DDE and DDT in Breast Adipose Tissue and Risk of Female Breast Cancer

Tongzhang Zheng,1 Theodore R. Holford,1 Susan T. Mayne,1 Barbara Ward,1 Darryl Carter,1 Patricia H. Owens,1 Robert Dubrow,1 Sheila H. Zahm,2 Peter Boyle,3 Shannon Archibeque,4 and John Tessar4

A case-control study was conducted in Connecticut from 1994 to 1997 to investigate the relation between dichlorodiphenyldichloroethane (DDE) and dichlorodiphenyltrichloroethane (DDT) exposure and breast cancer risk. Cases and controls were women aged 40-79 years, who had breast-related surgery at the Yale-New Haven Hospital and from whose surgical specimen the authors could obtain at least 0.4 g of breast adipose tissue for chemical analyses. A total of 304 incident breast cancer cases (including 62 in situ carcinomas) and 186 benign breast disease controls were recruited into the study. Tissue levels of DDE and DDT were measured using gas chromatography. Statistical significance for comparisons of mean levels of DDE and DDT was calculated using analysis of variance and rank sum tests. A logistic regression model was used to estimate the association and to control confounding. The age-adjusted geometric mean tissue level of DDE for cases (736.5 ppb) was similar to that for the controls (784.1 ppb). DDT levels were also similar for cases (51.8 ppb) and controls (55.6 ppb). The adjusted odds ratio is 0.9 (95% confidence interval: 0.5, 1.5) for DDE and 0.8 (95% confidence interval: 0.5, 1.5) for DDT when the highest quartile was compared with the lowest. These results do not support an association between adipose tissue levels of DDE and DDT and breast cancer risk. Am J Epidemiol 1999;150:453-8.

breast neoplasms; case-control studies; DDE; DDT

Dichlorodiphenyltrichloroethane (DDT) and its most stable metabolite, dichlorodiphenyldichloroethane (DDE), have been suggested to be associated with an increased risk of female breast cancer because of their reported estrogenic activity and ability to induce p450 enzymes, which are intimately involved in steroid hormone metabolism (1, 2). Epidemiologic studies relating DDE and DDT to breast cancer risk, however, have provided provocative but inconclusive results. A study with 20 breast cancer cases and 20 benign breast disease controls reported that a 10-ppb increase in adipose tissue levels of DDE corresponds to a 1 percent increase in breast cancer risk (3). Another study by Dewailly et al. (4) estimated an 8.9-fold increase in breast cancer risk among women whose DDE levels were above 1,292 ppb in adipose tissue compared with those with a level of 238 ppb or lower, based on a study of 18 breast cancer cases and 17 benign breast disease controls. A nested case-control study with 58 cases (5) reported a fourfold increase in risk of breast cancer for an elevation of serum DDE concentrations from 2.0 ng/ml (10th percentile) to 19.1 ng/ml (90th percentile). In another nested case-control study involving 50 White, 50 Black, and 50 Asian women with breast cancer, a two- to almost fourfold increased risk of breast cancer was reported for Black women and White women when comparing women in the third tertile with those in the lowest tertile of DDE in serum. No increased risk with higher serum DDE levels, however, was observed for Asian women in this study (6).

More recent studies with larger sample sizes, ranging from 141 to 265 breast cancer cases, however, do not support an association (7-10). In fact, the study by van't Veer et al. from Europe (9) reported a significant inverse association between the adipose tissue level of DDE and the risk of breast cancer.

Considering the inconclusive data and the ubiquitous exposure to DDE and DDT through the food chain, there exists an urgent need to further examine this association. Since DDE and DDT are lipophilic and highly persistent, they accumulate in fat tissue. Therefore, adipose tissue levels of DDT and DDE are generally considered to be the best indicator for lifetime environmental exposure in humans. Adipose tissue levels of DDE and DDT are also typically much higher than serum levels, thus providing a matrix in which differences are easier to observe. This case-control study,
which directly compares DDE and DDT levels in breast adipose tissue from incident breast cancer cases and controls, examines the hypothesis that environmental exposure to DDE and DDT increases the risk of female breast cancer.

