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ORIGINAL PAPER

# Serum organochlorines and breast cancer risk in Japanese women: a case–control study

**Hiroaki Itoh · Motoki Iwasaki · Tomoyuki Hanaoka · Yoshio Kasuga · Shiro Yokoyama · Hiroshi Onuma · Hideki Nishimura · Ritsu Kusama · Shoichiro Tsugane**

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**Abstract**
*Objective* Most epidemiological studies of the association between breast cancer risk and exposure to organochlorine pesticides or polychlorinated biphenyls (PCBs), which are suspected endocrine disrupters and potential risk factors for human breast cancer, have been conducted in western countries, and the majority of results have been null and the rest inconsistent. Here, we examined these associations in Japanese women in the largest study in Asian women to date.

*Methods* The study was a matched case–control study of breast cancer with 403 eligible matched pairs from May 2001 to September 2005 at four hospitals in Nagano Prefecture, Japan.

*Measurements* Serum samples were measured for PCBs and nine pesticide-related organochlorines, including dichlorodiphenyltrichloroethane (DDT). Odds ratios of breast cancer or its hormone-receptor-defined subtypes according to serum organochlorines were calculated.

*Results* No increase in the risk of breast cancer was seen among women with higher serum concentrations of any organochlorine: *o,p′*-DDT, *p,p′*-DDT, *p,p′*-dichlorodiphenyldichloroethylene, hexachlorobenzene, *β*-hexachlorocyclohexane, *trans*-nonachlor, *cis*-nonachlor, oxychlordane, mirex, or PCBs. Rather, higher serum levels of *cis*-nonachlor, mirex, or total PCBs were associated with a decreased risk of breast cancer

*Conclusions* Overall, these results suggest that breast cancer risk in Japan, a low-incidence country, is similar to that in western countries in terms of organochlorine exposure.

**Keywords** Hormone receptor · PCBs · DDT · DDE · Mirex

H. Itoh · M. Iwasaki (✉) · T. Hanaoka · S. Tsugane
Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: moiwasak@ncc.go.jp

Y. Kasuga
Department of Surgery, Nagano Matsushiro General Hospital, 183 Matsushiro, Matsushiro-cho, Nagano City, Nagano Prefecture 381-1231, Japan

Y. Kasuga
Department of Surgery, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano Prefecture 390-8621, Japan

S. Yokoyama · H. Onuma
Department of Breast and Thyroid Surgery, Nagano Red Cross Hospital, 5-22-1 Wakasato, Nagano City, Nagano Prefecture 380-8582, Japan

H. Nishimura
Department of Respiratory Surgery, Nagano Municipal Hospital, 1333-1 Tomitake, Nagano City, Nagano Prefecture 381-8551, Japan

R. Kusama
Department of Surgery, Hokushin General Hospital, 1-5-63 Nishi, Nakano City, Nagano Prefecture 383-8505, Japan

## Introduction

Breast cancer is the most frequent malignant disease among women in many western countries, and also in Japan [1]. Epidemiological evidence for the occurrence of breast cancer has suggested an association with several estrogen-dependent factors, namely early menarche, lower parity, late age at first childbirth, postmenopausal hormone use, taller height, and obesity [2–5]. This hormonal

dependency of breast cancer plays an important role in its development.

Because established risk factors for breast cancer account for only about half of its incidence [6–8]; however, epidemiological studies have also examined certain organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs), which have shown estrogenicity or anti-estrogenicity in experimental studies [9], as potential risk factors. Results have been mainly null, with several inconsistent exceptions [6, 10]. Some of these agents persist in the environment and are bio-accumulative. Release of organochlorine pollutants into the environment during part of the last century has resulted in exposure in humans, mainly via food intake, particularly fish [11]. Although the human body burden of traditional organochlorine compounds such as DDTs and PCBs has decreased in recent decades following bans on their use, they remain detectable in biologic samples [12].

Most studies of associations of breast cancer risk with particular organochlorine compounds to date have been conducted among Caucasians in western countries. Such associations among relatively high-risk Caucasians in these countries may not always be consistent with those among lower-risk Asian women in Asian countries such as Japan, however [13]. Indeed, the possibility that differences in background, such as in lifestyle, internal hormonal milieu, body burden of organochlorines, and hormone-receptor subtypes, might influence the associations have now made race, ethnicity, and area specificity issues in this field. In recent years, three studies have assessed the association between breast cancer and organochlorines in African-American women [14–16], one of which also included Asian subjects (50 pairs) [15], but all reported null results; and apart from one small study in India (25 pairs), no study has yet been conducted in an Asian country, including Japan [17].

Here, we conducted a matched case–control study to investigate the association between breast cancer risk and serum organochlorines in Japanese women. This is the first large-scale study to examine the association between breast cancer and DDTs, PCBs, and other organochlorine pesticides in an Asian country and an Asian population.

## Subjects and methods

### Study subjects

This multicenter, hospital-based case–control study was conducted from May 2001 to September 2005 at four hospitals in Nagano Prefecture, Japan. Cases were a consecutive series of women aged 20–74 years with newly arising, histologically confirmed invasive breast cancer admitted to the four hospitals during the survey period. Out of the 412 eligible patients, 405 (98%) agreed to participate. Healthy controls were selected from among medical checkup examinees in two of the hospitals, who were confirmed as not having cancer, with one control matched for each case by age (within 3 years) and residential area during the study period. Among potential control subjects, one declined to participate. Consequently, we obtained written informed consent from 405 matched pairs. Because two control subjects refused to provide blood samples, the analysis was finally restricted to 403 matched pairs. The study protocol was approved by the institutional review board of the National Cancer Center (Tokyo, Japan).

### Data collection

#### *Questionnaire survey*

Participants were asked to complete a self-administered questionnaire which included questions on demographic characteristics, anthropometric factors, smoking habits, family history of cancer, physical activity, medical history, and menstrual and reproductive history. Dietary habits were investigated using a 136-item semi-quantitative food-frequency questionnaire (FFQ) which was developed and validated in Japanese population [18]. The FFQ enquired about the frequency of consumption of individual food items, with response choices of never or less than once/month, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, once/day, 2–3 times/day, 4–6 times/day, and 7 times/day or more; as well as relative sizes compared to standard portions, expressed as small (50% smaller than standard), medium (same as standard), and large (50% larger). These data were then used to calculate average rates of consumption for each food group (g/day) and nutrients (mg/day).

#### *Clinical data*

Estrogen receptor (ER) and progesterone receptor (PR) status in breast cancer tissue of breast cancer patients were obtained from medical records. Hormone receptor status was determined using enzyme-linked immunoassay or immunohistochemical assay. Hormone receptor positivity values were determined either as specified by the laboratory that performed the assay, or in accordance with the laboratory's written interpretation thereof, or both.

