

Organochlorines and Endometrial Cancer Risk¹

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Abstract

There is concern that persistent environmental pollutants such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) increase breast cancer risk, at least partially through estrogenic effects. Because the endometrium is more sensitive to estrogenic stimulation than the breast, such a carcinogenic effect should be more pronounced in the endometrium than the breast. In a population-based case-control study in Sweden, we measured serum concentrations of 10 chlorinated pesticides and 10 PCB congeners in 154 endometrial cancer cases and 205 population controls. Information on potential confounders was obtained by mailed questionnaires. We used logistic regression to calculate odds ratios (ORs) as measures of relative risk. We performed analyses for lipid-adjusted concentrations of each individual substance and after grouping substances according to putative hormonal effects. We found no significant associations of increasing levels of pesticide or PCB exposure with endometrial cancer risk. The multivariate OR was 1.0 (95% confidence interval, 0.6–2.0; *P* for trend, 0.78) for the highest compared with the lowest quartile of dichlorodiphenyldichloroethylene (DDE), the predominant dichlorodiphenyltrichloroethane metabolite. Corresponding ORs were 1.0 for hexachlorobenzene, 0.9 for β -hexachlorocyclohexane, 1.4 for oxychlorane, and 1.2 for *trans*-nonachlor. Analyses of substances grouped by putative hormonal effect also showed no associations with endometrial cancer risk. For all estrogenic compounds, the OR for the highest compared with the lowest quartile was 1.1 (95% confidence interval, 0.6–2.2; *P* for trend, 0.90). Our data do not support the hypothesis that the organochlorine

exposure studied increases the risk for endometrial cancer.

Introduction

The concern that environmental organochlorine pollutants in food may cause cancer in humans is widespread (1). The theory that such effects would arise from weakly estrogenic effects of some organochlorines has been tested almost exclusively in epidemiological studies of breast cancer. Although some relatively small studies were supportive (2–7), two larger carefully conducted prospective studies (8, 9) and at least three recently published case-control studies (10–12) showed no convincing association between exposure to organochlorines (mainly DDT³ or PCBs) and breast cancer risk.

Despite accumulating reassuring evidence, public concern continues, as does scientific investigation focused largely on breast cancer. However, if certain organochlorines do affect the cancer risk through estrogenic mechanisms, their effects should be detected more easily in the endometrium. Whereas breast cancer risk is affected to only a relatively small extent by oral estrogens (13), the risk for endometrial cancer increases markedly after just a few years of use (14).

To our knowledge, to date only one study has addressed the association between organochlorine pesticides or PCBs and the risk of endometrial cancer, and the results were negative (15). To test the hypothesis that elevated blood concentrations of specific organochlorines are associated with an increased risk of endometrial cancer, we carried out a population-based study of 10 organochlorine pesticides and 10 PCB congeners.

Subjects and Methods

Study Population. Our study focused on women 50–74 years of age, resident between February 1996 and November 1997 in 12 Swedish counties on the coasts of the Gulf of Bothnia, the Baltic Sea, and the largest Swedish lakes. We assumed that the intake of organochlorine compounds through ingestion of possibly contaminated fish would be higher in these counties than elsewhere in Sweden. Women were eligible if they were born in Sweden, had no prior hysterectomy, and had never used hormone replacement therapy (except topical estriol, dienestrol, or estradiol); the use of such compounds could mask an effect of substances with less potent hormone-like effects, such as organochlorine compounds.

Women with incident histopathologically confirmed endometrial cancer diagnosed between February 1996 and November 1997 were identified through a network of personnel at the departments of gynecology and gynecological oncology in the study area. (One of the 26 departments did not collaborate.)

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³ The abbreviations used are: DDT, dichlorodiphenyltrichloroethane; PCB, polychlorinated biphenyl; BMI, body mass index; HCB, hexachlorobenzene; DDE, dichlorodiphenyldichloroethylene; LOD, limit of detection; HCH, hexachlorocyclohexane; DDD, dichlorodiphenyldichloroethane; CB, chlorinated biphenyl; OR, odds ratio; CI, confidence interval.