**MATERIALS AND METHODS**

**Study subjects**

All procedures were performed in accordance with a protocol approved by the Yale Human Investigations Committee. In order to be eligible, study subjects had to be women aged 40–79 years who had breast-related surgery at Yale-New Haven Hospital, New Haven, Connecticut, and from whose breast pathology specimen we could collect at least 0.4 g of residual breast adipose tissue for chemical analyses. Breast adipose tissue not needed for diagnostic purposes was collected and placed into a glass vial on ice by personnel from the Tissue Retrieval Facility at the Yale Cancer Center. The samples were then coded and frozen within 30 minutes of being excised and stored at –84°C. Study subjects were enrolled consecutively from January 1, 1994, to December 30, 1997.

The study pathologist (D. C.), who is responsible for almost all of the breast tissue diagnoses made at Yale-New Haven Hospital, classified the potential participants as either potential cases or controls and staged carcinomas according to the tumor-nodes-metastasis system (11). Potential cases were histologically confirmed incident primary breast cancer patients (International Classification of Diseases for Oncology (ICD-O) codes 174.0–174.9). Potential controls were patients with histologically confirmed incident benign breast disease (excluding atypical hyperplasia; including a diagnosis of normal breast tissue). Benign breast diseases were classified and grouped according to the 1985 Pathologists’ Consensus Statement as proliferative benign breast disease without atypia or nonproliferative disease.

After approval by the subjects’ physician, potential participants were approached by letter and then by phone, and those who consented were interviewed in person, generally in the woman’s home or in another convenient location. A standardized, structured questionnaire was used to obtain information on major known or suspected risk factors for breast cancer, including reproductive history, lactation history, past medical history, occupation, and demographic factors. The dietary information was collected through a scannable semiquantitative food frequency questionnaire developed by the Fred Hutchinson Cancer Research Center to optimize estimation of fat intake. Each subject was asked to characterize her usual diet in the year before she had the biopsy. Potential cases and controls were excluded if they had a previous diagnosis of cancer, with the exception of nonmelanoma skin cancer.

**Adipose tissue and laboratory analysis**

Frozen breast adipose tissue samples were sent in batches to the study laboratory at Colorado State University, where they remained frozen until analysis. Tissue samples were analyzed in batches of 12, with each batch having approximately six cases, four controls, and two quality control samples. Samples were batched and coded at Yale; laboratory personnel in Colorado were blind to the case-control status of the samples being analyzed.

The laboratory method for analyzing DDT and DDE in breast adipose tissue was described elsewhere (12). Briefly, the method involved the following: extraction in hexane; separation of organochlorine pesticides from polychlorinated biphenyls and purification of the sample using Florisil chromatography (U. S. Silica Company, Berkeley Springs, West Virginia); and identification and quantification of the compounds using gas chromatography. The quantitation limits of this method were 50 ppb for DDT and 25 ppb for DDE. All analyses were conducted under an established quality control/quality assessment program including method spikes, reagent blanks, and quality control windows. The estimated recovery of the various analytes exceeded 95 percent, and the coefficient of variation was 9.8 percent for DDE and 12.1 percent for DDT. Adipose tissue levels of DDE and DDT were reported as parts per billion (ppb), which is equivalent to nanograms of DDE or DDT per gram of lipid. The amount of lipid in the sample was quantified gravimetrically. Lipid adjustment is necessary since DDT and DDE are lipid soluble, and tissue sample lipid content varied among subjects. Lipid adjustment facilitates comparability of the results among individuals and across studies (13).

**Statistical analysis**

The primary analyses involved comparisons of lipid-adjusted breast adipose tissue levels of DDT and DDE between all cases and all controls. The statistical significance for multiple means of adipose tissue levels of DDT and DDE was calculated using analysis of variance and rank sum tests, and analysis of covariance was used to adjust for potential confounders. Because the distribution of exposures was skewed, we present the median as a measure of location and the first and third quartiles as summaries of the degree of variability. The log transformation was used to better approximate the

*Am J Epidemiol* Vol. 150, No. 5, 1999
normality assumption, and thus the antilog of the resulting adjusted means, that is, the adjusted geometric mean, was used as a summary statistic.