#### *Laboratory analysis*

Blood specimens were collected from all cancer patients prior to surgery. Aliquots of serum samples were shipped to a commercial laboratory, Shimadzu Techno Research

Inc. (Kyoto, Japan), for analysis of *o,p′*-DDT, *p,p′*-DDT, *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), hexachlorobenzene (HCB), *β*-hexachlorocyclohexane (*β*-HCH), *trans*-nonachlor, *cis*-nonachlor, oxychlordane, mirex, and more than 41 PCB congeners. An approximately 1.5-g aliquot of each serum sample was gravimetrically measured and then spiked with respective $^{13}$C-labelled internal standards, except for some PCB congeners. Compounds were fractionated and purified using two-stage liquid–liquid extraction with dichloromethane, ethanol and saturated ammonium sulfate aqueous solution, and florisil column chromatography.

Organochlorine pesticides and PCBs were detected and measured using a high-resolution mass spectrometer (Autospec Ultima, Micromass, Manchester, UK) with selected ion monitoring connected to a gas chromatograph (HP6890, Hewlett Packard) equipped with a capillary column (HT8-PCB fused silica capillary column 60 m × 0.25-mm id for PCBs; DB-17HT fused silica capillary column 30 m × 0.32 mm id, 0.15 μm for the organochlorine pesticides) based on isotope-dilution mass spectrometry.

Lower limits of detection (LODs), determined from a signal-to-noise ratio of 3, were 1.0 pg/g wet for organochlorine pesticides and 0.6 pg/g wet for PCB congeners. Measurement values below the LOD were assigned a value equal to the LOD. Total PCBs was calculated as the sum concentration of all PCB congeners measured. Cases and matched controls were assayed in the same batch by laboratory analysts who did not know the case–control status. Some compounds were detected in the method blank but were disregarded because of their sufficiently low contribution to measured values in serum concentrations or the sum of PCBs. Intraclass correlation coefficients derived from duplicate measures of five serum samples at usual concentration ranges were >0.99, except for that for HCB, which was 0.87.

To standardize serum concentrations of lipophilic organochlorines, measurement values were divided by serum total lipid concentration (TL), which was estimated using serum concentrations of total cholesterol (TC) and triglyceride (TG), and the equation TL [g/l] = 2.27 × TC [g/l] + TG [g/l] + 0.623, as proposed by Phillips et al. [19], and then divided by serum density (g/l). TC and TG values were enzymatically measured at Kyoto Biken Laboratories, Inc. (Kyoto, Japan).

### Statistical analysis

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). All statistical tests and 95% confidence intervals (CIs) were two-sided and considered significant at the 0.05 level. Serum lipid-adjusted organochlorine concentrations were categorized into quartile groups based on the distribution of controls. Missing variables were handled by complete case analysis. Twelve pairs which included women with a total energy intake of less than 500 kcal or 4,000 kcal or more were excluded from adjustment of odds ratios (ORs) for fish or vegetable consumption.

ORs and 95% CIs of breast cancer according to quartile of serum organochlorine concentration were calculated by conditional logistic regression analysis using the PHREG procedure with the STRATA statement in SAS. Stratified analyses by menopausal status or age were performed using unconditional logistic regression analysis. ORs were adjusted for total lipid concentration in serum, body-mass index, menopausal status and age at menopause, smoking status, fish consumption, vegetable consumption, family history of breast cancer in a first-degree relative, age at first childbirth, parity, age at menarche, history of breast cancer screening, and history of breast feeding. These adjusted variables were established risk factors for breast cancer or were correlated with breast cancer risk and serum organochlorines in this study. Owing to the significant inverse association between risk and total lipid concentration in serum, logistic regression models were additionally adjusted for serum lipids. Because lactation results in the excretion of organochlorines, a longer total duration of lactation may be associated with increased excretion of organochlorines [20]. In addition, the unconditional logistic regression models used for stratified analyses were also adjusted for the matching factors of age and area of residence.

To evaluate differences in the effect of organochlorines on breast cancer by hormone-receptor subtype, each breast cancer case was categorized by the combined classification of ER and PR status as follows: ER−PR−, ER−PR+, ER+PR−, or ER+PR+. ER−PR+ cases were excluded owing to their small number ($n = 12$). Polytomous logistic regression was performed based on a generalized logit model using the LOGIST procedure in SAS. The difference in the beta coefficient for ordinal variables used in the trend test by hormone receptor status was simultaneously tested using the Wald test in polytomous logistic regression models.

Along with the logistic regression analyses described above, we also performed linear trend tests to examine the monotonicity of the dose–response relationship based on median value in each quartile category of serum organochlorine concentration as an ordinal variable.

## Results

Case and control characteristics are summarized in Table 1. The cases included a higher percentage of premenopausal women than the matched controls. Among

**Table 1** Characteristics of breast cancer case subjects and their matched controls

| Characteristic | Cases ($n = 403$)[a] | | Controls ($n = 403$)[a] | | $P$ for difference[b] |
|---|---|---|---|---|---|
| Age (years), mean (SD) | 53.7 | (0.52) | 53.9 | (0.51) | 0.62 |
| Age at menarche (years), mean (SE) | 13.4 | (0.09) | 13.2 | (0.08) | 0.14 |
| Age at first childbirth (years), mean (SE) | 26.8 | (0.19) | 26.4 | (0.17) | 0.11 |
| Age at menopause (years), mean (SE)[c] | 49.0 | (0.29) | 49.4 | (0.26) | 0.10 |
| Number of live births, mean (SE) | 1.9 | (0.05) | 2.0 | (0.05) | 0.04 |
| Height (cm), mean (SE) | 155.4 | (0.29) | 155.6 | (0.29) | 0.88 |
| Body-mass index (kg/m$^2$), mean (SE) | 22.9 | (0.21) | 23.0 | (0.16) | 0.08 |
| Total lipid concentration in serum (%; w/w), mean (SE) | 0.617 | (0.01) | 0.632 | (0.01) | 0.005 |
| Recent fish consumption (g/day), mean (SE)[d] | 87.7 | (2.75) | 94.7 | (2.97) | 0.08 |
| Recent vegetable consumption (g/day), mean (SE)[d] | 256.6 | (8.09) | 310.8 | (10.3) | 0.0001 |
| Recent meat consumption (g/day), mean (SE)[d] | 58.2 | (1.96) | 57.6 | (2.04) | 0.56 |
| Recent fruit consumption (g/day), mean (SE)[d] | 287.9 | (10.7) | 287.7 | (10.2) | 0.86 |
| Recent total energy intake (kcal/day), mean (SE)[d] | 1882 | (27.3) | 1949 | (27.7) | 0.07 |
| Recent alcohol intake (one day per week or more), $n$ (%) | 74 | (26.6) | 101 | (30.6) | 0.50 |
| Premenopausal women, $n$ (%) | 183 | (45.4) | 141 | (35.0) | <0.0001 |
| Previous benign breast diseases, $n$ (%) | 46 | (12.0) | 30 | (7.5) | 0.04[e] |
| Previous breast feeding, $n$ (%) | 317 | (80.9) | 331 | (82.1) | 0.64[e] |
| Breast cancer in a first-degree relative, $n$ (%) | 31 | (7.8) | 23 | (5.7) | 0.48[e] |
| Previous breast cancer screening, $n$ (%) | | | | | <0.0001[e] |
| 1–2 times in the past five years | 92 | (23.9) | 78 | (19.5) | |
| 3–4 times in the past five years | 41 | (10.7) | 64 | (16.0) | |
| One or more times per year in the past five years | 62 | (16.1) | 215 | (53.8) | |
| Education (college degree or higher), $n$ (%) | 21 | (5.3) | 85 | (21.1) | <0.0001[e] |
| Smoking status, $n$ (%) | | | | | <0.0001[e] |
| Former | 50 | (12.5) | 8 | (2.0) | |
| Current | 34 | (8.5) | 23 | (5.7) | |
| Physical activity (moderate, ≥1/week, past 5 years), $n$ (%) | 91 | (22.9) | 127 | (31.5) | 0.053[e] |

