Environmental Exposure to Hexachlorobenzene (HCB) and Risk of Female Breast Cancer in Connecticut

Introduction

There have been growing concerns that environmental exposure to organochlorine compounds, including organochlorine pesticides, may increase the risk of female breast cancer (1). These concerns arise from the observations that these environmental pollutants have hormonal activity and induce mixed-function p-450 enzymes, which are also closely associated with the metabolism of steroid hormones (2–8).

HCB is an organochlorine fungicide that had been widely used for several decades for agricultural purposes, mainly as a seed protectant on grain, wheat, and field crops (9). HCB is no longer produced as a commercial product in the United States; since the mid 1970s, most HCBs in the United States have been formed as a byproduct in the production of chlorinated solvents, pesticides, and other chlorinated compounds (10–12). Due to its resistance to chemical and biological breakdown, HCB has contaminated almost all environmental media including air, water, soil, and plants. It has been detected in aquatic biota, mammals, and humans. Human exposure to HCB at present is primarily through the food chain (10–12).

Three earlier small studies (13–15) found no significant difference in adipose tissue levels of HCB between breast cancer patients and controls. A recent case-control study by Moysich et al. (16), however, reported an OR of 1.8 (95% CI, 0.6–5.4) for those with the highest serum levels of HCB when compared with those with the lowest serum levels among parous women (46 cases and 61 controls) who never lactated. Contrary to the suggestion of a positive association between HCB exposure and breast cancer risk among women who never lactated is an apparent inverse association among parous women (85 cases and 106 controls) who ever lactated (16). An OR of 0.3 (95% CI, 0.1–0.7) and an OR of 0.5 (95% CI, 0.2–1.1) were observed for those with medium and the highest serum levels of HCB when compared with those with the lowest serum levels.

Considering the ubiquitous human exposure to HCB and its reported hormone activities (12, 17, 18), the results reported by Moysich et al. (16) need to be examined in different populations with a larger sample size. We report the results from a case-control study in Connecticut evaluating the association between adipose tissue levels of HCB and risk of female breast cancer.

Materials and Methods

Potentially eligible cases and controls from YNHH (New Haven, CT) were identified using computerized patient infor-
From the YNHH Surgical Pathology Department, where records of all newly completed breast-related surgeries are kept. All procedures for subject selection were performed in accordance with a protocol approved by the Yale Human Investigations Committee. To be eligible, study subjects had to be women, ages 40–79 years, who had breast-related surgery at YNHH between January 1, 1994, and December 31, 1997, and from whose breast pathology specimen we could collect at least 0.4 g of residual breast adipose tissue for chemical analyses. Cases were histologically confirmed, incident breast cancer patients (ICD-O, 174.0–174.9) who had no previous diagnosis of cancer, with the exception of nonmelanoma skin cancer, and who were alive at the time of interview. We consecutively selected all breast cancer patients who met the study eligibility requirements. A sufficient amount of residual breast adipose tissue was available from a total of 385 incident breast cancer cases identified from YNHH, whereas 304 cases participated in the study (representing 79% of the eligible cases).

From the computerized files, we identified 251 potential controls who had had breast-related surgery at YNHH and were histologically diagnosed with BBDs, and from whose breast tissue samples we could collect at least 0.4 gram of adipose tissue for chemical analyses. As with the cases, controls had no previous diagnosis of cancer, with the exception of nonmelanoma skin cancer, and were alive at the time of interview. A total of 186 controls agreed to participate in the study (representing 74% of the eligible controls). Of the 186 such controls, 91 subjects were diagnosed with proliferative BBD without atypia and 95 subjects were diagnosed with nonproliferative disease (21 patients with normal breast tissue, 25 with fibroadenoma, and 49 with other nonproliferative disease). Women with diagnoses of atypical hyperplasia were excluded. The study pathologist (D. C.) was responsible for histological confirmation of all breast cancer patients and BBD controls and for classification of stage for breast cancer patients, according to the tumor-node-metastasis system (19).

