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PLASMA ORGANOCHLORINE LEVELS AND THE RISK OF BREAST CANCER

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ABSTRACT

Background Exposure to "environmental estrogens" such as organochlorines in pesticides and industrial chemicals has been proposed as a cause of increasing rates of breast cancer. Several studies have reported higher blood levels of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) and polychlorinated biphenyls (PCBs) in patients with breast cancer than in controls.

Methods We measured plasma levels of DDE and PCBs prospectively among 240 women who gave a blood sample in 1989 or 1990 and who were subsequently given a diagnosis of breast cancer before June 1, 1992. We compared these levels with those measured in matched control women in whom breast cancer did not develop. Data on DDE were available for 236 pairs, and data on PCBs were available for 230 pairs.

Results The median level of DDE was lower among case patients than among controls (4.71 vs. 5.35 parts per billion, $P=0.14$), as was the median level of PCBs (4.49 vs. 4.68 parts per billion, $P=0.72$). The multivariate relative risk of breast cancer for women in the highest quintile of exposure as compared with women in the lowest quintile was 0.72 for DDE (95 percent confidence interval, 0.37 to 1.40) and 0.66 for PCBs (95 percent confidence interval, 0.32 to 1.37). Exposure to high levels of both DDE and PCBs was associated with a nonsignificantly lower risk of breast cancer (relative risk for women in the highest quintiles of both DDE and PCBs as compared with women in the lowest, 0.43; 95 percent confidence interval, 0.13 to 1.44).

Conclusions Our data do not support the hypothesis that exposure to 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) and PCBs increases the risk of breast cancer. (N Engl J Med 1997;337:1253-8.)

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THE fivefold variation in the rates of breast cancer around the world,¹ combined with the observation that the daughters of women who migrate from a country with a low incidence of breast cancer to a country with a high incidence acquire the breast-cancer risk prevailing in the high-incidence country,² strongly suggests that environmental and lifestyle factors are the major causes of breast cancer. The incidence of breast cancer in the United States has risen by 1 percent per year since 1940,³ and there is uncertainty about the extent to which established risk factors can explain the increase. Environmental pollutants have been suggested as potential causes.^{4,5}

The hypothesis that among these pollutants, hormonally active organochlorine chemicals may be responsible has garnered wide attention. Many pesticides and industrial chemicals have the potential to act as "environmental estrogens" and have been shown to affect wildlife adversely. The most abundant organochlorine contaminants are the pesticide 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) and certain polychlorinated biphenyls (PCBs). DDT, which was introduced in the United States in 1945 and banned in 1972,⁶ has been implicated as the cause of eggshell thinning in bald eagles,⁷ and certain PCBs used in a wide variety of industrial products and manufactured between 1929 and 1977⁶ can alter sex determination in animals.⁸ In vitro assays demonstrate that 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), the main metabolite of

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DDT, and certain PCB congeners have estrogen-like activity.⁹

Many of these compounds accumulate in the body because of their lipid solubility and resistance to metabolism. They are also present in human adipose tissue and breast milk.¹⁰ In a nationwide study of breast milk in the 1970s, 99 percent of samples had detectable levels of DDT and PCBs.¹¹ DDT promotes the growth of mammary tumors in some rodent models.^{12,13} Limited data are available to assess possible associations with breast cancer in humans. Two small case-control studies reported higher levels of DDE among women with breast cancer than among controls.^{14,15} In another study the association was limited to women with estrogen-receptor-positive breast cancer.¹⁶ One of these studies¹⁵ also found higher levels of PCBs among the women with breast cancer than among controls. In a recent case-control study from Europe, concentrations of DDE in adipose tissue were lower in patients with breast cancer than in controls.¹⁷ A prospective study of 58 cases of breast cancer in New York¹⁸ found a significant increase in the risk of breast cancer with higher serum levels of DDE and a nonsignificant positive association with PCBs. In a larger prospective study of 150 cases in the San Francisco Bay area, Krieger et al.¹⁹ observed no overall elevation in risk with higher serum levels of either DDE or PCBs, but some have argued that the findings were not clearly null.²⁰

To test the hypothesis that higher blood levels of DDE or PCBs are associated with an increased risk of breast cancer, we measured levels of these organochlorines in 240 women with breast cancer and 240 control women in the Nurses' Health Study, using blood samples prospectively collected from 1989 to 1990.

