BRIEF COMMUNICATION

Organochlorine Pesticide Content of Breast Adipose Tissue From Women With Breast Cancer and Control Subjects

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The suggestion that organochlorine compounds used as pesticides may be related to environment-induced breast cancer has been based on several observations. DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane], its metabolite DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], similar pesticides, and related chemicals (PCBs [polychlorinated biphenyls] and PBBs [polybrominated biphenyls]) are known animal carcinogens *(1,2).* Both DDT and PCBs have been shown to be tumor promoters *(2)* and to have estrogenic activity *(3).* It is important to note that some studies *(4–6)* have suggested that organochlorines may inhibit rather than promote tumor growth and may even have antiestrogenic effects. Organochlorines have become ubiquitous in the environment and in human tissues because of their long half-life in the environment, their inefficient metabolism, and their high solubility in lipids, which leads to long-term sequestration in adipose tissues. However, since the use of DDT has been banned in the United States since 1972, the total-body burden of DDT and of its metabolites in the population has fallen with time.

Case–control studies of organochlorine levels in serum or adipose tissue *(7–21)* have yielded conflicting data regarding the connection between exposure to these chemicals and breast cancer. A pilot study conducted by Falck et al. *(14)* showed 50% higher concentrations of DDT, of its metabolite DDE, and of PCBs in mammary adipose tissue from women with cancer (n = 20) compared with women with benign breast disease (n = 20). A relative risk of approximately 3 was estimated for women in the highest tertile of exposure. Although preliminary, these results obtained from a small sample size suggested a role of these organochlorines in the causation of breast cancer. In contrast, a much larger European case–control study *(10),* which utilized gluteal adipose tissue, found no association between higher organochlorine content and the presence of breast cancer.

In this study, we carried out, to our knowledge, the largest study of the organochlorine content of breast adipose tissue reported to date.

The study population was derived from the Kaiser Permanente Medical Center in Woodland Hills, CA, during the period from January 1995 through December 1996. The study was approved by the Institutional Review Board of the Kaiser Permanente Medical Care Program. All patients were consecutively recruited and gave written informed consent to be included in the study. Of the 146 women studied, 73 had breast cancer. The control group comprised 73 women undergoing reduction mammoplasty for mastomegaly. Information regarding the patient's age, height, weight, menopausal status, and family history of breast cancer was obtained from the medical records.

At the time of biopsy, 0.2–1.0 g of breast adipose tissue was collected for organochlorine analysis *(18).* For the determinations of DDT and its metabolites in the breast adipose tissues, 200–300 mg of adipose tissue was accurately weighed with the use of a microbalance, treated with methanol (2 mL), and extracted three times with 2.5 mL of a mixture of diethylether and hexane (1 : 1). The combined extracts were reduced to 0.5 mL under nitrogen and chromatographed on a 2.5-g Florisil column (Supelco, Bellefonte, PA). The use of the Florisil column chromatography was in accordance with the method of the U.S. Environmental Protection Agency to separate pesticide residues. Two fractions were eluted; the first (13 mL of hexane) contained nonpolar residues (including DDE and DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane]), and the second (13 mL of 6% diethylether–hexane [1 : 1]) contained polar residues (including DDT). Each fraction was evaporated under reduced pressure with the use of a rotary evaporator to 0.50 mL for gas chromatographic analysis. Both fractions were analyzed by gas chromatography with electron-capture detection (^{63}Ni) and autosampler injection; external standards were used for quantitation. Gas chromatographic conditions were as follows: glass column (30 m × 0.32 mm inner diameter × 1-μm film thickness) containing 5% phenyl polysiloxane–95% methyl polysiloxane; a 50-mL/minute mixture of argon and 5% methane; and column temperature of 180 °C, inlet temperature of 260 °C, and detector temperature of 300 °C. Identification was based on retention time relative to pure standards of DDE, DDT, and DDD. External standard quantitation was done with the use of integrated peak areas with standard concentrations from 0.25 to 500 ng/mL.

The concentrations of DDT and of its metabolites expressed on a lipid basis were logarithmically transformed before any statistical tests were conducted. Statistical comparisons of organochlorine levels in patients and control subjects were made by two-sided Student's *t* test. A multivariate logistic regression analysis was performed to evaluate the relationship between organochlorines and the probability of breast cancer. We included covariates in the multivariate models if they were well-established risk factors for breast cancer and/or appeared to be possible confounders in preliminary univariate analyses. The covariates included the patient's age, body mass index (i.e., weight [in kilograms] divided by height [in meters] squared), menopausal status at the time of diagnosis of breast cancer, and family history of breast cancer. Preliminary analyses showed age to be a statistically significant predictor for breast cancer; therefore, in the final logistic regression model, age was treated as a confounding factor. The results with adjustment of age with the use of several age catego-

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ries were essentially the same as those with a single adjustment for a continuous variable; we are presenting the results with a single adjustment for a continuous variable. To examine the association between age and organochlorines, we carried out a regression analysis. We fit the data with quadratic curves for breast cancer patients and control subjects and examined the difference between the two curves.

