endrin) and PCBs]; (*b*) the acid wash of the eluted fraction was omitted; and (*c*) the eluted fraction was not further eluted through silica gel. The eluted fraction was assayed by gas chromatography with electron capture detection per Brock *et al.* (31). PCBs congeners were referred to by a standard numbering scheme as described by Ballschmitter and Zell (33). Samples were simultaneously assayed for total cholesterol and triglyceride levels, and lipid-adjusted values were calculated using the formula described by Philips *et al.* (34).

Thirty-one quality-control sample sets consisting of pooled sera were included, with one quality-control set approximately every 10th case-control set. One laboratory quality-control sample consisting of spiked bovine serum and one reagent blank were also assayed with every batch of ten samples. If either of these samples were of the normal controls limits, the samples were reassayed. Twenty-three of the CLUE I serum samples were reextracted for repeat assays. In these instances, the matched sample in the set was not reassayed at the same time. All of the sets were included in the final analysis because separate analyses with these case-control sets excluded were similar to the overall findings. Quality-control samples of pooled sera were used to calculate coefficients of variation. Intra-assay coefficients of variations for 1974 serum samples were 9.4% for total PCBs and 14.7% for DDE; for 1989, the coefficients were 17.4% for total PCBs and 8.4% for DDE. Interset coefficients of variations for 1974 serum samples were 19.7% for total PCBs and 12.8% for DDE; for 1989, the coefficients were 20.0% for PCBs and 6.7% for DDE.

Genotyping. The buffy coat was kept frozen until thawed for DNA extraction for this study. DNA was extracted from the thawed WBC fraction from each study subject by high-salt fractionation (35) followed by chloroform/isoamyl alcohol extraction (36). Concentration of DNA was adjusted to 100 μg/ml and stored at −70°C until genotype analysis. *GSTM1*, *GSTT1*, and *GSTP1* genotype could be determined for 110 cases and 113 controls. *GSTM1* and *GSTT1* genotypes were determined using the multiplex PCR method of Chen *et al.* (37), which does not distinguish between heterozygote and homozygote *GSTM1* or *GSTT1* positive genotypes but conclusively identifies null genotypes. *GSTP1* (Ile105Val) genotype was determined using the PCR-RFLP method of Watson *et al.* (38). *CYP17* genotype could be determined on 109 cases and 113 controls. The *CYP17* genotype was determined using the PCR-RFLP method of Carey *et al.* (39), in which restriction digestion by *Msp*AI identifies the presence of the *A2* allele. *COMT* genotype was determined by a PCR-based RFLP assay (29) for 112 matched case-control pairs.

Statistical Analyses

Statistical anlayses were performed separately for CLUE I and CLUE II cohort participants and for lipid-adjusted and -unadjusted DDE and PCB data. For CLUE I, total DDE was defined as the sum of *o,p′*DDT + *p,p′*DDT + *o,p′*DDE + and *p,p′*DDE; total PCB was defined as the sum of the following measured PCB congeners: 28, 52, 56, 74, 101, 105, 110, 118, 138, 146, 153, 156, 170, 172, 177, 178, 180, 183, 187, 189, 193, 194, 195, 201, 203, and 206. For CLUE II, total DDE was defined as the sum of *o,p′*DDE, and *p,p′*DDE; total PCB was defined as the sum of the following measured PCB congeners: 52, 66, 101, 105, 110. 118, 138, 146, 153, 156, 170, 172, 177, 178, 180, 183, 187, 189, 193, 195, 201, and 203. Differences in definition of total DDE and PCBs between the two cohorts reflect the inability to detect certain congeners and metabolites in the 1989 samples, in which overall concentrations were lower compared with 1974.