After approval by the subject’s physician, each participant was 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 (Seattle, WA), designed to optimize estimation of fat intake. Each subject was asked to characterize her usual diet in the year before she had the biopsy. Breast adipose tissue not needed for diagnostic purposes in all subsequent analyses. Although the mean or proportion for other baseline risk factors in Table 1 did not show a clear association with breast cancer risk, further stratification by sample using Florisil chromatography; and identification and quantification of the compounds using gas chromatography. Total lipid was quantified gravimetrically. The quantitation limit of this method is 5 ppb for HCB. All analyses were conducted under an established quality control/quality assessment program, including method spikes, reagent blanks, and quality control windows. Quality control mean recovery for HCB was 131.1% with a CV of 15.3%. Adipose tissue levels of HCB were reported as ppb, which is equivalent to ng of HCB/g of lipid.

Breast adipose tissue levels of HCB were compared between all cases and all controls, among pre- and postmenopausal women, based on parity and lactation history, breast cancer histology (lobular versus ductal carcinoma), type of BBD (proliferative versus nonproliferative disease), and stage of diagnosis (stage 0-II versus III/IV). Because earlier studies suggest that environmental estrogens may only affect the incidence of hormone-responsive breast cancer (15), adipose tissue levels of HCB were also compared based on the cases’ ER/PR status. Both ER and PR status were considered to be positive with an H-score >75, as described by McCarty et al. (21).

Quartiles of adipose tissue levels of HCB were formed based on the frequency distribution of controls. The statistical significance for multiple means of adipose tissue levels of HCB was calculated using ANOVA, and analysis of covariance was used to adjust for potential confounders. A linear logistic regression model was used to adjust for confounders when estimating the exposure/disease association. The variables included in the final model were age, BMI (kg/m²), lifetime months of lactation, age at menarche, age at first full-term pregnancy (nulliparous, <25 years and ≥25 years), number of live births (none, <3 and ≥3), fat intake, race (white, black, and other), and income 10 years before the disease diagnosis. An analysis that included an adjustment for adipose tissue level of PCBs and DDE was also conducted, but we found that the adjustment for PCBs and DDE did not bring any material change in the results. Therefore, we only reported the results without adjusting for PCBs and DDE. ORs and 95% CIs were calculated using Statistical Analysis Software (22).

### Table 1. Means or proportions for characteristics of cases and controls.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.3</td>
<td>52.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2</td>
<td>27.1</td>
<td>0.41</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>12.6</td>
<td>12.5</td>
<td>0.52</td>
</tr>
<tr>
<td>Nulliparous (%)</td>
<td>12.2</td>
<td>15.1</td>
<td>0.36</td>
</tr>
<tr>
<td>Age at first full pregnancy (years)</td>
<td>25.1</td>
<td>24.9</td>
<td>0.26</td>
</tr>
<tr>
<td>Lactation (months)</td>
<td>5.3</td>
<td>6.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Fat intake (g/day)</td>
<td>63.6</td>
<td>62.2</td>
<td>0.53</td>
</tr>
<tr>
<td>Annual income ($)</td>
<td>17,517</td>
<td>16,847</td>
<td>0.62</td>
</tr>
<tr>
<td>Race (% white)</td>
<td>87.5</td>
<td>84.4</td>
<td>0.33</td>
</tr>
</tbody>
</table>

* x² test for proportions and Wilcoxon two-sample test for means.

* Among parous women only.
these factors indicated that women with an earlier age at menarche (defined as ages ≤12 and 13 and 14) had nonsignificantly higher risk for breast cancer. Compared with those having a first full-term pregnancy before age 20, women with a later age (≥26 years) at first full-term pregnancy showed a higher risk (OR = 2.2; 95% CI, 1.2–3.9). Those having a lifetime duration of lactation of more than 12 months experienced a reduced risk (OR = 0.7; 95% CI, 0.4–1.1) compared with those who never lactated. Dietary fat intake at the second tertile, but not the third tertile, was associated with a 60% increased risk of breast cancer (OR = 1.6; 95% CI, 1.0–2.6).