[a] Sample size varied among variables because of missing information. Percentages were calculated among case or control subjects who provided answers

[b] Wilcoxon rank-sum test for continuous variables; Cochran-Mantel-Haenszel test based on rank scores for categorical variables

[c] Postmenopausal women only

[d] For 391 matched pairs

[e] Subjects with unknown or missing values were excluded from analysis

variables, positive or inverse associations with breast cancer were seen for a history of breast cancer screening, number of live births, menopausal status, educational level, smoking status, history of benign breast disease, vegetable consumption, and serum total lipid concentration.

Median serum organochlorine concentrations were not as high among cases as among controls (Table 2). All organochlorines, including *o,p′*-DDT and mirex, were detected in 100% of serum samples, except for 14 PCB peaks. Based on comparison of median values, average total PCBs among control participants consisted of the following PCB congeners: 153 (23.5%), 180 (13.4%), 138 (12.0%), 182/187 (6.7%), 118 (6.2%), 164/163 (5.0%), 170 (4.4%), 74 (3.7%), 99 (3.4%), 146 (3.0%), 156 (2.2%), 194 (1.9%), 198/199 (1.8%), 183 (1.6%), 177 (1.3%), 105 (1.2%), 203 (1.1%), and the remaining PCBs. PCB77 was not detected in any serum sample.

Table 3 shows that none of the serum organochlorines, including DDTs, *p,p′*-DDE, and total PCBs, was associated with an increased risk of breast cancer. In fact, risk was inversely associated with serum concentrations of *cis*-nonachlor, mirex and total PCBs, but not with those of the other compounds. For example, adjusted ORs (95%CIs; *P*-values for trend) of breast cancer risk for the highest vs. lowest quartile of exposure for total PCBs, mirex, and *cis*-nonachlor were 0.33 (0.14–0.78; *P* for trend = 0.008), 0.40 (0.19–0.84; *P* for trend = 0.02), and 0.41 (0.19–0.91; *P* for trend = 0.07), respectively. Adjustment for possible confounding variables attenuated the results for *o,p′*-DDT, *p,p′*-DDT, and *p,p′*-DDE and widened the 95% CI range for

**Table 2** Lipid-adjusted serum organochlorine concentrations (ng/g lipid) in breast cancer case subjects and matched controls

| Compound | Median (interquartile range) | | | | *P* for |
|---|---|---|---|---|---|
| | Cases ($n = 403$) | | Controls ($n = 403$) | | difference[a] |
| *o,p'*-DDT | 1.5 | (1.0, 2.3) | 1.6 | (1.1, 2.7) | 0.02 |
| *p,p'*-DDT | 9.3 | (6.2, 15) | 9.9 | (7.2, 16) | 0.03 |
| *p,p'*-DDE | 360 | (190, 620) | 370 | (220, 660) | 0.10 |
| *trans*-Nonachlor | 22 | (15, 30) | 23 | (17, 32) | 0.02 |
| *cis*-Nonachlor | 3.5 | (2.3, 5.0) | 3.9 | (2.7, 5.4) | 0.004 |
| Oxychlordane | 8.2 | (5.9, 11) | 8.6 | (6.6, 11) | 0.02 |
| HCB | 27 | (22, 33) | 27 | (23, 34) | 0.22 |
| Mirex | 1.9 | (1.5, 2.5) | 2.1 | (1.6, 2.8) | <0.0001 |
| *β*-HCH | 65 | (41, 110) | 64 | (41, 110) | 0.75 |
| Total PCBs[b] | 170 | (120, 220) | 180 | (140, 240) | 0.004 |

[a] Wilcoxon rank-sum test

[b] Sum of the 41 PCB peaks (International Union of Pure and Applied Chemistry numbers 17, 28, 51, 52/69, 43/49, 48/47, 44, 74, 66, 77, 90/101, 99, 123, 118, 114, 105, 126, 146, 153, 164/163, 138, 128/162, 167, 156, 169, 182/187, 183, 183, 174, 177, 180, 170, 189, 202, 201, 198/199, 196, 203, 194, 208, 206, and 209)

every compound, whereas the results for mirex and the PCBs were substantially changed. The main contributors to these changes were adjustment for history of breast cancer screening and smoking status. Additional analyses in the 349 complete pairs showed no substantial changes in ORs, indicating that these attenuations of estimates were not caused by either (or both) the reduced sample size or nonuniform lack of data.

Additional conditional logistic analysis for 34 individual PCB congeners (7 congeners were not analyzed here because of their lower detection frequency) showed no association with risk for any congener. To the contrary, half were associated with a significant decrease in risk: adjusted ORs (95% CIs; *P*-values for trend) for the highest vs. lowest quartile of exposure for PCB 153, 138, and 180, for example, were 0.40 (0.18–0.91; *P* for trend = 0.04), 0.61 (0.28–1.35; *P* for trend = 0.29), and 0.29 (0.13–0.66; *P* for trend = 0.004), respectively. In addition, the adjusted OR (95% CI; *P*-value for trend) for the highest vs. lowest quartile of exposure for PCB 48/47 was 0.45 (0.17–1.19; *P* for trend = 0.06). With regard to Wolff et al.'s functional groupings of PCBs [21] also, adjusted ORs (95% CIs; *P*-values for trend) for the highest vs. lowest quartile of exposure for PCB Group 1A (sum of PCB 44, 43/49 and 52/69), Group 1B (sum of PCB 90/101 and 182/187), Group 2A (sum of PCB 74, 66, 105 and 118), Group 2B (sum of PCB 128/162, 138 and 170), and Group 3 (sum of PCB 99, 153, 180, 196 and 203) were 0.53 (0.25–1.09; *P* for trend = 0.06), 0.28 (0.12–0.65; *P* for trend = 0.005), 0.82 (0.35–1.95; *P* for trend = 0.94), 0.28 (0.12–0.65; *P* for trend = 0.07), and 0.40 (0.18–0.91; *P* for trend = 0.03), respectively.