Concentrations of DDE and total PCBs between cases and controls were compared using the Wilcoxon signed-rank test. Conditional logistic regression models were used to assess the association between concentrations of DDE and PCBs and the risk of developing breast cancer. Concentrations of DDE and PCBs were categorized into fifths (CLUE I) or thirds (CLUE II) based on the distributions in the control groups. Thirds were used for CLUE II because of the number of observations for that program. ORs and the corresponding 95% CIs for each upper fifth (or third) compared with the lowest fifth (or third) were calculated. Adjustment for known or suspected risk factors for breast cancer other than the matching variables, such as family history of breast cancer, body mass index at age 20 or current, age at menarche, age at first birth, and duration of lactation, did not alter the point estimates of ORs. Therefore, only unadjusted analyses are presented. Trend tests were performed by conditional logistic regression analyses using median values of each fifth or third category among the controls as the independent variable.

Additional analyses included examining the association by individual PCB congener, the PCB congeners that contributed to most of the total, by structure-activity groups as proposed by Wolff *et al.* (40), and by strata of menopausal status at diagnosis, years since diagnosis, history of lactation (among parous women), estrogen receptor status, and genotype of *GSTM1*, *GSTT1*, *GSTP1*, *CYP17*, and *COMT*. Conditional logistic regression analyses were used in these analyses except for the stratification analyses of history and estrogen receptor status, in which unconditional logistic regression adjustment for matching factors (age and menopausal status at baseline) were used to preserve adequate numbers in the strata. Women with breast cancer were classified as postmenopausal at diagnosis based on the response to the questionnaire regarding the date of last menstrual period as well as the history of hysterectomy and oophorectomy. Women with missing information regarding the last menstrual period or women with a history of hysterectomy without oophorectomy were considered to be postmenopausal if diagnosed at age 51 or older. A two-sided *P* less than 0.05 was considered statistically significant.

Results

Selected characteristics of cases and controls are presented in Table 2. Cases and controls were closely matched on age. The distribution of risk factors were in the direction expected based on previous research with cases more likely than controls to have a family history of breast cancer, later age at first birth or nulliparity, earlier age at menarche (CLUE II only), higher education, higher weekly consumption of alcoholic drinks, and fewer months of breast feeding. Only the associations with family history of breast cancer ($P = 0.01$) and >12 months of lactation ($P = 0.01$) were statistically significant. Mean (SD) for cholesterol concentrations were: 204.5 (48.6) for cases and 217.5 (54.7) for controls in the CLUE I study and 180.3 (38.4) for cases and 189.6 (39.0) for controls in the CLUE II study.

Table 2 Characteristics of cases and controls by cohort participation

	1974		1989	
	% cases (N = 235)	% controls (N = 235)	% cases (N = 105)	% controls (N = 105)
Age at donation				
≤40	20.9	20.4	3.8	2.9
41–50	23.8	26.0	21.0	22.9
51–60	33.6	31.9	18.1	15.2
≥61	21.7	21.7	57.1	59.1
Education				
<12 years	38.3	43.0	24.8	31.4
≥12 years	61.7	57.0	75.2	68.6
Family history of breast cancer (define: mother, sister, grandmother)				
No	79.2	91.1	73.3	87.6
Yes	20.9	8.9	26.7	12.4
Age at first birth				
<20	18.7	19.2	22.9	22.9
20–29	40.4	35.7	51.4	49.5
≥30	6.8	4.3	1.0	3.8
Nulliparous	11.9	10.2	13.3	9.5
Missing	22.1	30.6	11.4	14.3
Age at menarche				
<12	13.2	14.5	19.1	14.3
12–13	40.4	31.9	54.3	49.5
≥14	17.5	17.9	16.2	22.9
Missing	28.9	35.7	10.5	13.3
Lactation (months)				
None	51.9	40.0	61.9	47.6
1–12	16.6	14.9	19.1	20.0
>12	6.0	9.8	5.7	17.1
Missing	25.5	35.3	13.3	15.2
Hormone replacement therapy				
Never	58.3	48.5	65.7	54.3
Ever	16.6	14.9	20.0	24.8
Missing	25.1	36.6	14.3	21.0
Alcohol consumption (no. of drinks/week)				
Never	48.1	46.8	47.6	50.5
<1	12.8	8.1	17.1	12.4
1–3	7.7	8.1	14.3	15.2
≥4	13.2	7.2	14.3	7.6
Missing	18.3	29.8	6.7	14.3
Cigarette smoking (at donation)				
Never	60.0	57.5	64.8	63.8
Former	16.6	15.7	23.8	21.0
Current	23.4	26.8	11.4	15.2
Time to diagnosis				
<2			48.6	
3–5 years	12.3	NA[a]	51.4	NA
6–10	17.9			
11–15	28.5			
≥16	41.3			
Estrogen receptor status				
ER-negative	11.5	NA	19.0	NA
ER-positive	33.2		66.7	
Unknown	55.3		14.3	