The median and mean adipose tissue levels of HCB were quite comparable between cases and controls overall and by menopausal status (Table 2). However, the mean adipose tissue levels of HCB were significantly higher (P < 0.001) among postmenopausal control women (20.2 ppb) than among premenopausal control women (17.3 ppb).

Cases and controls did not differ significantly by mean adipose tissue levels of HCB when cases were stratified by ER or PR status (Table 3). There were also no significant differences in mean HCB levels between ER+ and ER− patients (P = 0.21), nor between PR+ and PR− patients (P = 0.17).

Among parous women who lactated, adipose tissue levels of HCB were quite comparable between the 107 cases (20.9 ppb) and 79 controls (19.4 ppb). Among parous women who never lactated, the mean adipose tissue levels of HCB were also comparable for the 160 cases (19.3 ppb) and 77 controls (18.6 ppb). Among nulliparous women, although the mean adipose tissue level of HCB was nonsignificantly higher (P = 0.16) in 37 cases (28.9 ppb) than in 30 controls (19.2 ppb), the median levels were quite comparable between these cases (18.8 ppb) and controls (17.4 ppb).

The mean adipose tissue level of HCB for the breast cancer cases was not significantly different from that of controls with proliferative BBDs, nor from that of controls with nonproliferative BBDs/normal tissue. Further analyses by breast cancer histology show that the mean adipose tissue level of HCB for women with ductal carcinomas was not significantly different from that of women with lobular carcinomas, nor from the controls. The mean adipose tissue levels of HCB were also not significantly different between stage 0-II and stage III/IV disease (data not shown).

The risk of breast cancer by HCB level is presented in Table 4. There was no clear association between adipose tissue levels of HCB and breast cancer risk among study subjects overall, nor among pre- or postmenopausal women in either age-adjusted or covariate-adjusted analyses.

Among parous women who reported ever breast feeding (Table 5), an OR of 0.5 (95% CI, 0.2–1.4) was observed when the highest quartile was compared with the lowest quartile. There was no association between adipose tissue levels of HCB and the risk of breast cancer among parous women who reported never breast feeding (OR = 0.7; 95% CI, 0.3–1.7 for the fourth quartile). For multiparous women, the covariate adjusted OR was 0.5 (95% CI, 0.1–2.1) for the second tertile, whereas the covariate adjusted OR was 2.1 (95% CI, 0.5–8.8) for the third tertile. The results, however, are based on only 37 cases and 30 controls.

**Discussion**

The possibility that chronic, low-level environmental exposure to estrogenic organochlorine pesticides increases the risk of female breast cancer has been hotly debated in recent years (1, 2). The results from recent epidemiological studies of organochlorine pesticides and breast cancer risk, however, are largely negative. Although several earlier small studies suggested an increased risk of breast cancer associated with DDT/DDE (14, 15, 23), more recent studies with larger sample sizes found no association (24–26) or an inverse association between DDT/DDE and breast cancer risk (27).

Among the studies that investigated the association between environmental exposure to HCB and female breast cancer risk, a study from Finland (13), with breast adipose tissue samples collected between 1985 and 1986, found no significant difference (P = 0.48) in mean breast adipose tissue levels of HCB between 44 breast cancer patients (140 ppb) and 33 noncancer control women (110 ppb). In a study from the United States with breast adipose tissue samples collected in 1987, Falck et al. (14) reported that the mean breast adipose tissue level of HCB was 28 ppb for 20 breast cancer cases and 26 ppb for 20 BBD controls on a lipid basis (P = 0.54). In another study from Canada that collected breast adipose tissue samples between 1991 and 1992, Dewailly et al. (15) reported a mean breast adipose tissue level of 33.4 ppb for 17 controls, 31.1 ppb for nine ER-negative breast cancer patients, and 41.7 ppb for nine ER-positive breast cancer patients. None of the three groups in this study was significantly different from another at the 5% significance level.