Further, no significant association was seen between serum organochlorines and an increased risk of breast cancer by hormone-receptor subtype (Table 4). The decrease in risk with increased serum concentration of *trans*-nonachlor or *cis*-nonachlor was greater in ER−PR− than ER+PR+ cases (*P* for heterogeneity = 0.01 or 0.04, respectively). A significant association was seen between increased serum concentrations of mirex or total PCBs and decreased risk of ER+PR− breast cancer, and this subtype was more sensitive to mirex than the ER+PR+ or ER−PR− subtypes (*P* for heterogeneity = 0.007 or 0.004, respectively).

Stratified analysis showed different patterns of association by menopausal status (Table 5). Postmenopausal women had a statistically significant decrease in breast cancer risk with increased serum concentration of *trans*-nonachlor. In contrast, point estimates of ORs for *o,p′*-DDT or *p,p′*-DDT were higher than 1.0 among postmenopausal women, albeit the trends were not linear. A marginal decrease in risk with increased serum concentrations of *o,p′*-DDT or *p,p′*-DDT was observed in premenopausal women but did not reach statistical significance. These associations are not shown in Table 3. Serum mirex or total PCBs were inversely associated with breast cancer risk regardless of menopausal status. Similar patterns were observed on stratification by the median age of controls (data not shown).

## Discussion

In this study, we found no increase in the risk of breast cancer among women with higher serum concentrations of any organochlorine, including DDTs and PCBs. To the contrary, we found statistically significant inverse associations between risk and total PCBs, *cis*-nonachlor, and mirex. This finding contrasts with that of the Seveso Italy study, which reported an association between breast cancer risk and an organochlorine, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [22]. Our findings suggest that, as in other countries, organochlorines are not related to an increased risk of breast cancer in Japan, a low-incidence country. Our lack of association with DDE is consistent with the null results of a nested case–control study of associations between serum DDE and PCBs and breast cancer risk in Asian women by Krieger et al. [15] (50 pairs), whereas our statistically significant inverse association for PCBs is not consistent with the null result for PCBs in most previous studies. Krieger et al. [15] also showed an insignificant positive association between serum DDE and breast cancer in white and black women, which is inconsistent with the majority of previous studies [6, 23]. The interethnic

**Table 3** Odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer according to quartiles of serum lipid-adjusted organochlorine concentration

| Compound | Quartile median (ng/g lipid) | No. of cases | No. of controls | Simple OR[a] (403 matched pairs) | | Adjusted OR[a,b] (349 matched pairs) | |
|---|---|---|---|---|---|---|---|
| | | | | OR | 95% CI | OR | 95% CI |
| *o,p'*-DDT | 0.90 | 103 | 81 | 1.00 | (referent) | 1.00 | (referent) |
| | 1.3 | 100 | 104 | 0.72 | 0.47–1.10 | 0.57 | 0.25–1.29 |
| | 2.0 | 122 | 109 | 0.81 | 0.54–1.22 | 1.13 | 0.53–2.38 |
| | 4.1 | 78 | 109 | 0.51 | 0.33–0.81 | 0.67 | 0.30–1.52 |
| | *P* for trend | | | | 0.007 | | 0.48 |
| *p,p'*-DDT | 5.6 | 136 | 100 | 1.00 | (referent) | 1.00 | (referent) |
| | 8.5 | 79 | 101 | 0.53 | 0.35–0.80 | 0.58 | 0.27–1.25 |
| | 12.0 | 97 | 95 | 0.68 | 0.45–1.03 | 0.99 | 0.47–2.07 |
| | 23.0 | 91 | 107 | 0.55 | 0.35–0.84 | 0.58 | 0.27–1.25 |
| | *P* for trend | | | | 0.06 | | 0.33 |
| *p,p'*-DDE | 160 | 116 | 97 | 1.00 | (referent) | 1.00 | (referent) |
| | 300 | 89 | 99 | 0.75 | 0.51–1.11 | 0.47 | 0.24–0.92 |
| | 490 | 107 | 103 | 0.84 | 0.57–1.26 | 0.99 | 0.48–2.02 |
| | 1100 | 91 | 104 | 0.68 | 0.44–1.06 | 1.02 | 0.46–2.26 |
| | *P* for trend | | | | 0.17 | | 0.46 |
| *trans*-Nonachlor | 13 | 126 | 89 | 1.00 | (referent) | 1.00 | (referent) |
| | 20 | 89 | 103 | 0.52 | 0.34–0.81 | 0.69 | 0.33–1.46 |
| | 27 | 96 | 104 | 0.54 | 0.34–0.84 | 0.72 | 0.33–1.57 |
| | 41 | 92 | 107 | 0.50 | 0.32–0.79 | 0.49 | 0.22–1.06 |
| | *P* for trend | | | | 0.02 | | 0.08 |
| *cis*-Nonachlor | 2.0 | 132 | 94 | 1.00 | (referent) | 1.00 | (referent) |
| | 3.3 | 98 | 107 | 0.58 | 0.38–0.87 | 0.51 | 0.25–1.06 |
| | 4.7 | 90 | 96 | 0.56 | 0.36–0.86 | 0.69 | 0.33–1.47 |
| | 7.0 | 83 | 106 | 0.45 | 0.28–0.71 | 0.41 | 0.19–0.91 |
| | *P* for trend | | | | 0.002 | | 0.07 |
| Oxychlordane | 5.4 | 128 | 100 | 1.00 | (referent) | 1.00 | (referent) |
| | 7.8 | 92 | 95 | 0.66 | 0.43–1.02 | 0.73 | 0.35–1.51 |
| | 9.7 | 77 | 93 | 0.57 | 0.37–0.89 | 0.60 | 0.28–1.31 |
| | 15 | 106 | 115 | 0.63 | 0.41–0.97 | 0.65 | 0.31–1.38 |
| | *P* for trend | | | | 0.09 | | 0.33 |
| HCB | 20 | 107 | 95 | 1.00 | (referent) | 1.00 | (referent) |
| | 25 | 92 | 86 | 0.90 | 0.59–1.38 | 0.67 | 0.32–1.37 |
| | 30 | 110 | 120 | 0.77 | 0.50–1.17 | 0.91 | 0.43–1.92 |
| | 38 | 94 | 102 | 0.75 | 0.47–1.20 | 0.95 | 0.43–2.11 |
| | *P* for trend | | | | 0.18 | | 0.90 |
| Mirex | 1.4 | 130 | 87 | 1.00 | (referent) | 1.00 | (referent) |
| | 1.9 | 109 | 98 | 0.73 | 0.50–1.08 | 0.56 | 0.28–1.13 |
| | 2.4 | 86 | 112 | 0.50 | 0.34–0.75 | 0.60 | 0.30–1.19 |
| | 3.5 | 78 | 106 | 0.48 | 0.32–0.73 | 0.40 | 0.19–0.84 |
| | *P* for trend | | | | 0.0003 | | 0.02 |
| $\beta$-HCH | 26 | 96 | 98 | 1.00 | (referent) | 1.00 | (referent) |
| | 52 | 100 | 102 | 1.01 | 0.66–1.53 | 0.81 | 0.39–1.72 |
| | 82 | 99 | 91 | 1.12 | 0.71–1.78 | 0.72 | 0.31–1.69 |
| | 160 | 108 | 112 | 1.00 | 0.61–1.62 | 1.04 | 0.43–2.52 |
| | *P* for trend | | | | 0.91 | | 0.63 |

