ORIGINAL ARTICLE

Breast cancer and serum organochlorine residues

C Charlier, A Albert, P Herman, E Hamoir, U Gaspard, M Meurisse, G Plomteux

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See end of article for authors' affiliations

Correspondence to: Dr C Charlier, Laboratoire de Toxicologie clinique, CHU Sart Tilman, 4000 Liege, Belgique; C.CHARLIER@chu.ulg.ac.be

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Background: Controversy still exists about the breast carcinogenic properties in humans of environmental xenoestrogens (organochlorines), justifying new investigations.
Aims: To compare the blood levels of total dichlorodiphenyltrichloroethane (DDT) and hexachlorobenzene (HCB) in samples collected at the time of breast cancer discovery, in order to avoid the potential consequences of body weight change (after chemotherapy or radiotherapy) on the pesticide residue levels.
Methods: Blood levels of HCB and total DDT (we calculated total DDT concentrations by adding all DDT and DDE isomers) were compared in 159 women with breast cancer and 250 presumably healthy controls. Risk of breast cancer associated with organochlorine concentration was evaluated.
Results: Mean levels of total DDT and HCB were significantly higher for breast cancer patients than for controls. No differences in serum levels of total DDT or HCB were found between oestrogen receptor positive and oestrogen receptor negative patients with breast cancer.
Conclusions: These results add to the growing evidence that certain persistent pollutants may occur in higher concentrations in blood samples from breast cancer patients than controls.

Breast cancer is the most common cancer in women, affecting one out of every 10 women in Europe and the USA. If some of the newly detected breast cancers can be explained by improved detection, it is also true that the incidence of the disease cannot completely be elucidated in such a way.

Over the past decade, there has been increasing interest in the so called environmental endocrine disrupters, in particular when such compounds are acting as oestrogens or antiandrogens.[1] Current knowledge provides limited evidence for linking human exposure to endocrine disrupters and risk of cancer development.[1] Little is known about the human consequences of long term exposure to mixtures of multiple chemicals, even in small amounts. Important questions concern the lifetime period of exposure (in utero, neonatal period, infancy, and adulthood), the long latency between exposure and the potential event, and the cumulative risk of exposure to multiple products during different time periods. On the other hand, a marked increase in the incidence of cancer has been observed during the past decades, especially for breast, prostate, and testis cancer. It is well known that the aetiology of breast cancer has a strong hormonal component. The relation between exposure to oestrogenic compounds and risk of breast cancer was highlighted by the evidence of development of carcinoma of the vagina and cervix in daughters of women treated with pharmacological doses of diethylstilboestrol (DES) during pregnancy.[2] The mothers themselves developed 35% more breast cancers.

Some pesticides, especially organochlorines, are known to induce biological responses comparable to those of endogenous oestrogens, such as increased uterine weight and vaginal epithelial cornification.[3] Several experimental studies have shown the promoting activity of organochlorine compounds in the development of oestrogen related tumours in animals.[4 5] Studies of human organochlorine exposure have been mostly conducted among populations with known or suspected occupational or accidental exposure.[3 6] However, almost every single individual is daily exposed to much lower concentrations of environmental compounds through dietary habits, water source, and the food chain.

Several epidemiological studies have been published on the risk of breast cancer in association with blood or fat concentrations of organochlorines, especially p,p'-DDE (1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene) the main metabolite of the 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) insecticide, whose use is prohibited today in the USA, Canada, and Europe, but which was largely employed in the past and is still used extensively for mosquito control in developing countries. In a study published by Wolff *et al* in 1993,[7] breast cancer was strongly associated with the concentration of DDE in serum. The author reported a fourfold increase in the relative risk of developing breast cancer when serum DDE concentrations rose from 2.0 ppb to 19.1 ppb. In 1994, another study[8] suggested that women with oestrogen receptor positive breast tumours had a higher body burden of DDE than women with benign breast diseases or oestrogen receptor negative breast tumours. In the case-control study of Krieger and colleagues,[9] no difference was found between the average DDE serum concentrations of cases and controls, thus rejecting the hypothesis of a link between exposure to DDT and risk of breast cancer. Interestingly, when restricting the material of Krieger *et al* to blacks and whites separately, the results were positive (tendency towards significance) for white women. Conversely, Hunter and colleagues[10] did not observe any evidence of an increased risk of breast cancer among women with relatively high concentrations of plasma DDE.