They reported 396 cases (~95% of the expected number; Ref. 16). Of these, 288 (73%) volunteered to donate blood samples and complete the study questionnaire; 41 patients refused to participate, and 67 cases were not approached (due to forgetfulness of the medical staff). Subsequently, 134 case women were excluded because they had used hormone replacement therapy, leaving 154 in the study.

Population controls were recruited from the same 12 counties as the cases. They were randomly selected from a continuously up-dated population register, and were frequency-matched to the cases by 5-year age groups. Controls were not matched to cases by geographic area of residence (county) or any other characteristic.

The period of control recruitment coincided with that of cases because we sampled and enrolled controls in four phases: the spring of 1996; the fall of 1996; the spring of 1997, and the fall of 1997. Of 742 control women selected, 559 (75.3%) responded to the study questionnaire, and 492 (66.3%) also agreed to donate blood samples. After the exclusion of 287 women because of prior hysterectomy ($n = 46$) or use of hormone replacement therapy ($n = 241$), 205 control women were included in the study. Subjects who were excluded (because of refusal to give a blood sample, a previous hysterectomy, or the use of hormone replacement) did not differ meaningfully from those who were included in the study in terms of age and BMI (*i.e.*, the main variables associated with pesticide and PCB levels. See "Statistical Methods").

The self-administrated study questionnaire requested information on weight, height, reproductive history, diet, hormone use, smoking, physical activity, and medical history, among others. Missing information—mostly details on dates of use and brands of hormones taken as oral contraceptives and hormone replacement therapy—was supplemented by a telephone interview in ~50% of cases and controls.

The local Ethics Committee approved the design of this study.

Blood Sampling. Blood samples from fasting case patients were drawn at the hospital departments before any cancer treatment and from control subjects at a primary health care unit or at home. Fifteen control subjects (but no case patients) failed to fast. Serum was separated within 2 h of collection, and frozen at -20°C until shipment to the Swedish National Food Administration laboratory for analysis.

Analysis of Organochlorine Compounds in Human Serum. We analyzed the lipid portion of serum samples for the 10 organochlorine pesticides and 10 PCB congeners (Table 2) chosen *a priori* because of their likelihood of being present in the food chain in Sweden. The method used is described in detail in Atuma and Aune (17). Serum samples (4 g) were mixed with methanol and a mixture of internal standards was added to correct for recovery and ensure quality control. The samples were then extracted three times with *n*-hexane-diethyl ether (1:1, v/v). After evaporation of the solvents, the fat content was determined gravimetrically. The fat was redissolved in *n*-hexane and treated with concentrated sulfuric acid. The PCB congeners were separated from the bulk of the chlorinated pesticides by elution through a silica gel column (4.5 g of 3% water-deactivated silica gel). The first fraction, containing the PCB congeners, HCB and *p,p'*-DDE, was eluted with ~30 ml of *n*-hexane, and the second fraction, containing mainly chlorinated pesticides, was eluted with 40 ml of a *n*-hexane-diethyl ether mixture (3:1, v/v). Analysis of the two fractions was performed on a gas chromatograph with dual capillary columns and electron capture detectors (^{63}Ni). The columns

were of different polarity to ease identification of analytes, which was based on retention times relative to internal standards. Quantification was performed using multilevel calibration curves obtained by injection of standard solutions of at least three different concentrations.

The LOD was determined as 3 SD above the value for the blank and varied between 1 and 7 pg/g serum (not lipid-adjusted) for the PCB congeners and between 2 and 7 pg/g serum for the chlorinated pesticides.