METHODS

Study Population

In 1976, 121,700 married registered nurses from 11 states were enrolled in the Nurses' Health Study and subsequently followed by questionnaire every two years. Self-reported diagnoses of breast cancer are confirmed by a review of medical records.²¹ The completeness of follow-up as a proportion of potential person-years through 1992 is 95 percent.²¹ Information on risk factors for breast cancer, such as family history (updated in 1982 and 1988) and reproductive history (updated until 1984), is obtained by questionnaire. Menopausal status was defined on the basis of a woman's response to the question whether her periods had ceased permanently. Women who had had a hysterectomy with one or both ovaries left intact were classified as premenopausal until the age at which 10 percent of the cohort had undergone natural menopause (46 years for smokers and 48 years for nonsmokers) and as postmenopausal at the age at which 90 percent of the cohort had undergone natural menopause (54 for smokers and 56 for nonsmokers); in the intervening years these women were classified as being of uncertain menopausal status and excluded from menopause-specific analyses.

From 1989 to 1990, 32,826 women sent us a blood sample, which was separated into aliquots of plasma, red cells, and buffy coat. Women who sent a blood sample were very similar to other women in the cohort with respect to reproductive risk factors for

breast cancer such as age at menarche, parity, and age at the birth of their first child. Women who gave a blood specimen were slightly more likely to have a history of benign breast disease or a family history of breast cancer. These differences should not influence the internal validity of comparisons between case patients and controls in the subcohort of women who gave a blood specimen.

We defined case patients as women who did not have a diagnosis of cancer (other than nonmelanoma skin cancer) when they sent in the blood specimen and in whom breast cancer was subsequently diagnosed before June 1, 1992. There were 240 eligible case patients: 200 women had invasive cancer, 39 had carcinoma in situ, and 1 had cancer with uncertain histologic features. For each case patient we matched a control subject who had not reported a diagnosis of cancer according to the year of birth, menopausal status at the time of blood sampling, month in which the blood sample was returned, time of day that the blood sample was obtained, fasting status at blood sampling, and for postmenopausal women, postmenopausal hormone use.

Laboratory Analyses

The laboratory methods have been described in detail elsewhere.^{18,22} Briefly, a polar extract of plasma lipids was further treated with a step involving chromatographic cleanup and enrichment of the column and then analyzed by gas chromatography with electron-capture detection. All steps were scaled appropriately for 0.50-ml aliquot volumes. We have previously demonstrated using Nurses' Health Study specimens that the precision with the use of this volume and an optimized analytic procedure is similar to that with previous procedures using 1-ml and 2-ml aliquots.²³ The amount of methanol was optimized (0.3 ml) to create a good interface between the aqueous layer and the ether-hexane extractant (1.25 ml). Results are reported as parts per billion (ppb) of DDE (which is equivalent to nanograms of DDE per milliliter) and of the sum of the higher PCB congeners — compounds with retention times longer than that of DDE (pentachlorobiphenyls, hexachlorobiphenyls, and heptachlorobiphenyls). The limits of detection were less than 1 ppb for both DDE and PCBs, on the basis of a value that was three times the standard deviation²⁴ of 24 determinations over the course of sample analyses of a quality-control plasma pool with approximately 1 ppb of both DDE and PCBs. Both DDE and PCBs are stable in frozen blood; organochlorine levels in serum frozen at -20°C were unchanged over a period of one year²² (and unpublished data). Plasma cholesterol was determined with the procedure of Alain et al.²⁵

Serum samples from pairs of case patients and controls (with the order of samples randomized) were sent to the laboratory in batches of 12 pairs; each batch included 2 unidentifiable split samples from pooled plasma from premenopausal or postmenopausal women. For each batch we calculated the coefficient of variation; the median coefficient of variation was 4.3 percent for DDE and 13.2 percent for PCBs. DDE values were missing for one member of four case-control pairs, and PCB values were missing for one member of an additional six pairs because the samples were lost or contaminated.