The average age of breast cancer patients was significantly higher than that of the control subjects (mean [standard deviation] = 57.5 years [31.1 years] for breast cancer patients and 42.7 years [15.5 years] for control subjects; $P = .0001$). The mean degree of adiposity, as measured by body mass index, did not differ significantly between the breast cancer patients and the control subjects ($P = .40$). The mean adipose tissue concentrations of DDT and of its metabolites DDE and DDD were expressed on a wet-weight basis as well as on a lipid basis (Table 1, A). DDE concentrations were higher than DDT concentrations in both patients and control subjects. Because DDT is rapidly metabolized to DDE or DDD, individual variations in the rate of conversion of DDT to its metabolites may play a role in determining the strength of the link between DDT exposure and breast cancer *(5)*. We, therefore, examined the mean levels of DDT alone, of DDE alone, and of the sum of DDT, DDE, and DDD (Table 1, A). In our samples, similar to what has been seen in the majority of the previously reported studies of human serum and adipose tissue levels of DDT, DDE, and DDD, the predominant isomers were *p,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD. There was no statistically significant difference in the mean breast adipose tissue concentration of DDT between patients and control subjects. The mean concentrations of DDE were statistically significantly higher in patients than in control subjects ($P = .006$). The sum of DDT and its metabolites was also statistically significantly elevated in patients compared with control subjects.

There were differences in the age distribution between the breast cancer patients and the control subjects; therefore,

Table 1, A. Unadjusted mean concentrations of DDT and of its metabolites in breast adipose tissue from patients with breast cancer and from control subjects

Pesticides*	Control subjects (n = 73)	Breast cancer patients (n = 73)	P†
	Wet weight basis, ng/g‡		
DDT	197.6 (96.3–298.9)	231.4 (130.1–332.8)	.22
DDE	642.1 (382.7–901.4)	693.6 (570.2–816.9)	.005
DDD	21.7 (1.5–41.9)	9.2 (2.4–16.0)	.80
DDT + DDE + DDD	861.4 (569.5–1153.2)	934.3 (742.3–1126.3)	.02
	Lipid basis, ng/g‡		
DDT	267.3 (95.4–431.3)	261.6 (114.3–408.8)	.23
DDE	709.1 (457.7–960.5)	800.0 (656.7–943.3)	.006
DDD	24.0 (2.4–45.6)	9.8 (2.6–17.0)	.79
DDT + DDE + DDD	1000.4 (649.5–1351.3)	1071.4 (839.6–1303.2)	.04

Table 1, B. Logistic regression coefficients for testing association between organochlorines and breast cancer after adjustment with age

Variable*	Parameter estimate§ β	Standard error	Test for $H_o = \beta = 0$, two-sided *P* value‖	Odds ratio¶ (95% confidence interval)
Testing for DDT				
Intercept	−3.364	1.308	.01	
Age	0.082	0.021	.0001	1.085 (1.042–1.131)
DDT	0.051	0.063	.42	1.052 (0.930–1.191)
Testing for DDE				
Intercept	−3.646	1.469	.01	
Age	0.076	0.023	.001	1.079 (1.031–1.129)
DDE	0.119	0.180	.50	1.126 (0.792–1.603)
Testing for DDT + DDE + DDD				
Intercept	−3.145	1.28	.01	
Age	0.083	0.021	.0001	1.086 (1.043–1.132)
DDT + DDE + DDD	−0.101	0.122	.41	0.904 (0.712–1.148)

*DDT = 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; DDE = 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDD = 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane.

†*P* value was calculated by use of a two-sided Student's *t* test.

‡Mean (95% confidence interval).

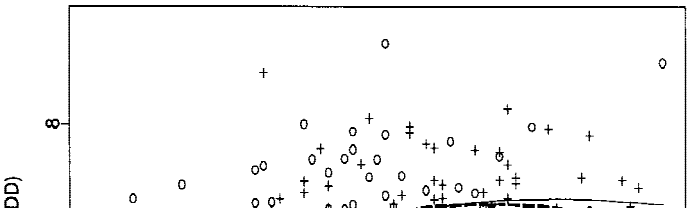
§Estimate of intercept and the regression coefficient.

‖Determined by Wald test.

¶Odds ratio = exponent $^{\beta}$.

logistic regression models were used to study the relationship between the content of organochlorines and breast cancer, independent of the potential confounding factor of age. When adjusted for age, there was no statistically significant relationship between either DDT, DDE, or DDT + DDE + DDD concentrations and breast cancer (Table 1, B). Although the mean levels of DDE were statistically higher in breast cancer patients than in control subjects, this association was completely explained by accumulation of organochlorines with age. As shown in Fig. 1, there was no statistically significant difference in the quadratic curves fit between the case patients and control subjects for organochlorine concentrations.

There are several possible explanations for our study's failure to confirm the hypothetical link between organochlorine pesticides and breast cancer. Unlike previous studies, the breast cancer patients and control subjects in our study were not age matched but were sequentially selected on the basis of the presence of fat tissue adequate for chemical analysis. This lack of matching resulted in statistically significant difference in age distribution between the two groups, reflecting a difference in DDE accumulation due to age. Another weakness of our study was the nature of the control group patients. The assumption that patients requiring reduction mammoplasty are representative of healthy women without breast cancer can be challenged. Because there has been no reported increase in the incidence of breast cancer associated with macromastia, we believe that our control group is at least as appropriate as women undergoing breast biopsies for benign breast disease or for suspected cancer, a group that has been utilized in previous case–control studies. Finally, the study may have had insufficient power to detect an association that actually exists in nature, although it was larger than previous adipose tissue studies showing positive association. We believe that this is unlikely because our data, when adjusted for age, do not suggest higher levels of DDE or DDT + DDE + DDD in breast cancer patients than in control subjects. The results of our study are in agreement with other large studies that have found no association between organochlorine levels in plasma and gluteal adipose tissue and breast cancer risk. However, to our knowledge, our study is the first study to be in disagreement with previously published small studies that have found a positive association with organochlorine levels in breast adipose tissue. It has been postulated that organochlorines are associated with the development of breast cancer when they are present in the microenvironment of the breast *(22);* however, on the basis of the observations in our study, there seems to be no clear association between organochlorines exposure and risk of breast cancer.



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NOTES

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