[a] NA, not applicable; ER, estrogen receptor.

Mean (SD) for triglyceride concentrations were: 152.3 (64.0) for cases and 153.6 (69.3) for controls in the CLUE I study and 149.6 (60.1) for cases and 148.2 (66.7) for controls in the CLUE II study.

Mean and median concentrations of DDE and total PCBs, unadjusted and adjusted for serum lipid concentrations, are shown in Table 3. In both CLUE I and CLUE II, prediagnostic mean and median concentrations of DDE were lower among cases than controls. PCB levels were similar for cases and controls. The concentrations of both organochlorine compounds were higher in 1974 than in 1989. For example, when considering the concentrations without lipid adjustment, the median values of the control groups in 1974 were 59% higher for DDE and 147% higher for PCBs.

The risk of breast cancer did not increase with increasing serum concentrations of DDE or PCBs (Table 4). In fact, the risk of breast cancer tended to be lowest among women with the highest concentrations of DDE or PCB. The associations were closer to the null when concentrations were adjusted for lipid levels but provided no evidence of increased risk.

The association between PCBs and breast cancer was further investigated by performing congener-specific analyses, combining the main PCBs (for 1974, PCBs 28, 74, 118, 138, 153, 156, 170, and 180; for 1989, PCBs 118, 138, 153, 170, 180, 189, 195, 201, and 203) and according to structure-activity groups proposed by Wolff *et al.* (40). None of the individually measured congeners were associated with a statistically significant increased risk of breast cancer, and, in general, the risk of breast cancer tended to be lower among women with higher concentrations of the congeners (data not shown). Results of the analysis limited to the main PCBs were similar to the results with total PCBs. Three structure-activity groupings of PCB congeners have been proposed (40). Group 1 congeners are potentially estrogenic (Group IA: weak phenobarbital inducers, estrogenic, not persistent; Group IB: weak phenobarbital inducers, persistent); Group 2 congeners are potentially antiestrogenic and immunotoxic, dioxin-like (Group 2A: non-*ortho* and mono-*ortho* substituted; Group 2B: di-*ortho* substituted); and Groups 3 are neither estrogenic nor antiestrogenic but induce enzymes (CYP1A, CYP2B, and phenobarbital). The risk of breast cancer tended to decrease with increasing concentrations of Group 3 congeners. None of the structure-activity groupings were associated with an increased risk of breast cancer (data not shown).

Analysis of the association by time from blood sampling to the time of diagnosis was conducted to assure that the results are not influenced by the presence of occult disease at the time of blood drawing. The results for the different follow-up intervals were consistent with the overall findings. Even for cases diagnosed 16–20 years after blood sampling, the risk of breast cancer among the highest third of the distribution of DDE compared with the lowest third was 0.37 (95% CI, 0.18–0.78); for PCBs, it was 0.51 (95% CI, 0.25–1.05).