Statistical Analysis

Since both DDE and PCBs are correlated with blood lipid content,²⁶ linear regression analysis of log-transformed DDE and PCB values was performed to adjust for plasma cholesterol concentration. We used these adjusted values in our principal analyses; we also used the unadjusted values in supplementary analyses.

We assessed the relations of plasma DDE and PCBs using Spearman correlation coefficients for continuous variables and by examining the distribution of risk factors for breast cancer within thirds of plasma organochlorine levels among the controls, testing for statistical significance with the Kruskal-Wallis test.²⁷ We used the Wilcoxon signed-rank test for paired data and the Wilcoxon rank-sum test for unpaired data to compare plasma DDE and

PCB levels between case patients and controls.²⁷ We divided the control distribution into quintiles and calculated the relative risk and 95 percent confidence interval for each quintile relative to the lowest quintile using conditional logistic regression,²⁸ controlling for established risk factors for breast cancer in addition to the matched factors. To assess the potential synergism between organochlorine compounds, we compared women in the highest quintile of both DDE and PCBs with women in the lowest quintile of both. To examine whether the associations between organochlorines were modified by conventional risk factors for breast cancer, we conducted unconditional analyses within strata of the other risk factors for breast cancer, controlling for the matched variables. All P values are two-sided.

RESULTS

The median age of the subjects was 59 years (range, 43 to 69), 68 percent of both case patients and controls were postmenopausal, and the median age at menopause was 49 years for the case patients and 50 years for the controls. Differences in other risk factors for breast cancer between case patients

and controls were not statistically significant, with the exception of maternal history of breast cancer (reported by 11 percent of case patients and 5 percent of controls, P=0.01), history of breast cancer in a sister (8 percent of case patients and 3 percent of controls, P=0.03), and history of benign breast disease (56 percent of case patients and 41 percent of controls, P=0.001).

Plasma levels of both DDE (r=0.31, P<0.001 by Spearman rank correlation) and PCBs (r=0.25, P<0.001) increased with age (Table 1). The only statistically significant association of either DDE or PCBs with established or suspected risk factors for breast cancer (Table 1) was a positive association between body-mass index and plasma DDE levels. Among parous women, more women in the lowest thirds than in the highest thirds of DDE and PCB levels had breast-fed their children for more than six months; however, these associations were not sig-

TABLE 1. RELATION BETWEEN ESTABLISHED OR SUSPECTED RISK FACTORS FOR BREAST CANCER AND PLASMA LEVELS OF DDE AND PCBs AMONG 236 NURSES' HEALTH STUDY PARTICIPANTS WITHOUT DIAGNOSED BREAST CANCER.*

RISK FACTOR	LOWER THIRD, ≤4.02 ppb OF DDE	MIDDLE THIRD, >4.02-7.32 ppb OF DDE	UPPER THIRD, >7.32 ppb OF DDE	P VALUE
	Median age (yr)	54	59	
Median age at menarche (yr)	12	12	13	0.28†
Median no. of children	3	3	3	0.67†
Median age at birth of 1st child (yr)‡	24	24	25	0.05†
Median body-mass index§	23.2	24.3	25.0	0.01†
Lactation for >6 mo total (%)‡	31	32	17	0.25¶
Family history of breast cancer (%)	8	9	6	0.87¶
History of benign breast disease (%)	36	46	40	0.48¶
	LOWER THIRD, ≤3.99 ppb OF PCBs	MIDDLE THIRD, >3.99-5.49 ppb OF PCBs	UPPER THIRD, >5.49 ppb OF PCBs	
Median age (yr)	55	59	62	<0.001†
Median age at menarche (yr)	12	12	13	0.56†
Median no. of children	3	3	3	0.09†
Median age at birth of 1st child (yr)‡	24	25	24	0.23†
Median body-mass index§	24.4	24.1	24.9	0.43†
Lactation for >6 mo total (%)‡	37	15	29	0.08¶
Family history of breast cancer (%)	5	7	12	0.31¶
History of benign breast disease (%)	44	41	36	0.57¶