**Table 3** continued

| Compound | Quartile median (ng/g lipid) | No. of cases | No. of controls | Simple OR[a] (403 matched pairs) | | Adjusted OR[a,b] (349 matched pairs) | |
|---|---|---|---|---|---|---|---|
| | | | | OR | 95% CI | OR | 95% CI |
| Total PCBs | 110 | 126 | 99 | 1.00 | (referent) | 1.00 | (referent) |
| | 160 | 96 | 85 | 0.82 | 0.53–1.26 | 0.79 | 0.36–1.72 |
| | 200 | 102 | 116 | 0.61 | 0.40–0.92 | 0.57 | 0.28–1.15 |
| | 290 | 79 | 103 | 0.48 | 0.30–0.77 | 0.33 | 0.14–0.78 |
| | *P* for trend | | | | 0.002 | | 0.008 |

[a] Cases and controls were matched for age and area

[b] Adjusted for total lipid concentration in serum (<0.5409%, 0.5409–0.6144%, 0.6145–0.701%, or ≥0.702%); body-mass index (<20.93, 20.93–22.59, 22.6–24.88, or >24.88 kg/m$^2$); menopausal status and age at menopause (premenopause, <48, 48–50, 51–52, or ≥53 years); smoking status (never, former, or current); fish consumption (<54.9, 54.9–82.2, 82.4–115.4, or ≥115.9 g/day); vegetable consumption (<177.27, 177.27–260.2, 261.2–378.3, or ≥379.1 g/day); family history of breast cancer in a first-degree relative (yes or no); age at first childbirth (nulliparous, <25, 25–26, 27–28, ≥29 years)—Ordinal variable; parity (nulliparous, 1, 2, or ≥3); age at menarche (<12, 12, 13, 14, or ≥15 years); history of breast cancer screening (never, 1–2 times in the past five years, 3–4 times in the past five years, or one or more times per year in the past five years); and history of breast feeding (yes or no)

Subjects with missing values in any of the variables included in the models were not used, nor was the corresponding subject in the matched case–control pair

variation between Caucasian and Asian women suggested by Krieger et al. [15] has not been confirmed.

Our null finding for *p,p′*-DDE is consistent with the results of a previous meta-analysis [23] and inconsistent with our marginal decrease in risk of ER+PR+ breast cancer or breast cancer in premenopausal women for *o,p′*-DDT. This difference in effects between *p,p′*-DDE and *o,p′*-DDT might be partly explained as follows. First, *p,p′*-DDE is androgenic but only weakly estrogenic or negative, whereas *o,p′*-DDT is the most estrogenic of all DDT-related compounds [9]. Second, it is unclear whether serum *p,p′*-DDE represents *o,p′*-DDT intake because most serum *p,p′*-DDE results from *p,p′*-DDE intake, because of the slow conversion of ingested DDT to *p,p′*-DDE in humans [24]. The association of breast cancer risk with the specific serum levels of *o,p′*-DDT, *p,p′*-DDT, or *p,p′*-DDE was not always consistent, but in some analyses showed similar patterns owing to the correlation of their serum concentrations (Spearman correlation coefficient among controls = 0.57–0.86). Further, no previous study with a small sample size has found an increased risk of breast cancer in relation to *o,p′*-DDT exposure [17, 25]. In addition, the lack of association with *p,p′*-DDT is consistent with the majority of previous studies [17, 20, 25–40], including three nested case–control studies [26–28], whereas other studies found significant or marginal inverse [41, 42] or positive [43–47] associations.

Our inverse association between serum total PCBs and breast cancer is inconsistent with the majority of previous studies, which had null results [10]. This difference may be due in part to the lower blood levels of total PCBs in our subjects than in any previous population studied (mean, median, or geometric mean in controls 257.1–2885.8 ng/g lipid) [10, 16, 46] and to the lower sex hormone levels in Asian women [48]. Given that some PCB congeners did not show the same risk pattern as total PCBs, the effect of total PCBs might also depend on the congener pattern. The inverse associations were consistent across Wolff et al.'s functional PCB groups, except for Group 2A (moderately persistent; potentially antiestrogenic and immunotoxic, dioxin-like PCBs); but despite the lack of association for this group, the association of total PCBs with decreased risk remained because the major components of total PCBs were PCB 153 and 180 (Group 3). At least two prospective studies which found inverse associations for total PCBs also found inverse associations for the PCBs of Group 3 (enzyme inducers) [28, 42].

Among other agents, no increase in the risk of breast cancer was seen for serum levels of chlordane-related compounds. This finding is in general agreement with previous studies [28, 29, 32, 39, 42, 45, 47, 49, 50], as follows. Oxychlordane's lack of association or only insignificant inverse association with breast cancer is consistent with most previous studies [28, 29, 32, 45, 49, 50], with a significant inverse association seen in a recent nested case–control study in postmenopausal women only [42]. An inverse association for *cis*-nonachlor was also found in two case–control studies, but was insignificant in both [29, 49], whereas a third case–control and a prospective study reported no association [42, 45]; while *trans*-nonachlor's lack of association is consistent with previous studies [28, 32, 39, 42, 47, 50].

In contrast to the consistency of findings between the present and past studies for chlordane-related compounds,

**Table 4** Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of hormone receptor-defined breast cancer according to lipid-adjusted organochlorine concentration in serum