Because of the estrogen-like activity of some organochlorine compounds, analyses were performed considering both menopausal status at the time of diagnosis and tumor estrogen receptor status. Tumor estrogen receptor status was available for 86% of cases from the 1989 cohort but for only 45% of the cases from the 1974 cohort because routine tumor estrogen receptor assays were not performed until after 1980. The risk of breast cancer in association with concentrations of DDE and PCBs varied slightly by menopausal status at diagnosis and tumor estrogen receptor status (Table 5). Whether the breast cancer cases were diagnosed when pre- or postmenopausal or with an estrogen receptor-negative or -positive tumor, there was no evidence of a consistent increase in risk with higher levels of organochlorine components. The only significant trend in risk was in the inverse direction among women from the 1974 cohort with postmenopausal onset of breast cancer.

We examined the association with DDE and PCBs stratifying by lactation history among parous women because of previously reported associations between PCBs and DDE and

Table 3 Mean and median DDE and PCB concentrations according to cohort participation

	1974					1989				
	Mean (SD)		Median		P^a	Mean (SD)		Median		P^a
	Cases	Controls	Cases	Controls		Cases	Controls	Cases	Controls	
Total DDE										
Unadjusted (ng/ml)	11.5 (7.1)	13.6 (10.6)	9.8	11.1	0.06	7.9 (6.4)	9.7 (3.6)	6.4	7.0	0.41
Lipid-adjusted ng/gm	1698.9 (929.3)	1920.3 (1409.0)	1463.9	1668.4	0.20	1311.9 (1036.5)	1586.3 (1557.4)	1119.2	1181.7	0.56
Total PCBs										
Unadjusted (ng/ml)	4.9 (3.8)	4.7 (2.3)	3.9	4.2	0.21	2.1 (2.0)	2.2 (1.9)	1.6	1.7	0.37
Lipid-adjusted (ng/gm)	735.3 (644.8)	663.6 (322.5)	595.4	607.3	0.48	327.7 (306.3)	332.9 (279.6)	252.0	270.3	0.58

[a] Wilcoxon signed rank test.

Table 4 Association between DDE and PCBs and breast cancer risk

	1974					1989				
	Fifths	Cases	Controls	OR	(95% CI)	Thirds	Cases	Controls	OR	(95% CI)
Total DDE										
Unadjusted	1. <6.93	62	47	1.0		1. <4.34	29	35	1.0	
ng/ml	2. 6.94–9.60	52	47	0.88	(0.52–1.50)	2. 4.35–10.53	59	34	1.80	(0.97–3.33)
	3. 9.61–12.86	48	46	0.83	(0.49–1.43)	3. 10.54–57.16	17	36	0.53	(0.24–1.17)
	4. 12.87–17.74	43	48	0.70	(0.40–1.22)					
	5. 17.75–80.33	30	47	0.50	(0.27–0.89)					
				(P_{trend} = 0.02)					(P_{trend} = 0.08)	
Lipid-adjusted	1. <1017.19	49	47	1.0		1. <816.3	38	35	1.0	
ng/g	2. 1,017.20–1,425.39	61	47	1.24	(0.72–2.13)	2. 816.4–1,595.1	44	35	1.18	(0.65–2.13)
	3. 1,425.40–1,864.57	47	47	0.96	(0.55–1.67)	3. 1,595.2–10,065.6	23	35	0.58	(0.29–1.17)
	4. 1,864.58–2,446.69	42	47	0.86	(0.49–1.51)					
	5. 2,446.70–10,795.91	36	47	0.73	(0.40–1.32)					
				(P_{trend} = 0.13)					(P_{trend} = 0.15)	
Total PCBs										
Unadjusted	1. <2.83	53	47	1.0						
ng/ml	2. 2.84–3.71	56	47	1.07	(0.61–1.89)	1. <1.30	42	34	1.0	
	3. 3.72–4.77	44	47	0.80	(0.44–1.47)	2. 1.31–2.17	29	35	0.65	(0.33–1.30)
	4. 4.78–6.28	42	47	0.75	(0.42–1.34)	3. 2.18–14.63	34	36	0.73	(0.37–1.46)
	5. 6.29–32.60	40	47	0.68	(0.36–1.29)					
				(P_{trend} = 0.16)					(P_{trend} = 0.56)	
Lipid-adjusted	1. <394.47	42	47	1.0						
ng/g	2. 394.48–558.72	59	47	1.41	(0.79–2.50)	1. 13.6–191.8	40	34	1.0	
	3. 558.73–669.46	41	47	0.94	(0.49–1.77)	2. 191.9–333.5	32	35	0.78	(0.41–1.47)
	4. 669.47–852.22	45	47	1.08	(0.59–2.01)	3. 333.6–2,007.9	33	36	0.76	(0.38–1.51)
	5. 852.23–6,460.04	48	47	1.12	(0.59–2.15)					
				(P_{trend} = 0.44)					(P_{trend} = 0.60)	