The study by Moysich et al. (16) reported comparable mean serum levels of HCB for 154 cases (0.41 ppb) and 192 controls (0.42 ppb). Among women who never lactated in their study, the mean serum levels of HCB were 0.45 ppb for 46 breast cancer cases and 0.39 ppb for 61 controls (P > 0.05). A nonsignificant OR of 1.8 (95% CI, 0.6–5.4) was observed for those with the highest serum levels of HCB when compared with those with the lowest serum levels based on very few.

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**Table 2** Lipid-adjusted adipose tissue levels of HCB (ppb) among breast cancer cases and BBD controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Median</th>
<th>Mean ± SD</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>304</td>
<td>16.6</td>
<td>21.0 ± 17.7</td>
<td>0.21</td>
</tr>
<tr>
<td>Controls</td>
<td>186</td>
<td>15.9</td>
<td>19.1 ± 15.0</td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>87</td>
<td>14.4</td>
<td>18.3 ± 15.0</td>
<td>0.65</td>
</tr>
<tr>
<td>Controls</td>
<td>75</td>
<td>15.4</td>
<td>17.3 ± 12.8</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>217</td>
<td>17.4</td>
<td>22.1 ± 18.6</td>
<td>0.37</td>
</tr>
<tr>
<td>Controls</td>
<td>111</td>
<td>17.0</td>
<td>20.2 ± 16.3</td>
<td></td>
</tr>
</tbody>
</table>

*Ps for test of means between cases and controls adjusting for age using analysis of covariance.

**Table 3** Lipid-adjusted adipose tissue of HCB (ppb) in breast cancer cases and controls by ER and PR status

<table>
<thead>
<tr>
<th>Hormone status</th>
<th>Number of subjects</th>
<th>Median</th>
<th>Mean ± SD</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+</td>
<td>157</td>
<td>17.1</td>
<td>22.3 ± 20.7</td>
<td>0.10</td>
</tr>
<tr>
<td>ER−</td>
<td>126</td>
<td>16.2</td>
<td>19.6 ± 13.2</td>
<td>0.75</td>
</tr>
<tr>
<td>Unknown</td>
<td>21</td>
<td>16.7</td>
<td>20.2 ± 16.9</td>
<td>0.75</td>
</tr>
<tr>
<td>Cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR+</td>
<td>116</td>
<td>17.2</td>
<td>23.1 ± 23.9</td>
<td>0.07</td>
</tr>
<tr>
<td>PR−</td>
<td>128</td>
<td>16.4</td>
<td>19.8 ± 12.2</td>
<td>0.66</td>
</tr>
<tr>
<td>Unknown</td>
<td>60</td>
<td>16.3</td>
<td>19.7 ± 12.9</td>
<td>0.77</td>
</tr>
</tbody>
</table>

*Ps for test of means between controls and each type of cases adjusting for age using analysis of covariance.
subjects, with an average of 15 cases/strata. Among parous women who ever lactated, Moysich et al. (16) found a nonsignificantly lower mean serum level of HCB for the 85 cases (0.39 ppb) than that for the 106 controls (0.44 ppb). Further analyses by serum levels of HCB, in fact, show a reduced risk of breast cancer among those with medium (OR = 0.3; 95% CI, 0.1–0.7) and the highest serum levels of HCB (OR = 0.5; 95% CI, 0.2–1.1) when compared with the lowest serum levels.