Based on combined analysis of the studies which report DDE concentrations in breast cancer and control patients, Safe[11] concluded that the hypothesised linkage between environmental oestrogens and the increased incidence of breast cancer is unproven. Although many agree with this,[12–14] others estimate that there is a pattern of epidemiological data[15–18] that offers a possible resolution to several anomalies in breast cancer research. Consequently, reductions in exposure will provide an opportunity for primary prevention of this disease. Clearly, it appears that more investigations are required to evaluate the interactions between environmental exposure and established risk factors.

Abbreviations: DDE, dichlorochlorophenylethylene; DDT, dichlorodiphenyltrichloroethane; DES, diethylstilboestrol; HCB, hexachlorobenzene; OR, odds ratio; SD, standard deviation

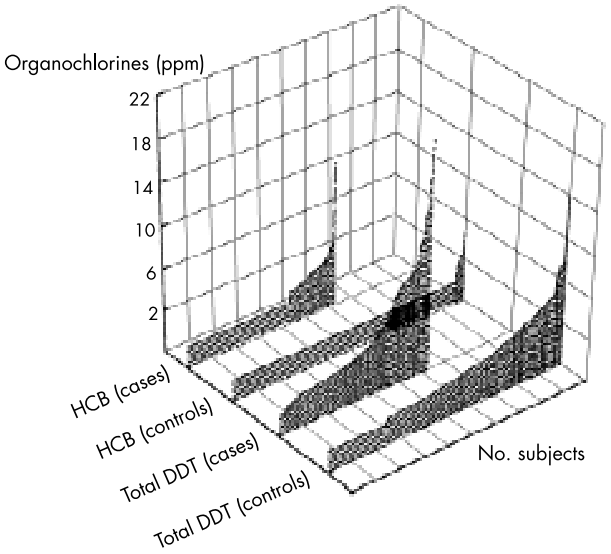

Figure 1 Total DDT and HCB distribution in controls (n = 250) and cases (n = 159).

In the present study, blood samples were collected from two groups of women. The first group (cases) consisted of patients with confirmed breast cancer, and the second group (controls) included presumably healthy women. We focused on two pesticides with oestrogenic activity: total DDT (calculated by adding all DDT isomers: op′-DDT, pp′-DDT, op′-DDE, and pp′-DDE) and HCB (hexachlorobenzene) were quantified simultaneously using a gas chromatographic analyser coupled to an ion trap mass spectrometer detector. Serum concentrations of detected organochlorines in cases and controls were compared.

METHODS

Subjects

The present retrospective study involved 600 women who underwent a medical examination between September 1999 and February 2000 in the Department of Gynaecology or Department of Endocrine Surgery. These women were referred to hospital after a doubtful mammographic preventive screening. Women who had a palpable breast mass or mammographic abnormality underwent a biopsy, and from this initial group, 159 women (54.21 (12.12) years of age) suffering from breast cancer and hospitalised for a mastectomy or tumourectomy were considered as cases. Control subjects were selected at random in a population of women free of any known cancer consulting for routine vaginal cytological examinations at Sart Tilman University Hospital. For each case patient, we matched at least one control subject according to the year of birth, menopausal status, reproductive history (no child or at least one child), and date of blood sampling. All patients gave their informed consent for participating in the study. For controls (250 women, 53.29 (12.35) years of age), blood specimens were taken at the time of the examination, whereas for women with breast cancer, samples were collected prior to surgical intervention. Blood samples were immediately centrifuged and serum specimens kept frozen at −18°C until assay (within one week).

For each subject, information regarding age, smoking habits, living environment (urban or rural), pregnancy, breast feeding, and menopausal status was recorded from a questionnaire. For women with breast cancer, tumour size and oestrogen receptor status were determined from laboratory and medical examinations. Other data, such as specific dietary histories, were unavailable.