A linear logistic regression model was used to adjust for potential confounders when estimating the exposure-disease association. Quartiles of adipose tissue levels of DDE and DDT were formed based on the frequency distribution of controls. The variables included in the final model were age, body mass index (<22, 22–24.9, 25–29.9, ≥30 kg/m²), lifetime months of lactation (0, 1–6, >6), age at menarche (<13, 13–15, ≥16 years), age at first full-term pregnancy (nulliparous, <25, ≥25 years), menopausal status, race (Whites, Blacks, and others), and income 10 years before the disease diagnosis or interview (<$10,000, $10,000–14,999, $15,000–24,999, ≥$25,000). An analysis that included an adjustment for the adipose tissue level of polychlorinated biphenyls was also conducted, but these results are not discussed here because they did not make any material change in the results. Odds ratios and 95 percent confidence intervals were calculated using SAS statistical software (14).

RESULTS

Interviews were completed with 79 percent of the potentially eligible cases (n = 304) and 74 percent of the potentially eligible controls (n = 186). Of the 304 breast cancer cases, 62 were diagnosed as carcinoma in situ, 207 were stage I/II disease, 19 were stage III/IV disease, and 16 had missing stage information. The 186 women in the control group were classified as breast cancer cases, 62 were diagnosed as carcinoma in situ, 207 were stage I/II disease, 19 with fibroadenoma, and 49 with other nonproliferative disease (including 21 patients with normal breast tissue, 25 without atypia and 95 with nonproliferative disease). The adipose tissue levels of DDE and DDT also did not differ between cases and the controls. The adipose tissue levels of DDE and DDT between breast cancer cases and the controls. The adipose tissue levels of DDE and DDT when the study population was stratified by menopausal status (n = 162 for premenopausal women and n = 328 for postmenopausal women, data not shown).

Age- and covariate-adjusted odds ratios for the association between DDE and DDT and breast cancer risk are presented in Table 3. There was no increased risk of breast cancer associated with increasing adipose tissue levels of either DDE or DDT. The covariate-adjusted odds ratio was 0.9 (95 percent confidence interval: 0.5, 1.5) for DDE and 0.8 (95 percent confidence interval: 0.5, 1.5) for DDT when the highest quartile was compared with the lowest quartile. The trends were not statistically significant.

DISCUSSION

Our results show no differences in breast adipose tissue levels of DDE and DDT between breast cancer cases and the controls. The adipose tissue levels of DDE and DDT also did not differ between cases and controls among either pre- or postmenopausal women. Therefore, our study does not support the hypothesis that increasing adipose tissue levels of DDT and DDE are associated with an increase in the risk of female breast cancer.

This study had several strengths, particularly a relatively large sample size and use of adipose tissue rather than serum to quantitate exposure. However, the

<table>
<thead>
<tr>
<th>TABLE 1. Mean values for selected characteristics of cases and controls, Connecticut, 1994–1997</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong> (years)</td>
</tr>
<tr>
<td>Cases (n = 304)</td>
</tr>
<tr>
<td>Controls (n = 186)</td>
</tr>
<tr>
<td><em>p value</em></td>
</tr>
</tbody>
</table>

* Among parous women only.
† χ² test for proportions and Wilcoxon two-sample test for means.