It may be argued that the observed lack of association between HCB and breast cancer risk in the current study could be due to the use of patients with BBD as controls. If there was an association between HCB and BBD, this could lead to an underestimate of the true relative risk for breast cancer. However, although the possibility exists, it is unlikely that the lack of association of HCB with breast cancer risk could be entirely attributable to the use of BBD patients as controls because previously observed positive associations between organochlorine compounds and female breast cancer risk came from two studies that used patients with BBD as controls (14, 15). In the present study, the mean adipose tissue level of HCB for the breast cancer cases (21.0 ppb) was not significantly different from 91 controls with proliferative BBD (19.9 ppb), nor from 95 controls with nonproliferative BBBDs/nominal tissue (18.2 ppb).

Another potential consideration regarding the observed lack of association between HCB and breast cancer risk in this study involves the process for selecting cases and controls based on the availability of at least 0.4 g of residual breast adipose tissue for chemical analyses. For diagnostic purposes, many women at YNHH undergo fine-needle biopsy, and these women would not be eligible for the study because fine-needle biopsy specimens are typically very small, therefore, insufficient for chemical analyses. Cases, but not controls, however, would be more likely to undergo subsequent surgical procedures that would produce an adequate amount of tissue for the study. Thus, more cases than controls would be considered potentially eligible for this study. However, in order for this study to introduce bias, it would require that the decision to use fine-needle biopsy for diagnostic purposes be related to body burden of HCB, a scenario that is extremely unlikely.

It is also a concern that breast adipose tissue levels of HCB may be affected by the case status. Particularly, the tissue levels of HCB for late stage patients may be affected due to mobilization of energy from fat stores (28). However, a recent follow-up study (25) of organochlorine compounds and breast cancer risk did not support that disease stage at diagnosis significantly impacts serum levels of these compounds. In our study, the mean adipose tissue levels of HCB were also not significantly different between the 186 controls (19.9 ppb) and the 269 women with stage 0-II disease (20.0 ppb), and the 19 women with stage III/IV disease (24.9 ppb). Exclusion of breast cancer patients diagnosed with stage III/IV disease and 16 patients whose information on stage at diagnosis was missing did not result in any material change to the conclusion.

The failure of epidemiological studies to observe an increase in the risk of breast cancer from organochlorine pesticides is at odds with laboratory studies demonstrating estrogenic effects of these compounds. One potential explanation is that most pesticides and other environmental estrogens are only very weak estrogens, usually hundreds to thousands of times less active than estradiol (4). Therefore, there is little chance that environmental estrogens could exert an important estrogenic effect (1). But others (3) argue that, unlike endogenous estrogen, environmental estrogens may be able to more freely enter cells. This would greatly increase the availability and biological activity of environmental estrogens relative to similar blood concentrations of endogenous estrogen, most of which is inhibited from entering cells by binding to estrogen-binding plasma protein (29).

Another potential explanation for the overall negative association is that, other than the ubiquitous synthetic estrogens in the human environment, there is also a sea of natural and synthetic antiestrogens that may negate any effects of environmental estrogens, hence, the net effect may be zero (1). But
again, others argue that antiestrogens can be potent hormone modulators themselves (1), and exposure and extent of exposure to various environmental estrogens and antiestrogens vary by populations and by the actual exposures (13–16, 23–27, 30–32). Therefore, these effects may not cancel out. The potential unbalanced exposure to these estrogens and antiestrogens may still increase or decrease the risk of breast cancer.

In summary, no significant difference in breast adipose tissue levels of HCB was observed between breast cancer patients and controls in this case-control study. The risk also did not vary by menopausal status, parity, lactation status, ER or PR status of the breast cancer cases, breast cancer histology, stage of diagnosis, or type of BBD. Therefore, our study does not support a positive association between environmental exposure to HCB and risk of breast cancer. However, as also pointed out by others (3, 25, 33, 34), humans have good reasons to avoid release of and exposure to HCB and other organochlorine compounds, because these compounds have the potential to act as "environmental estrogens" and have been shown to affect wildlife and human health, including decreases in sperm count, decreases in the duration of lactation, and increase in the frequencies of preterm births and congenital malformations.

Acknowledgments
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