*The plasma levels of DDE and PCBs were measured after adjustment for plasma cholesterol concentrations. The subjects ranged in age from 43 to 69 years. Levels of DDE and PCBs were divided into thirds. Data on PCBs were based on 230 women.

†The Kruskal-Wallis test was used.

‡Only parous women were included in the analysis.

§The body-mass index is calculated as the weight in kilograms divided by the square of the height in meters.

¶The chi-square test was used.

TABLE 2. PLASMA LEVELS OF DDE AND PCBs AMONG CASE PATIENTS WITH BREAST CANCER AND CONTROLS IN THE NURSES' HEALTH STUDY.*

	CASE PATIENTS AND CONTROL†	MEAN (±SD) VALUE	MEDIAN VALUE	P VALUE‡
	no.	parts per billion		
DDE				
Case patients	236	6.01±4.56	4.71	0.14
Controls	236	6.97±5.99	5.35	
PCBs				
Case patients	230	5.08±2.51	4.49	0.72
Controls	230	5.16±2.26	4.68	

*The plasma levels of DDE and PCBs were measured after adjustment for plasma cholesterol concentrations.

†DDE values were missing for one member of four case-control pairs, and PCB values were missing for an additional six pairs due to lost samples or evidence of contamination.

‡The Wilcoxon signed-rank test was used.

nificant. Results were similar in analyses that were not adjusted for plasma cholesterol concentration.

Women who were given a diagnosis of breast cancer after providing a blood sample in 1989 or 1990 had lower levels of plasma DDE than controls (Table 2); the median level was 4.71 ppb in case patients and 5.35 ppb in controls ($P=0.14$). Plasma PCB levels were essentially the same in case patients and controls. Results were similar in analyses that were not adjusted for plasma cholesterol concentration: the unadjusted median for DDE was 5.07 ppb in case patients and 5.59 ppb in controls ($P=0.14$); the unadjusted median for PCBs was 4.58 ppb in case patients and 4.73 ppb in controls ($P=0.60$). Levels of DDE and PCBs were similar among women with and those without axillary-lymph-node involvement at diagnosis. The exclusion of 101 pairs in which the case patient was given a diagnosis of breast cancer within one year after blood sampling had little effect on these findings. After restriction of the analyses to 197 patients with invasive cancer and their controls for whom data were available, the median value for DDE among case patients was 5.02 ppb, as compared with 5.60 ppb among controls ($P=0.20$). For PCBs the median value among both patients with invasive cancer and controls was 4.69 ppb ($P=0.87$). In analyses restricted to 139 case patients with estrogen-receptor-positive disease and their controls, the results were similar.

We found no evidence of a positive association between high levels of plasma DDE or PCBs and a risk of breast cancer (Table 3). The multivariate relative risk for the highest decile of plasma DDE levels as compared with the lowest decile was 0.38 (95 percent confidence interval, 0.13 to 1.09); for PCBs the

risk was 0.44 (95 percent confidence interval, 0.15 to 1.29). Even among women with high levels of both DDE and PCBs, there was still no evidence of a positive association. As compared with women who were in the lowest quintiles of both DDE and PCBs, women in the highest quintiles were at non-significantly lower risk of breast cancer (multivariate relative risk=0.43; 95 percent confidence interval, 0.13 to 1.44).