Am J Epidemiol Vol. 150, No. 5, 1999
TABLE 2. Lipid-adjusted adipose tissue levels of dichlorodiphenylchloroethane (DDE) and dichlorodiphenyltrichloroethane (DDT) in breast cancer cases and controls, Connecticut, 1994–1997

<table>
<thead>
<tr>
<th></th>
<th>DDE</th>
<th></th>
<th>DDT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>First quartile</td>
<td>Median</td>
<td>Third quartile</td>
</tr>
<tr>
<td>Controls</td>
<td>186</td>
<td>412.6</td>
<td>772.8</td>
<td>1,356.0</td>
</tr>
<tr>
<td>Cases</td>
<td>304</td>
<td>455.6</td>
<td>789.5</td>
<td>1,450.1</td>
</tr>
<tr>
<td>DDE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>186</td>
<td>28.7</td>
<td>49.4</td>
<td>78.1</td>
</tr>
<tr>
<td>Cases</td>
<td>304</td>
<td>28.5</td>
<td>49.5</td>
<td>84.8</td>
</tr>
</tbody>
</table>

* p value for geometric mean difference and adjusted for age using analysis of covariance.

study also had some possible limitations. The impact of each of these potential limitations on the study results is discussed below.

First, age, an important potential confounder in this study, was different in cases (56.3 years) versus controls (52.6 years), with cases significantly older than controls. However, the age difference between the cases and the controls in this study cannot be used to explain the observed lack of association. Age was found to be positively associated with body burden of DDE (r = 0.34) and DDT (r = 0.32) and, therefore, was controlled for throughout the data analyses. If residual confounding from an age difference between the cases and controls has any impact on the observed effect, it should cause a false positive association rather than a null one.

Another possible limitation concerns the use of benign breast disease controls. The risk factors for benign breast disease are currently poorly understood as reviewed by Ernster (15), and it is possible that some of the reproductive and demographic variables shown in Table 1 are risk factors for benign breast diseases as well as for the breast cancer. Many of the variables in Table 1 that have previously been associated with breast cancer (16) were not significantly associated with breast cancer in this study. This could be a function of sample size; however, a similar lack of association for established risk factors has been seen in a recent prospective follow-up study (6) and a case-control study (10) that investigated the association between DDE and breast cancer risk. Even though there are the potential limitations in our control group, it is unlikely that the lack of association of DDE and DDT with breast cancer risk is due to the use of benign breast disease patients as controls, since the adipose tissue levels of DDE and DDT were quite similar for 91 women diagnosed with proliferative benign breast disease and 95 women with nonproliferative disease or

TABLE 3. Odds ratio (OR) for breast cancer by dichlorodiphenylchloroethane (DDE) and dichlorodiphenyltrichloroethane (DDT) levels in breast adipose tissue, Connecticut, 1994–1997

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>DDE (ppb)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>No. of controls</td>
<td>OR,*</td>
<td>95% CI*</td>
<td>OR,*</td>
<td>95% CI</td>
</tr>
<tr>
<td></td>
<td>&lt;412.6</td>
<td>65</td>
<td>46</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>412.6–779.2</td>
<td>85</td>
<td>47</td>
<td>1.2</td>
<td>0.7, 2.1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>779.3–1,355.9</td>
<td>71</td>
<td>46</td>
<td>0.9</td>
<td>0.5, 1.5</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>≥1,356.0</td>
<td>83</td>
<td>47</td>
<td>0.9</td>
<td>0.5, 1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>p trend (df = 1)</td>
<td></td>
<td></td>
<td>0.40</td>
<td></td>
<td>0.46</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>DDT (ppb)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>No. of controls</td>
<td>OR,*</td>
<td>95% CI*</td>
<td>OR,*</td>
<td>95% CI</td>
</tr>
<tr>
<td></td>
<td>&lt;28.7</td>
<td>77</td>
<td>45</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28.7–49.4</td>
<td>74</td>
<td>48</td>
<td>0.9</td>
<td>0.5, 1.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>49.5–78.0</td>
<td>62</td>
<td>46</td>
<td>0.7</td>
<td>0.4, 1.2</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>≥78.1</td>
<td>91</td>
<td>47</td>
<td>0.8</td>
<td>0.5, 1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>p trend (df = 1)</td>
<td></td>
<td></td>
<td>0.31</td>
<td></td>
<td>0.38</td>
<td></td>
</tr>
</tbody>
</table>