Samples with concentrations at LODs 3 SD above the blank have a 99% probability of being non-zero. To increase this probability, the quantification limits were set at higher levels than the LODs. In this case, the lowest standard concentration was used: 10 pg/g serum for the PCB congeners, HCH isomers, and chlordanes; 20 pg/g for *p,p'*-DDD, *p,p'*-DDT, and *o,p'*-DDT; 50 pg/g for HCB; and 200 pg/g for *p,p'*-DDE. The reproducibility of the method was demonstrated by 21 replicate determinations using an in-house control serum sample included among the analytical batches during the course of the study. The mean concentrations of the PCB congeners ranged from <10 pg/g serum (CB 52 and 101) to 1310 pg/g serum (CB 153). The mean chlorinated pesticide concentrations ranged from <10–20 pg/g serum (α -HCH, γ -HCH, *p,p'*-DDD and *o,p'*-DDD) to 5260 pg/g serum (*p,p'*-DDE). The coefficients of variation were <13% for most of the compounds except the PCB congeners CB 28 (22%) and CB 105 (20%). The coefficient of variation for fat content was 4%.

The possibility of elimination of some of the compounds during the evaporation step was studied in a standard addition experiment. The recovery qualification criteria were set at 70–120%, depending on the substances. The average recoveries of the different PCB congeners added to serum samples were $98 \pm 12\%$ and $94 \pm 8\%$ for 0.1 and 0.8 ppb, respectively. The recoveries for the chlorinated pesticides varied from 78 to 118%. This shows that the loss of compounds during the analytical process was negligible. The results reported were not corrected for recovery.

The study analysts were blinded to the case-control status of the samples. Because concentrations of compounds are dependent on the amount of lipid in serum at the time of sampling, we expressed results in ng/g lipid in the serum, without further adjustment for lipid contents in the statistical analyses (18). When concentrations were below the quantification limit, they were set to 50% of that limit in all statistical analysis.

Grouping of Organochlorines. In addition to analyses of individual compounds, we considered groups of substances according to their possible hormonal activity. The grouping was based on a literature review by an outside expert (K. Moysich), blinded to any study findings. In grouping compounds, we added molar concentrations to compensate for differences in molecular weight (unit of measurement, nmol/g lipid).

Thus, we grouped the compounds as follows:

(a) estrogenic: *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT, β -HCH, γ -HCH, *trans*-nonachlor, oxychlordane, and CBs 28, 52, 101, and 153 (19–31);

(b) antiestrogenic: CBs 105, 118, 156, and 167 (30); and

(c) no known estrogenic effect: α -HCH, HCB, *p,p'*-DDE, CBs 138 and 180 (19, 30).

The hormonal activity of PCB congeners CB 101, 138, 153, and 180 has barely been studied. However, CB 101 and 153 seem to be weakly estrogenic (26, 28–31).

Because *p,p'*-DDE has been reported to have antiandrogenic effects (32), we also analyzed the group of compounds

Table 1 Distribution of established or suspected risk factors for endometrial cancer among 154 patients with endometrial cancer and 205 population controls

A.	Characteristic	No. of Cases/ controls ^a	Cases, mean (SD)	Controls, mean (SD)	P (t test)
	Age (yr)	154/205	64.6 (7.2)	62.8 (7.4)	0.02
	Age at menarche (yr)	154/205	13.3 (1.3)	13.6 (1.3)	0.03
	Age at menopause (yr)	134/181	51.4 (3.8)	50.0 (4.0)	0.002
	Parity among parous (132 cases/179 controls)	154/205	2.0 (1.3)	2.1 (1.3)	0.28
	Age at first birth (yr)	132/179	24.5 (4.2)	24.4 (4.4)	0.85
	Age at last birth (yr)	132/179	29.8 (5.6)	30.3 (5.3)	0.38
	Breast-feeding ^b (months)	132/179	8.1 (7.9)	10.5 (10.3)	0.03
	Height (cm)	154/205	163.9 (5.4)	164.0 (5.0)	0.89
	BMI (kg/m ²)	154/205	28.4 (5.1)	26.0 (4.2)	0.0001
	Fish consumption (portions per month)				
	All fish	142/205	5.0 (2.6)	5.2 (3.1)	0.55
	Fatty fish ^c	142/204	3.2 (1.7)	3.1 (1.1)	0.32
B.			Proportions		P (χ^2)
	Premenopausal	151/205	8.0	9.8	0.55
	Nulliparous	154/205	14.3	12.7	0.66
	Ever used oral contraceptives	154/205	16.9	27.8	0.02
	Ever use of topic estriol, dienestrol, estradiol	154/205	17.5	3.4	0.001
	First-degree relative with endometrial cancer	154/205	4.5	6.3	0.46
	Ever smoked regularly	154/205	26.0	33.2	0.14
	Diabetes mellitus	154/205	13.0	3.4	<0.001
	Hypertension	154/205	34.4	19.0	0.001