| Compound | Quartile median (ng/g lipid) | Adjusted ORs[a] | | | | | | *P* for heterogeneity | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ER−PR− (75 cases) | | ER+PR− (64 cases) | | ER+PR+ (203 cases) | | | | |
| | | OR | 95% CI | OR | 95% CI | OR | 95% CI | $P^b$ | $P^c$ | $P^d$ |
| *o,p'*-DDT | 0.90 | 1.00 | (referent) | 1.00 | (referent) | 1.00 | (referent) | | | |
| | 1.3 | 1.00 | 0.41–2.41 | 0.59 | 0.23–1.56 | 0.69 | 0.38–1.27 | | | |
| | 2.0 | 1.43 | 0.61–3.35 | 1.15 | 0.49–2.68 | 0.93 | 0.51–1.69 | | | |
| | 4.1 | 0.96 | 0.37–2.47 | 0.78 | 0.30–2.04 | 0.55 | 0.28–1.08 | | | |
| | *P* for trend | | 0.79 | | 0.82 | | 0.12 | 0.98 | 0.40 | 0.38 |
| *p,p'*-DDT | 5.6 | 1.00 | (referent) | 1.00 | (referent) | 1.00 | (referent) | | | |
| | 8.5 | 0.60 | 0.26–1.36 | 1.42 | 0.58–3.47 | 0.68 | 0.37–1.26 | | | |
| | 12 | 0.66 | 0.28–1.54 | 1.26 | 0.50–3.19 | 0.99 | 0.54–1.81 | | | |
| | 23 | 0.53 | 0.23–1.25 | 0.94 | 0.36–2.49 | 0.91 | 0.48–1.72 | | | |
| | *P* for trend | | 0.25 | | 0.60 | | 0.96 | 0.64 | 0.59 | 0.24 |
| *p,p'*-DDE | 160 | 1.00 | (referent) | 1.00 | (referent) | 1.00 | (referent) | | | |
| | 300 | 0.68 | 0.29–1.58 | 0.40 | 0.16–1.00 | 0.68 | 0.37–1.26 | | | |
| | 490 | 0.81 | 0.35–1.92 | 0.70 | 0.30–1.62 | 0.93 | 0.51–1.67 | | | |
| | 1100 | 1.06 | 0.42–2.64 | 0.49 | 0.19–1.27 | 0.95 | 0.49–1.85 | | | |
| | *P* for trend | | 0.55 | | 0.40 | | 0.75 | 0.24 | 0.31 | 0.72 |
| *trans*-Nonachlor | 13 | 1.00 | (referent) | 1.00 | (referent) | 1.00 | (referent) | | | |
| | 20 | 0.64 | 0.28–1.44 | 0.93 | 0.37–2.31 | 0.66 | 0.35–1.23 | | | |
| | 27 | 0.48 | 0.19–1.17 | 1.11 | 0.42–2.92 | 0.81 | 0.42–1.56 | | | |
| | 41 | 0.26 | 0.10–0.69 | 0.53 | 0.18–1.52 | 0.79 | 0.41–1.54 | | | |
| | *P* for trend | | 0.006 | | 0.17 | | 0.73 | 0.29 | 0.27 | 0.01 |
| *cis*-Nonachlor | 2.0 | 1.00 | (referent) | 1.00 | (referent) | 1.00 | (referent) | | | |
| | 3.3 | 1.10 | 0.49–2.45 | 0.90 | 0.37–2.18 | 0.55 | 0.30–1.02 | | | |
| | 4.7 | 0.63 | 0.25–1.56 | 0.72 | 0.28–1.89 | 0.85 | 0.45–1.60 | | | |
| | 7.0 | 0.35 | 0.13–0.95 | 0.57 | 0.21–1.52 | 0.70 | 0.36–1.36 | | | |
| | *P* for trend | | 0.01 | | 0.22 | | 0.50 | 0.31 | 0.46 | 0.04 |
| Oxychlordane | 5.4 | 1.00 | (referent) | 1.00 | (referent) | 1.00 | (referent) | | | |
| | 7.8 | 1.12 | 0.49–2.55 | 1.14 | 0.47–2.74 | 0.68 | 0.36–1.27 | | | |
| | 9.7 | 0.54 | 0.21–1.38 | 0.81 | 0.31–2.13 | 0.73 | 0.39–1.40 | | | |
| | 15 | 0.60 | 0.24–1.53 | 0.49 | 0.18–1.37 | 0.83 | 0.43–1.57 | | | |
| | *P* for trend | | 0.17 | | 0.08 | | 0.77 | 0.68 | 0.13 | 0.25 |
| HCB | 20 | 1.00 | (referent) | 1.00 | (referent) | 1.00 | (referent) | | | |
| | 25 | 0.81 | 0.35–1.89 | 0.75 | 0.30–1.87 | 0.62 | 0.33–1.15 | | | |
| | 30 | 0.80 | 0.34–1.88 | 1.05 | 0.43–2.53 | 1.01 | 0.55–1.83 | | | |
| | 38 | 0.80 | 0.31–2.04 | 0.58 | 0.21–1.56 | 1.03 | 0.53–2.00 | | | |
| | *P* for trend | | 0.64 | | 0.33 | | 0.65 | 0.67 | 0.22 | 0.44 |
| Mirex | 1.4 | 1.00 | (referent) | 1.00 | (referent) | 1.00 | (referent) | | | |
| | 1.9 | 0.86 | 0.36–2.04 | 0.43 | 0.19–1.00 | 0.89 | 0.50–1.60 | | | |
| | 2.4 | 0.97 | 0.41–2.29 | 0.30 | 0.13–0.71 | 0.46 | 0.25–0.86 | | | |
| | 3.5 | 0.69 | 0.27–1.75 | 0.10 | 0.03–0.32 | 0.57 | 0.29–1.10 | | | |
| | *P* for trend | | 0.43 | | <0.0001 | | 0.049 | 0.004 | 0.007 | 0.50 |
| *β*-HCH | 26 | 1.00 | (referent) | 1.00 | (referent) | 1.00 | (referent) | | | |
| | 52 | 1.86 | 0.79–4.38 | 2.21 | 0.86–5.67 | 1.09 | 0.58–2.04 | | | |
| | 82 | 0.79 | 0.30–2.11 | 1.45 | 0.55–3.86 | 1.29 | 0.67–2.47 | | | |
| | 160 | 1.19 | 0.43–3.25 | 0.91 | 0.30–2.80 | 1.10 | 0.54–2.24 | | | |
| | *P* for trend | | 0.77 | | 0.29 | | 0.90 | 0.52 | 0.27 | 0.71 |

**Table 4** continued

| Compound | Quartile median (ng/g lipid) | Adjusted ORs[a] | | | | | | *P* for heterogeneity | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ER−PR− (75 cases) | | ER+PR− (64 cases) | | ER+PR+ (203 cases) | | | | |
| | | OR | 95% CI | OR | 95% CI | OR | 95% CI | $P^b$ | $P^c$ | $P^d$ |
| Total PCBs | 110 | 1.00 | (referent) | 1.00 | (referent) | 1.00 | (referent) | | | |
| | 160 | 1.09 | 0.47–2.58 | 0.62 | 0.25–1.56 | 1.20 | 0.64–2.25 | | | |
| | 200 | 0.68 | 0.29–1.58 | 0.35 | 0.14–0.88 | 0.80 | 0.44–1.45 | | | |
| | 290 | 0.38 | 0.13–1.05 | 0.20 | 0.07–0.59 | 0.54 | 0.26–1.11 | | | |
| | *P* for trend | | 0.03 | | 0.003 | | 0.055 | 0.41 | 0.10 | 0.46 |