Methods

Total DDT and HCB in serum were identified and quantified using a gas chromatographic analyser coupled to an ion trap mass spectrometer detector (Saturn 2000, Varian). The analytical method is described elsewhere.[19] Briefly, sample preparation included a liquid-liquid extraction (petroleum ether:diethylether, 98:2) followed by a solid phase extraction (Bond Elut Certify, Varian). The eluate was evaporated to dryness, reconstituted, and then injected into the gas chromatograph (Saturn 2000, Varian). All solvents were pesticide grade quality. Reference standards of all pesticides were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). The calibration curve was constructed from 0 to 40 ppb and linearity applied for this concentration range. Endosulfan-d4 (0.5 ppb) was used as internal standard. Limits of quantification were defined as 10 times the standard deviation (SD) of the results from the lowest quality control serum pool over the course of the analyses (n = 15). These limits were approximately 0.5 ppb for all organochlorines (with coefficients of variation from 4.6% to 7.8%). Samples were analysed in duplicate in batches that included breast cancer and control samples, together with pooled serum quality control samples. Analytical personnel were blind to the nature of the samples. Two positive samples from the first batch (one from the cancer group and one from the control group) were quantified with every batch of new samples; the corresponding coefficients of variation were respectively 6.3% and 4.1%. Expression of the results on a lipid basis (total serum lipid content calculated from measurements of cholesterol and triglycerides at the time of analysis) was compared with non-adjusted results; as no difference could be found (all subjects were fasting individuals), only crude results are presented here.

Statistical analysis

Results were expressed as mean (SD). When the quantification of organochlorines gave results lower than our quantification limit, we recorded a "0" value in our data table and these results were included in all statistical analyses. Concentrations of total DDT and HCB between matched cases and controls were compared using the Mann-Whitney U test. The χ^2 test was used to compare the proportion of smokers and non-smokers, of breast feeding history, and of rural and urban women in the two groups. Spearman's correlation coefficient was completed to assess the relation between pesticide serum concentration and age, or, for women with breast cancer, tumour size and oestrogen receptor status. Odds ratios (ORs) were calculated for total DDT and HCB positive cases in order to test the association between organochlorine residues and breast cancer. Adjusted ORs were calculated using conditional logistic regression models in order to evaluate the influence on crude results of the simultaneous presence of DDT congeners and HCB, and also of breast feeding (either yes or no, duration of lactation not available). All results were considered to be significant at the 5% critical level.

RESULTS

The two groups of women were similar with respect to smoking habits (50% *v* 44% smokers, p = 0.53), living environment (56% *v* 52% urban, p = 0.66), and breast feeding report (48.1% *v* 49.4%).

The most frequently observed organochlorine was p,p′-DDE (83.54%). HCB was detected in 18.35% of samples. In the control group, 60 women (24.0%) were without any detectable pesticide residue, while there were only four (2.5%) in the breast cancer group. A DDT congener together with HCB was found simultaneously in two samples from the control group (0.8%) and in four samples from the cases (2.5%).

Figure 1 shows total DDT and HCB distributions in cases and controls. The mean concentration of total DDT was

Table 1 Breast cancer risk as estimated by crude and adjusted ORs for total DDT and HCB at 0.5 ppb threshold level (LOQ)

Above LOQ (0.5 ppb)	Cases (%)	Controls (%)	OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR‡ (95% CI)
Total DDT	95.57	72.30	5.36 (1.89 to 15.19)	5.66* (1.83 to 17.51)	5.64 (1.81 to 17.65)
HCB	31.65	4.00	8.68 (2.83 to 26.62)	9.06† (2.81 to 29.21)	9.14 (2.84 to 29.41)

OR, odds ratio; CI, confidence interval; LOQ, limit of quantification.
*Adjusted OR for total DDT when taking HCB presence into account; †adjusted OR for HCB when taking total DDT presence into account.
‡OR adjusted for breast feeding history.

significantly higher in women with breast cancer than in control subjects (3.94 (3.88) *v* 1.83 (1.98) ppb, $p < 0.0001$). The same conclusion applied for HCB (0.79 (1.65) ppb for cases and 0.09 (0.41) for controls, $p = 0.0005$).

We determined the ORs and confidence intervals for total DDT and HCB at the limit of quantification threshold concentration in order to measure the association between the presence of detectable concentrations of DDT congeners or HCB and breast cancer (see table 1). Results were not affected if a DDT congener and HCB were present simultaneously. After adjustment was made for breast feeding, crude results were not dramatically affected (see table 1).

Levels of total DDT were related to age ($r = 0.20$, $p = 0.02$), but HCB concentrations were not ($r = -0.04$, $p = 0.62$). The serum total DDT or HCB concentrations were independent from smoking habits (respectively, $p = 0.54$ and $p = 0.81$) or living environment (respectively, $p = 0.58$ and $p = 0.27$). In the breast cancer group, oestrogen receptor status was available for 102 women but was not correlated with the total DDT concentration ($r = 0.02$, $p = 0.88$) or the HCB concentration ($r = 0.09$, $p = 0.49$). Tumour size was recorded in 90 cases. No correlation was found between this parameter and serum total DDT concentrations ($r = 0.15$, $p = 0.20$) or serum HCB concentrations ($r = 0.05$, $p = 0.65$).