^a Number of cases and controls providing information.

^b Mean duration of breast-feeding (months) for all women, including nulliparous: cases, 6.9 (SD, 7.9); controls 9.2 (10.3).

^c Herring, Baltic herring, mackerel.

with no known hormonal effect excluding this substance. Finally, we also considered all PCB congeners (total PCB).

Statistical Analysis. Because many variables were strongly skewed, we used the nonparametric two-sample Wilcoxon test for unpaired data to conduct unadjusted comparisons of serum organochlorine concentrations in case and control women. Background variables were compared by *t* tests or χ^2 tests for homogeneity. In the main analyses ORs and 95% CIs were calculated using unconditional logistic regression models, fit by maximum likelihood (33). We considered the possible confounding effects of the following variables, which are known or hypothesized risk factors for endometrial cancer: age; menopausal status; ages at menarche, menopause, and first and last births; parity; breast-feeding; height; BMI [*i.e.*, ratio of weight to height squared (kg/m²)], use of oral contraceptives or topic estriol, dienestrol, or estradiol; family history of endometrial cancer; smoking; and clinical history of diabetes mellitus and hypertension. Only control for age and BMI affected risk estimates meaningfully and so were included in the final models. Tests for trend over categories were performed by the introduction of "semi-continuous" variables obtained by assigning consecutive integers to levels of categorized variables.

The organochlorine pesticides and PCB congeners were analyzed both in untransformed form (presented in "Results") and in logarithmically transformed form, with similar results. For most compounds, subjects were also grouped into quartiles according to the distribution among controls. For CBs 28, 52, and 101, a substantial number of subjects (32% of CB 28, 63% of CB 52, and 74% of CB 101) had values below the quantification limit. Therefore, we categorized these variables into three groups: women with values below the quantification limit as a referent, and those above quantification limits divided into two equal-sized groups among controls. More than 90% of women had values below the quantification limit for α -HCH, γ -HCH, *p,p'*-DDD, and *o,p'*-DDT; therefore, we subdivided these as below (referent) and above the quantification limit.

Because all subjects had *o,p'*-DDE concentrations below the quantification limit of 4 ng/g lipid in serum, we could not include this variable in any analysis. We also considered variables in continuous form in the analysis of substances grouped according to possible hormonal effects.

Results

Case-control differences largely reflected known epidemiological associations. There was no difference between cases and controls regarding fish consumption patterns, but controls reported more breast-feeding (a major excretory route for organochlorine compounds) than cases (Table 1).

We compared lipid-adjusted concentrations of pesticides and PCBs between fasting control subjects (*n* = 190) and nonfasting control subjects (*n* = 15), and between users (27 case patients and 7 control subjects) and nonusers (127 case patients and 198 control subjects) of topical estriol, dienestrol, or estradiol. We did so because we were concerned about potential differences in serum levels of organochlorine substances that could bias our results if these groups differed substantially regarding serum organochlorine levels. In both comparisons, no meaningful differences were observed, and we will present all results including these women in the analyses. The covariates remaining in the multivariate models, *i.e.*, age and BMI, were positively correlated with concentrations of several compounds [*e.g.*, for BMI and *p,p'*-DDT, the Spearman correlation coefficient (*r*²) was 0.39; for BMI and *p,p'*-DDE, *r*² = 0.24]. However, none of these correlation coefficients was >0.50 (for neither age nor BMI and any of the organochlorine compounds concentrations analyzed).