[a] Cases were stratified by combined estrogen and progesterone receptor status. Each analysis used 381 controls. ORs were adjusted for age (continuous); residential area (urban or rural); total lipid concentration in serum (<0.5409%, 0.5409–0.6144%, 0.6145–0.701%, or ≥0.702%); body-mass index (<20.93, 20.93–22.59, 22.6–24.88, or >24.88 kg/m$^2$); menopausal status and age at menopause (premenopause, <48, 48–50, 51–52, or ≥53 years); smoking status (never, former, or current); fish consumption (<54.9, 54.9–82.2, 82.4–115.4, or ≥115.9 g/day); vegetable consumption (<177.27, 177.27–260.2, 261.2–378.3, or ≥379.1 g/day); menopausal status and age at menopause (premenopause, <48, 48–50, 51–52, or ≥53 years); smoking status (never, former, or current); family history of breast cancer in a first-degree relative (yes or no); age at first childbirth (nulliparous, <25, 25–26, 27–28, ≥29 years)—Ordinal variable; parity (nulliparous, 1, 2, or ≥3); age at menarche (<12, 12, 13, 14, or ≥15 years); history of breast cancer screening (never, 1–2 times in the past five years, 3–4 times in the past five years, or one or more times per year in the past five years); and history of breast feeding (yes or no)

[b] *P* value for heterogeneity in odds ratios between ER−PR− and ER+PR−

[c] *P* value for heterogeneity in odds ratios between ER+PR− and ER+PR+

[d] *P* value for heterogeneity in odds ratios between ER−PR− and ER+PR+

however, our finding of an inverse association between mirex and breast cancer risk is inconsistent with past studies. Four previous hospital-based case–control studies in the US have assessed this association, with null results [29, 45, 51, 52], although one reported a borderline association in a group with no history of lactation ($OR = 2.42$, 95% CI 0.98–4.32) [52]. A second noted a higher range of mirex exposure (mean 0.037 ng/g serum among controls [52]) than that seen here, likely because of the history of use of this agent in the US versus no use in Japan [53]. Furthermore, Asian women have lower sex hormone levels [48] and higher dietary intake of phytoestrogens [54, 55] than Caucasian women in Western countries. These differences may partly explain why these previous results were not reproduced here.

The lack of association, in our study, of breast cancer risk with serum concentration of HCB or $\beta$-HCH is consistent with the majority of previous studies of HCB [29, 33, 35, 37, 45, 46, 49, 52, 56–58] and $\beta$-HCH [17, 28, 29, 31, 32, 35, 41, 43, 45, 47, 49, 59, 60], respectively. Moreover, a recent nested case–control study of HCB in postmenopausal women reported a significant inverse association [42]. In contrast, several case–control studies found positive associations between breast cancer risk and HCB [30, 61] or $\beta$-HCH [33, 37, 44], and also observed much higher blood concentrations of HCB (mean 0.79 ppb [61]; mean 0.11 μg/g lipid [30]) or $\beta$-HCH (mean 0.31 mg/l [44]) in breast cancer patients.

Many of our findings by hormone-receptor subtype are inconsistent with previous studies. Of interest, we found no significant increase in the risk of organochlorines on ER+PR+ breast cancer, which is suggested to be the most sensitive breast cancer to estrogen-related risk factors [62, 63]. Indeed, several organochlorines were inversely associated with ER+PR− or ER−PR− breast cancers, although these associations did not always agree with previous studies. In contrast to our results for total PCBs in breast cancer subtypes, most previous studies found no difference in risk by hormone receptor status [15, 28, 32, 39, 47, 49, 51, 56, 57, 64–68]. On the other hand, inverse associations between PCBs and ER−PR− breast cancer [34] or ER− breast cancer in postmenopausal women [42] have also been reported. Our findings of inverse associations between ER+PR− breast cancer and serum total PCBs or mirex are also inconsistent with previous studies [51, 67]. In addition, the inverse association between *trans*-nonachlor and ER−PR− breast cancer is consistent with a recent prospective study of ER− breast cancer in postmenopausal women [42], whereas the majority of other studies reported a null finding [28, 32, 39, 49–51]. Further, our inverse association between *cis*-nonachlor and ER−PR− breast cancer is inconsistent with the two previous studies of this risk, which observed no association [42, 49]. Finally, our finding of marginal inverse associations of ER+PR+ breast cancer with mirex or *o,p′*-DDT may be inconclusive, because no previous study has found them.

Stratification by menopausal status showed several different patterns of association between breast cancer and organochlorines. Similar patterns were seen on stratification by median age, suggesting that those by menopausal

**Table 5** Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer according to quartiles of serum lipid-adjusted organochlorine concentration by menopausal status

| Compound | Quartile median (ng/g lipid) | Adjusted OR[a] | | | |
|---|---|---|---|---|---|
| | | Premenopause | | Postmenopause[b] | |
| | | (164 cases; 134 controls) | | (193 cases; 247 controls) | |
| | | OR | 95% CI | OR | 95% CI |
| *o,p'*-DDT | 0.90 | 1.00 | (referent) | 1.00 | (referent) |
| | 1.3 | 0.46 | 0.22–0.98 | 1.07 | 0.44–2.65 |
| | 2.0 | 0.60 | 0.27–1.34 | 1.63 | 0.71–3.75 |
| | 4.1 | 0.46 | 0.17–1.26 | 1.03 | 0.44–2.42 |
| | *P* for trend | | 0.26 | | 0.71 |
| *p,p'*-DDT | 5.6 | 1.00 | (referent) | 1.00 | (referent) |
| | 8.5 | 0.54 | 0.25–1.16 | 1.53 | 0.64–3.68 |
| | 12 | 0.39 | 0.17–0.88 | 2.26 | 0.95–5.35 |
| | 23 | 0.45 | 0.17–1.17 | 1.55 | 0.68–3.52 |
| | *P* for trend | | 0.08 | | 0.67 |
| *p,p'*-DDE | 160 | 1.00 | (referent) | 1.00 | (referent) |
| | 300 | 0.64 | 0.30–1.36 | 0.58 | 0.24–1.40 |
| | 490 | 0.57 | 0.28–1.20 | 1.09 | 0.47–2.57 |
| | 1100 | 0.92 | 0.32–2.63 | 0.89 | 0.38–2.08 |
| | *P* for trend | | 0.72 | | 0.81 |
| *trans*-Nonachlor | 13 | 1.00 | (referent) | 1.00 | (referent) |
| | 20 | 0.54 | 0.26–1.16 | 0.54 | 0.20–1.40 |
| | 27 | 0.88 | 0.36–2.15 | 0.40 | 0.15–1.08 |
| | 41 | 0.78 | 0.31–1.97 | 0.35 | 0.13–0.93 |
| | *P* for trend | | 0.67 | | 0.06 |
| *cis*-Nonachlor | 2.0 | 1.00 | (referent) | 1.00 | (referent) |
| | 3.3 | 0.67 | 0.32–1.39 | 0.49 | 0.20–1.23 |
| | 4.7 | 0.78 | 0.32–1.93 | 0.52 | 0.20–1.32 |
| | 7.0 | 0.53 | 0.20–1.42 | 0.42 | 0.16–1.06 |
| | *P* for trend | | 0.21 | | 0.15 |
| Oxychlordane | 5.4 | 1.00 | (referent) | 1.00 | (referent) |
| | 7.8 | 0.57 | 0.26–1.27 | 0.90 | 0.36–2.27 |
| | 9.7 | 0.72 | 0.30–1.71 | 0.46 | 0.17–1.24 |
| | 15 | 1.01 | 0.41–2.47 | 0.50 | 0.19–1.32 |
| | *P* for trend | | 0.94 | | 0.11 |
| HCB | 20 | 1.00 | (referent) | 1.00 | (referent) |
| | 25 | 1.05 | 0.52–2.13 | 0.53 | 0.20–1.39 |
| | 30 | 0.95 | 0.44–2.06 | 0.91 | 0.38–2.17 |
| | 38 | 0.88 | 0.30–2.58 | 0.77 | 0.32–1.87 |
| | *P* for trend | | 0.80 | | 0.95 |
| Mirex | 1.4 | 1.00 | (referent) | 1.00 | (referent) |
| | 1.9 | 0.84 | 0.39–1.83 | 0.44 | 0.19–1.02 |
| | 2.4 | 0.43 | 0.19–0.98 | 0.29 | 0.13–0.66 |
| | 3.5 | 0.28 | 0.10–0.75 | 0.36 | 0.16–0.85 |
| | *P* for trend | | 0.005 | | 0.06 |