DISCUSSION

Endogenous hormones have been linked to the development of a wide variety of animal and human cancers. This is particularly true for breast cancer, where risk is increased by a prolonged reproductive life,[20] whereas a premature menopause is protective.[21] The association of DDT, an organochlorine insecticide, with egg fragility and reduced eggshell calcium in several types of birds, suggested that endocrine reproductive mechanisms in animals might be altered by ingested pesticides.[22] Widespread use of DDT began in the United States in 1946 and increased until 1959. It then declined steadily until it was effectively stopped in 1972.[23] DDT accumulates in the body, mainly in adipose tissue, and has a half life of 10–50 years.[1] DDT and its major metabolite, DDE, have been shown to have oestrogenic properties in vitro and also in vivo.[5 22 24] The potential link between an increased incidence of breast cancer and a synchronous period of widespread pesticide use requires clarification. Previous studies have investigated the possible association between the blood or fat concentrations of organochlorines and breast cancer, but controversy remains because of the conflicting results, especially because all the epidemiological studies differ by the detected compounds, the selected population, the time of blood sampling, and also the analytical methods used.[7–18 25–28]

In our study, samples were analysed at a maximum of one week following collection and results were not affected by conservation. As in the study of Krieger and colleagues,[9] our results were not affected by differences in serum lipids related to dietary intake immediately prior to sampling, since case patients and controls had undergone overnight fasting prior to venepuncture. A limitation of our study is that serum specimens were collected retrospective to the diagnosis of breast cancer. Cancer is known to induce changes in metabolism and body weight, and these are not accounted for in the present study, so that a misclassification of exposure cannot be excluded. However, breast cancer was always the primary tumour and the sampling occurred before surgery and treatments such as chemotherapy or radiotherapy, so that misclassification of exposure owing to treatment does not seem to occur.

The p,p′-DDE concentrations were similar to those observed by Wolff and colleagues,[7] even if the highest value obtained in our study was slightly lower (20 ppb) than that obtained in the study of Wolff *et al* (44.3 ppb). Serum concentrations were much higher in the study of Krieger and colleagues[9] for blood samples collected between 1964 and 1971. This probably reflects the progressive decline in DDE concentrations, as illustrated by many studies related to human milk.[29–31] This trend should accompany reduced risk of any disease associated with DDT exposure.

Our results show a significant difference of organochlorines concentrations between cases and controls. These observations are in agreement with the first results published by Wolff and colleagues[7] and Dewailly and colleagues,[8] but fail to establish a positive correlation between DDT concentrations and the oestrogen receptor status in the breast cancer group. In the study of Krieger and colleagues,[9] the authors concluded that, overall, there was no evidence of increased breast cancer risk for the higher DDE concentrations, while for the white subpopulation, a positive association was found. Since our study population comprised only white women, our finding of increased risk of breast cancer after DDT exposure is in agreement with results of Krieger *et al*, and strongly suggests that the interethnic variation showed by Krieger *et al* was not the result of chance. A potential explanation of the Krieger *et al* results could be the balance between breast cancer risk factors and antioestrogenic compounds (phytoestrogens, for example) in the Asian cohort. Identification and quantification of such protective compounds may have a pronostic significance.

An interesting result of the present study is the association between HCB and breast cancer. HCB has been shown to be present at higher concentrations in breast adipose tissue of women suffering from breast cancer than control tissues.[32 33] Although these data are also not proven,[34] a tumour promoting activity of HCB on human breast epithelial cells has been reported,[35] which is consistent with the present findings. No correlation between HCB concentration and oestrogen receptor status was shown.

These data warrant further analysis, and consideration of possible exposure routes or dietary intake. Carcinogenesis is a multifactorial event, and it is important to try to clarify the role of chemicals in cancer development.

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Authors' affiliations

C Charlier, G Plomteux, Clinical Toxicology Laboratory, Sart Tilman University Hospital, University of Liege, 4000 Liege, Belgium

A Albert, Biostatistics Department, Sart Tilman University Hospital
P Herman, U Gaspard, Gynaecology Department, Sart Tilman University Hospital
E Hamoir, M Meurisse, Endocrine Surgery Department, Sart Tilman University Hospital

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