breast cancer risk among parous women who never lactated (20). The risk of breast cancer was not increased among parous women; in fact, ORs were in the inverse direction with increasing concentrations of DDE and PCBs (data not shown).

The association between concentrations of DDE and total PCBs and the development of breast cancer among CLUE II participants was examined stratifying by polymorphisms of *GSTM1*, *GSTT1*, *GSTP1*, *COMT*, and *CYP17* (Table 6). Associations did not vary significantly by genetic polymorphisms.

Discussion

The results of this prospective study provide evidence against the hypothesis that organochlorine compounds are associated with the risk of developing breast cancer. Women with the highest serum concentrations of DDE had the lowest risk of developing breast cancer. This association held for both unadjusted and lipid-adjusted concentrations and was strongest among women with the longest follow-up interval (16–20 years). The risk of developing breast cancer also tended to decrease with increasing levels of PCBs. Our study has the advantage of being community-based, assessing exposure to DDE and PCBs years before the diagnosis of breast cancer, and examining exposure from two time periods.

We used cohorts from two time periods (1974 and 1989) and, thus, were able to examine the long-term effect of exposure to relatively high levels of the organochlorine compounds present in the population near the time that the compounds were banned as well as more recent, lower levels of exposure. The concentrations of DDE in 1974 were more than twice that observed in Mexico (19), where DDT is still used. Even among women exposed to high levels, we did not observe an increased risk of breast cancer from DDE or PCBs after a follow-up of up to 20 years.

Our study is the largest published to date, and the results are consistent with three previously published nested case-control studies (21–23). Krieger *et al.* examined serum specimens obtained before the banning of the DDE and PCBs. Mean

Table 5 Association between organochlorine components and the risk of breast cancer by menopausal status at diagnosis and estrogen receptor status

	1974				1989			
	Lower third	Middle third	Upper third	P_{trend}	Lower third	Middle third	Upper third	P_{trend}
DDE								
Premenopausal at diagnosis	1.0	0.42	0.86	(0.68)	1.0	4.28	1.42	(0.8)
Postmenopausal at diagnosis	1.0	0.75	0.52	(0.003)	1.0	1.57	0.50	(0.15)
Estrogen receptor-negative	1.0	1.10	1.67	(0.41)	1.0	0.74	0.17	(0.13)
Estrogen receptor-positive	1.0	0.86	0.80	(0.61)	1.0	2.28	0.59	(0.19)
PCB								
Premenopausal at diagnosis	1.0	0.65	2.21	(0.12)	1.0	1.45	2.12	(0.40)
Postmenopausal at diagnosis	1.0	0.67	0.62	(0.10)	1.0	0.83	0.74	(0.44)
Estrogen receptor-negative	1.0	1.13	1.34	(0.71)	1.0	0.19	0.07	(0.06)
Estrogen receptor-positive	1.0	0.98	0.83	(0.69)	1.0	0.91	1.19	(0.55)

Table 6 Association between organochlorine compounds and breast cancer according to genotype, CLUE II, 1989[a]