Among DDT compounds, *p,p'*-DDE (the principal metabolite of *p,p'*-DDT), had the highest mean concentrations (600–700 ng/g lipid). The average concentrations of the other pesticides were usually lower by a factor 10 or more. For all

Table 2 Serum levels of organochlorine compounds among 154 case patients with endometrial cancer and 205 control women^a

	Range		Median		Mean (SD)		<i>P</i> ^b
	Cases	Controls	Cases	Controls	Cases	Controls	
Pesticides (ng/g lipid)							
DDT							
<i>p,p'</i> -DDT	2–116	2–96.4	17.8	13.9	23.3 (19.6)	18.6 (16.7)	0.01
<i>o,p'</i> -DDT ^c	2–19.7	2–22.7	2	2	2.4 (1.9)	2.4 (2.1)	0.37
<i>p,p'</i> -DDE	15–2881	31.7–2542	582.5	497	702.8 (499.8)	623.6 (479.0)	0.04
<i>p,p'</i> -DDD ^c	2–30.2	2–25.7	2	2	2.7 (2.8)	2.7 (2.3)	0.84
HCB	16.5–175	14.6–351	66.8	64.9	70.3 (25.2)	66.2 (30.1)	0.08
HCH							
α -HCH ^c	1–8.0	1–7.4	1	1	1.1 (0.7)	1.1 (0.7)	0.81
β -HCH	13.2–781	7.4–744	57.8	51.1	71.8 (69.5)	62.7 (60.7)	0.02
γ -HCH ^c	1–10.7	1–13.4	1	1	1.4 (1.2)	1.4 (1.5)	0.17
Oxychlorthane	3.5–53.6	3.0–48.1	14.4	12.8	16.6 (9.0)	14.3 (7.6)	0.01
<i>trans</i> -Nonachlor	4.3–89.1	5.6–70	25.0	22.5	27.4 (13.4)	25.1 (12.2)	0.06
PCBs (ng/g lipid)							
CB 28 ^c	1–174	1–352	3.8	3.0	6.3 (16.0)	5.5 (24.8)	0.02
CB 52 ^c	1–11.2	1–12.3	1	1	2.2 (1.9)	2.0 (1.7)	0.45
CB 101 ^c	1–20.1	1–15.5	1	1	2.0 (2.9)	2.1 (2.3)	0.10
CB 105	1–28.9	1–21.1	6.1	5.6	6.8 (4.7)	6.2 (4.4)	0.14
CB 118	5.3–185	4.8–178	50.2	43.0	52.8 (25.3)	46.5 (24.2)	0.01
CB 138	4.5–259	17.5–264	107.5	101	112.5 (43.2)	108.5 (45.7)	0.30
CB 153	19.7–526	60.4–607	226.5	223	237.1 (85.3)	236.2 (91.9)	0.74
CB 156	7.4–54.1	6.3–58.2	17.4	18.1	19.2 (7.0)	19.6 (7.7)	0.75
CB 167	1–30.5	1–28.4	9.3	8.7	10.5 (4.9)	9.6 (4.6)	0.06
CB 180	40.2–497	55.6–397	147	152	162.2 (61.1)	163.4 (60.0)	0.80

^a Including women with levels below the quantification limit (half of the quantification limit was taken as an estimated value).

^b Wilcoxon test.

^c Variables with many values below quantification limit: *o,p'*-DDT (*n* = 336); *p,p'*-DDD (*n* = 312); α -HCH (*n* = 345); γ -HCH (*n* = 304); CB 28 (*n* = 114); CB 52 (*n* = 228); CB 101 (*n* = 262).