**Table 5** continued

| Compound | Quartile median (ng/g lipid) | Adjusted OR[a] | | | |
|---|---|---|---|---|---|
| | | Premenopause | | Postmenopause[b] | |
| | | (164 cases; 134 controls) | | (193 cases; 247 controls) | |
| | | OR | 95% CI | OR | 95% CI |
| $\beta$-HCH | 26 | 1.00 | (referent) | 1.00 | (referent) |
| | 52 | 2.06 | 0.98–4.32 | 1.21 | 0.44–3.31 |
| | 82 | 1.69 | 0.72–3.97 | 1.02 | 0.37–2.81 |
| | 160 | 0.63 | 0.21–1.90 | 0.93 | 0.33–2.60 |
| | *P* for trend | | 0.71 | | 0.58 |
| Total PCBs | 110 | 1.00 | (referent) | 1.00 | (referent) |
| | 160 | 1.62 | 0.73–3.60 | 0.53 | 0.21–1.35 |
| | 200 | 0.45 | 0.21–0.99 | 0.47 | 0.20–1.15 |
| | 290 | 0.31 | 0.08–1.16 | 0.30 | 0.12–0.75 |
| | *P* for trend | | 0.04 | | 0.01 |

[a] Adjusted for age (continuous); residential area (urban or rural); total lipid concentration in serum (<0.5409%, 0.5409–0.6144%, 0.6145–0.701%, or ≥0.702%); body-mass index (<20.93, 20.93–22.59, 22.6–24.88, or >24.88 kg/m$^2$); smoking status (never, former, or current); fish consumption (<54.9, 54.9–82.2, 82.4–115.4, or ≥115.9 g/day); vegetable consumption (<177.27, 177.27–260.2, 261.2–378.3, or ≥379.1 g/day); smoking status (never, former, or current); family history of breast cancer in a first-degree relative (yes or no); age at first childbirth (nulliparous, <25, 25–26, 27–28, ≥29 years)—Ordinal variable; parity (nulliparous, 1, 2, or ≥3); age at menarche (<12, 12, 13, 14, or ≥15 years); history of breast cancer screening (never, 1–2 times in the past five years, 3–4 times in the past five years, or one or more times per year in the past five years); and history of breast feeding (yes or no)

[b] Additionally adjusted for age at menopause (<48, 48–50, 51–52, or ≥53 years)

status might rather have resulted from the difference in age or period, at least in part, in addition to the difference in endogenous estrogen levels between menopausal statuses. Age may be a critical determinant of the association between serum organochlorines and breast cancer risk, as suggested by the most recent study [69], because the human body burden of persistent organochlorines is positively correlated with age, and has historically decreased in Japan [12] as well as in the US [53] and Norway [28], at the least. On this basis, age-stratified analysis may be essential to any risk evaluation of highly persistent substances.

Our study has four main strengths. First, owing to their biological persistence, serum concentrations of organochlorines reflect long-term cumulative exposure to the compounds and their individual differences, allowing a greater degree of certainty about the exposure at risk. Second, because surgery may change the blood levels of organochlorines, blood samples were collected from case patients before surgery [70]. Third, our use of measurement methods with low LODs allowed us to detect serum *o,p′*-DDT in serum with adequate frequency (100%) and directly assess its association with breast cancer. The inadequate LODs and subsequent low detection frequencies in most previous studies prevented them from explicitly assessing this association, notwithstanding the greater estrogenicity of *o,p′*-DDT than *p,p′*-DDT and *p,p′*-DDE [6]. Although some PCBs with a value between 0 and the LOD were assigned the LOD value, additional analysis showed that any subsequent misclassification was inconsequential. Even when we assigned no value (e.g. LOD/2 or LOD) for individuals with a PCB level below the LOD to ensure that values between 0 and the LOD were retained, the results for total PCBs were not substantially changed. The PCB congeners, including those with nondetectable values, made only a minor contribution to total PCBs. Fourth, almost all invited subjects participated in the study, likely eliminating the possibility of nonresponse bias. Moreover, our null result for DDE is consistent with the majority of previous nested case–control studies, although a certain discrepancy between the results of prospective and case–control studies has been noted [6, 23].

Several limitations of the study also warrant mention. First, although we considered a large number of covariates in all analyses, the observational design of the study means that unmeasured or residual confounding could not be completely excluded. Because serum concentrations of organochlorines are highly correlated with each other and can be correlated with unmeasured substances, the associations observed here might not always have represented the direct effect of organochlorines. As examples, the results for *trans*-nonachlor, *cis*-nonachlor, and oxychlordane, which are impurities or metabolites of chlordane, showed a similar but not always consistent pattern, whereas

their serum concentrations were highly correlated (Spearman correlation coefficient among controls = 0.83–0.97). Further, PCB48/47 and 51, which are usually not detected in biological samples, were frequently detected in serum. This may suggest the possibility of sample contamination during sampling or sample storage. In addition, although the substantially high participation rates among both eligible cases and controls minimized potential biases related to control selection, the use of controls from medical checkup examinees, whose distribution of risk factors for breast cancer may differ from the general population due to greater health consciousness, might have led to selection bias. This possibility is heightened by the lack of differences between patients and controls in the distribution of several established risk factors for breast cancer (family history, reproductive factors, etc.). Finally, samples collected from cases post-diagnosis may less likely reflect serum levels at the time relevant to carcinogenesis than those collected prospectively in cohort studies.

## Conclusions

In conclusion, our results do not support the hypothesis that higher serum organochlorine concentrations increase the risk of breast cancer in Japanese women. Overall, the present study suggests that breast cancer risk in Japan, a low-incidence country, is similar to that in western countries in terms of organochlorine exposure.

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