Among 48 premenopausal case patients and 53 controls with values for DDE, the median level was 3.72 ppb among case patients and 3.30 ppb among controls ($P=0.95$). For PCBs the median was 3.91 ppb among 47 premenopausal case patients and 4.11 ppb among 51 premenopausal controls ($P=0.54$). The results for postmenopausal women were similar to the overall results. The absence of an association between DDE, PCBs, and breast cancer was similar within strata of age, age at menarche, age at birth of first child, number of children, and history of lactation.

DISCUSSION

In this prospective study, we did not observe any evidence of an increased risk of breast cancer among women with relatively high levels of plasma DDE or PCBs. Most of the relative risks we observed for higher levels of exposure were less than 1, and the upper bounds of the 95 percent confidence intervals generally excluded all but small increases in risk. Moreover, women with high levels of both DDE and PCBs were not at higher risk than women with the lowest levels of these compounds.

The hypothesis that environmental organochlorine contaminants cause breast cancer is based largely on indirect evidence. Some but not all studies have shown that DDE and PCBs act as estrogens in vitro and in animals. Indeed, some PCB congeners and organochlorines, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, have antiestrogenic activity. In general, these compounds are very weak estrogens in in vitro assays, requiring concentrations of up to 100,000 times more than the natural estrogen 17 β -estradiol to achieve equivalent estrogenic activity.⁹ On the basis of these in vitro assays, it has been estimated that humans are exposed to naturally occurring estrogenic compounds in our diet in amounts that are many orders of magnitude higher than those of environmental organochlorine estrogens.²⁹ Nevertheless, given that organochlorines are fat soluble, persist in adipose tissue, and are excreted in breast milk,³⁰ it may be that ductal and other cells in the breast are exposed to these compounds over a period of many decades. Such prolonged exposure may counterbalance the low estrogenic potency of organochlorines.

Because they are highly lipophilic and metabolically resistant, DDE and PCBs undergo lifelong sequestration in human adipose tissue. Blood levels of

TABLE 3. RELATIVE RISK OF BREAST CANCER ACCORDING TO QUINTILE OF PLASMA DDE AND PCB LEVELS AT BASE LINE IN THE NURSES' HEALTH STUDY, 1989 TO 1992.*

VARIABLE	QUINTILE 1, ≤2.78 ppb OF DDE	QUINTILE 2, >2.78–4.54 ppb OF DDE	QUINTILE 3, >4.54–6.26 ppb OF DDE	QUINTILE 4, >6.26–9.46 ppb OF DDE	QUINTILE 5, >9.46 ppb OF DDE	P VALUE FOR TREND
	No. of case patients	61	54	35	43	
No. of controls	47	46	49	47	47	
Relative risk						
Matched (95% CI)†	1.0‡	0.88 (0.52–1.50)	0.56 (0.31–0.99)	0.73 (0.42–1.27)	0.66 (0.36–1.22)	0.22
Multivariate (95% CI)§	1.0‡	0.80 (0.45–1.43)	0.47 (0.25–0.90)	0.74 (0.40–1.36)	0.72 (0.37–1.40)	0.47
	QUINTILE 1, ≤3.59 ppb OF PCBs	QUINTILE 2, >3.59–4.28 ppb OF PCBs	QUINTILE 3, >4.28–5.12 ppb OF PCBs	QUINTILE 4, >5.12–6.31 ppb OF PCBs	QUINTILE 5, >6.31 ppb OF PCBs	
No. of case patients	64	40	39	41	46	
No. of controls	46	45	46	45	48	
Relative risk						
Matched (95% CI)†	1.0‡	0.57 (0.31–1.06)	0.54 (0.29–1.00)	0.55 (0.29–1.05)	0.59 (0.31–1.12)	0.26
Multivariate (95% CI)§	1.0‡	0.62 (0.32–1.20)	0.52 (0.25–1.06)	0.54 (0.26–1.10)	0.66 (0.32–1.37)	0.47

*The plasma levels of DDE and PCBs were measured after adjustment for plasma cholesterol concentrations. CI denotes confidence interval.