* OR, adjusted for age only; CI, confidence interval; OR, adjusted for age, body mass index (<22, 22–24.9, 25–29.9, ≥30 kg/m²), lifetime months of lactation (0, 1–6, >6), age at menarche (<13, 13–15, ≥16 years), age at first full-term pregnancy (nulliparous, <25, ≥25 years), menopausal status, race (Whites, Blacks, and others), income 10 years before the disease diagnosis or interview (<$10,000, $10,000–14,999, $15,000–24,999, ≥$25,000).
normal tissue. In addition, previously observed positive associations between DDE and DDT and female breast cancer risk came from two studies that used patients with benign breast disease as controls (3, 4).

A third possible limitation derives from the process for selecting potential cases and controls based upon the availability of 0.4 g of residual adipose tissue. At Yale-New Haven Hospital, many women undergo fine needle biopsy, and these women would not have been eligible for the study since fine-needle biopsy specimens are typically very small and therefore insufficient for chemical analyses. Cases, but not controls, would be more likely to undergo subsequent surgical procedures. Thus, more cases than controls would be considered potentially eligible for this study. This possible selection bias would influence the results only if the decision to use fine needle biopsy for diagnostic purposes were related to body burden of DDE or DDT, a scenario that is extremely unlikely.

There are two broad views regarding the effects of environmental exposure to organochlorine compounds, including DDT and DDE, on the risk of female breast cancer. One considers that humans now basically live in an environment that is a virtual sea of estrogens (17–19), and the long-term effect of low level exposure to these environmental estrogens could be to increase the risk of female breast cancer (1). The other, however, considers that exposure to relatively low levels of DDE and other weakly estrogenic organochlorine compounds may not have a significant effect on a woman's breast cancer risk (20–22).

Experimental studies have provided substantial evidence to conclude that DDE and DDT are not mammary carcinogens (23). Laboratory studies also show that the relative estrogenic potencies for most of the organochlorine pesticides are approximately $10^4$–$10^6$ times lower than that for the natural estrogen, 17β-estradiol (24). In fact, $p,p'$-DDE, the predominant environmental DDT pollutant, has not been consistently found to possess estrogenic activity, while its antiandrogenic activity is well recognized (25–27). Furthermore, women occupationally exposed to relatively high levels of DDE and DDT also did not show an increase in risk of breast cancer as reviewed by Safe (24). A recent epidemiologic study from Mexico, an area in which DDT has been widely used for agricultural purposes and malaria control, did not find an increased risk of breast cancer associated with serum levels of DDT and DDE (7).

Some descriptive epidemiologic features also do not support an association between DDT and DDE exposure and risk of breast cancer. For example, the risk of breast cancer is higher in White women than in Black women in the United States (28), while the body burden of DDE and DDT has been found to be much higher among Blacks (6, 29). In our current study, the mean adipose tissue level of DDE for Black women (1,926.3 ppb) was significantly higher ($p < 0.01$) than that for White women (917.0 ppb). The incidence rates of breast cancer in Connecticut, however, have been higher in Whites than Blacks for both carcinoma in situ and invasive breast cancer during the past two decades (T. Zheng et al., unpublished data).

In summary, the results from the present study did not show significant differences in breast adipose tissue levels of DDE and DDT between cases and controls or among either pre- or postmenopausal women. Our results also do not support the hypothesis that increasing adipose tissue levels of DDT and DDE are associated with an increase in the risk of female breast cancer.

ACKNOWLEDGMENTS

This study is supported by grant CA-62986 from the National Cancer Institute/National Institute of Environmental Health Science.

The authors thank the study interviewers, Donna Carrano, Melita Bosnyak, Heather Hutson, and Sylvia Ullman, for their high quality interviewing; Drs. Leticia DeDios and Christine Howe for their assistance in the collection of study materials; and Omar Dawood for data management. Special thanks go to Drs. Kumiko Iwamoto, Gwen Collman, and G. Iris Obrams at the National Institutes of Health for their support and guidance of the study.

REFERENCES