Genotype	DDE				PCBs			
	Low	Middle	High	P_{trend}	Low	Middle	High	P_{trend}
GSTM1								
Present	1.0	3.23	1.27	0.83	1.0	0.87	1.32	0.53
Null	1.0	1.49	0.27	0.01	1.0	0.59	0.50	0.16
T1								
Present	1.0	2.32	0.52	0.14	1.0	0.79	0.62	0.28
Null	1.0	1.20	0.51	0.36	1.0	0.19	0.93	0.78
P1								
Ile/Ile	1.0	4.20	0.52	0.30	1.0	0.64	0.58	0.34
Ile/Val or Val/Val	1.0	0.94	0.40	0.08	1.0	0.49	0.75	0.74
COMT								
HH	1.0	0.76	0.35	0.22	1.0	0.58	0.92	0.90
HL	1.0	1.76	0.63	0.44	1.0	1.07	0.48	0.17
LL	1.0	3.93	0.59	0.35	1.0	0.19	0.54	0.80
CYP17								
A1/A1	1.0	2.64	0.47	0.17	1.0	0.54	0.35	0.11
A1/A2	1.0	2.30	1.08	0.96	1.0	0.38	1.21	0.49
A2/A2	1.0	0.86	0.05	0.04	1.0	0.84	0.49	0.36

[a] Unconditional logistic regression adjusted for age and menopausal status at baseline.

levels were similar among cases and controls, and there was no evidence of a trend in risk with increasing serum concentrations. Hunter *et al.* compared DDE and PCB concentrations in breast cancer cases diagnosed within 3 years after blood collection in 1989 and 1990 to matched controls. Similar to our results, the risk was lowest among women in the highest category of DDE and PCB concentrations. A nested case-control study from Denmark found no association with concentrations of DDE or PCBs measured from blood samples collected in 1976 (23).

Our findings are also consistent with three of the four studies that have examined the association between serum concentrations of DDE and breast cancer measured after the diagnosis of breast cancer (18–20). Only the study by Wolff *et al.* (17) observed an association between the presence of breast cancer and higher DDE concentrations. Two of these studies also examined the association between concentration of PCBs and breast cancer. Results of the study by Wolff *et al.* (17) were suggestive of a threshold effect of PCBs. Moysich *et al.* (20) observed an increased risk of postmenopausal-onset breast cancer associated with total PCB concentrations and moderately chlorinated PCB congeners only among parous women who never lactated. We were not able to reproduce this observation in our study.

A potential concern in interpreting studies based on serum rather than adipose tissue measurement of exposure to DDE and PCBs is that serum measurements may not adequately reflect adipose tissue levels. Nevertheless, studies have shown good correlation between serum levels and adipose tissue levels (1, 41), and lipid-adjustment of serum is a good estimation of adipose tissue levels (34). In addition, the results of recent serum-based studies are consistent with the majority of published case-control studies involving DDE or PCB exposure based on adipose tissue measurements (Table 1; Refs. 10–16). The largest study published to date of adipose levels of DDE and breast cancer (265 cases, 341 controls) also found a lower risk of breast cancer for women in the highest *versus* the lowest fourth of the DDE distribution (OR, 0.73; 95% CI, 0.44–1.21); PCBs were not assayed (15).

Although we previously reported an association between breast cancer and the putative high-risk polymorphisms of *GSTM1*, *GSTT1*, *GSTP1*, and *COMT*, we found no evidence that these genetic factors influenced susceptibility to organochlorine compound effects. Thus, the search to identify the relevant environmental or endogenous exposures involved in the potential gene-environment interactions related to breast carcinogenesis must continue.

Of the three prospective studies of the association between exposure to DDE and PCBs, two studies examined levels before, or at the time of, banning of compounds and had a long

length of follow-up to the time of diagnosis. None of the three observed an excess risk of breast cancer among women with higher concentrations of DDE or PCBs. The balance of data from both case-control and prospective studies are reassuring that exposure to DDE and PCBs do not confer an increased risk of breast cancer. It now seems likely that whatever environmental exposures contribute to the risk of breast cancer, exposure to DDT and PCBs can be ruled out.

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Serum Concentrations of Organochlorine Compounds and the Subsequent Development of Breast Cancer

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