†Case patients and controls were matched for year of birth, menopausal status, month in which blood sample was returned, time of day blood sample was drawn, fasting status at blood sampling, and for postmenopausal women, postmenopausal hormone use.

‡This was the reference group.

§After control for matching variables, conditional logistic-regression analyses were adjusted for a history of breast cancer in a mother or sister, a history of benign breast disease, age at menarche (<11 years, 11 to 14, and ≥15), number of children and age at birth of first child (nulliparous, 1 to 2 children and age ≤24 at first birth, 1 to 2 children and age >24 at first birth, ≥3 children and age ≤24 at first birth, and ≥3 children and age >24 at first birth), duration of lactation (0, 1 to 6 months, and >6 months), and body-mass index (<21, 21 to 24.9, 25 to 29.9, and ≥30).

these compounds are among the most stable biologic markers of exposure known. In blood samples collected before and after treatment for breast cancer (an average of 56 days apart), the r values for the lipid-adjusted correlations between the first and second samples were 0.99 for DDE and 0.96 for PCBs.³¹ Among 31 healthy women who provided two blood samples two months apart, the r values were 0.96 for DDE and 0.89 for PCBs.³² The half-life of plasma DDE is approximately 10 years (Wolff M, Toniolo P: unpublished data). Among workers with occupational exposure to organochlorines, the length of time that highly chlorinated PCBs persist in the body varies widely; the most long-lived congeners have half-lives of 7 to 30 years.³³ These data, and the consistent correlation of DDE and PCBs with age, suggest that the blood levels of DDE and PCBs we measured reflect exposure that occurred over a period of many years.

Epidemiologic data regarding possible relations of organochlorines with breast cancer are limited. Several small case-control studies reported higher levels of

DDE¹⁴ or PCBs¹⁵ among case patients than controls. A recent European case-control study (264 case patients) reported a significant inverse trend between levels of adipose DDE and the risk of breast cancer; data on PCBs were not available.¹⁷ In a prospective study of 14,290 women in New York,¹⁸ levels in serum were compared in 58 women given a diagnosis of breast cancer within one to six months after blood collection in 1985 to 1991 and 171 controls. The adjusted relative risk for the highest quintile of DDE as compared with the lowest was 3.68 (95 percent confidence interval, 1.10 to 13.50); for PCBs the risk was 4.35 (95 percent confidence interval, 0.92 to 20.47). In the largest prospective study to date, Krieger et al.¹⁹ examined serum from 150 case patients selected from a cohort of 57,040 San Francisco Bay area women who had provided blood between 1964 and 1971. Little association was seen between organochlorine levels and the risk of breast cancer.

A strength of the three available prospective studies (including this study) is that the analyses of DDE and PCBs were all performed in the same laboratory;

neither laboratory technique nor a variation in accuracy is likely to account for differences in findings. The levels of DDE we observed in this study among women who provided blood samples in 1989 or 1990 were similar to those observed in samples obtained in 1985 to 1991 from New York women,¹⁸ but the median was six times lower than the medians for women in the San Francisco Bay area in the late 1960s.¹⁹ This finding is compatible with the long-term decline in DDE levels since the compound was banned in 1972 and suggests that there is no relation with breast cancer at either the levels currently prevailing or the higher levels that were present when DDT was still being used. The PCB levels we observed were similar to those observed in both of the previous prospective studies, underlining the persistence of these compounds in our environment even after production ceased in 1977.

Although we cannot exclude the possibility that exposure in utero or during childhood could increase the risk of breast cancer decades later, because DDT and PCBs were introduced into the environment largely in the 1940s and 1950s, exposure very early in life could not have accounted for most of the increase in the incidence of breast cancer over the past several decades, which has been greatest in postmenopausal women who were already adults when the compounds were being most widely used. The absence of an association with DDE and PCBs does not rule out the possibility that other pesticides and environmental contaminants may be associated with breast cancer. There are good ecologic reasons to avoid the release of DDT and PCBs into our environment, but on the basis of our results the use of these compounds does not explain the high and increasing rates of breast cancer.

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