Table 3 ORs with 95% CIs of developing endometrial cancer according to quartiles^a of serum organochlorine pesticide levels

Compound	Model ^b	OR (95% CI)				<i>P</i> for trend ^c
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
<i>p,p'</i> -DDT	Age-adjusted	1.0	1.3 (0.7–2.5)	1.1 (0.6–2.2)	1.8 (1.0–3.4)	0.08
	Multivariate	1.0	1.1 (0.6–2.2)	0.8 (0.4–1.6)	1.1 (0.5–2.1)	0.95
<i>p,p'</i> -DDE	Age-adjusted	1.0	1.0 (0.6–2.0)	1.4 (0.7–2.5)	1.4 (0.8–2.6)	0.17
	Multivariate	1.0	0.9 (0.5–1.8)	1.1 (0.6–2.0)	1.0 (0.6–2.0)	0.78
HCB	Age-adjusted	1.0	1.3 (0.7–2.4)	1.1 (0.6–2.1)	1.1 (0.6–2.2)	0.95
	Multivariate	1.0	1.2 (0.6–2.2)	1.0 (0.5–1.9)	1.0 (0.5–1.9)	0.76
β -HCH	Age-adjusted	1.0	1.0 (0.5–2.0)	1.4 (0.7–2.7)	1.3 (0.7–2.6)	0.29
	Multivariate	1.0	0.8 (0.4–1.5)	1.0 (0.5–2.0)	0.9 (0.5–1.9)	0.87
Oxychlorthane	Age-adjusted	1.0	1.3 (0.7–2.6)	1.2 (0.6–2.4)	1.8 (0.9–3.4)	0.12
	Multivariate	1.0	1.1 (0.6–2.2)	1.0 (0.5–2.0)	1.4 (0.7–2.8)	0.33
<i>trans</i> -Nonachlor	Age-adjusted	1.0	1.4 (0.7–2.7)	1.6 (0.8–3.1)	1.4 (0.7–2.8)	0.31
	Multivariate	1.0	1.2 (0.6–2.3)	1.3 (0.7–2.7)	1.2 (0.6–2.5)	0.56

^a Median values for quartile 1 (cases/controls); quartile 4 (cases/controls), in ng/g lipid: *p,p'*-DDT, 6.3/4.8; 37.3/34; *p,p'*-DDE, 209.5/181; 1072/1165; HCB, 40.8/40.2; 109.5/94.2; β -HCH, 26/21.9; 100/98.4; oxychlorthane, 6.7/6.9; 24/22.9; *trans*-nonachlor, 12.4/13; 40.4/39.1.

^b Multivariate adjusted for age and BMI.

^c Tests for trend were performed by assigning consecutive integers to levels of categorized variables.

compounds, the range of exposure was substantial both among case patients and among control women (Table 2).

In unadjusted analyses, median concentrations of *p,p'*-DDT, *p,p'*-DDE, β -HCH, and oxychlorthane were higher among case patients than among controls (Table 2). However, after adjustment for age and BMI in logistic regression, ORs were close to unity and there was no evidence of any trends in risk over quartiles of exposure (Table 3). Similarly, there were no substantial differences in risk between women with values above and below the quantification limit for *o,p'*-DDT (OR adjusted for age and BMI, 1.4; 95% CI, 0.6–3.5), *p,p'*-DDD (OR, 0.9; 95% CI, 0.5–1.7), α -HCH (OR, 1.2; 95% CI, 0.4–

3.7), or γ -HCH (OR 1.5; 95% CI, 0.8–2.8). In the analyses of organochlorines as continuous variables, we found no associations between risk for endometrial cancer and any of the 10 pesticides evaluated (data not shown).

Among the 10 PCB congeners, CB 153 had the highest concentrations (mean ~236 ng/g lipid), whereas CB 28, CB 52, and CB 101 often had concentrations below the quantification limit (2 ng/g lipid). As for the pesticides, we found a wide range of serum concentrations of PCBs among both cases and controls (Table 2).

Unadjusted mean concentrations of CB 28 and CB 118 were higher among cases than among controls (Table 2). How-

Table 4 ORs with 95% CIs of developing endometrial cancer according to quartiles of serum levels of specific PCB congeners^a

Congener	Model ^b	OR (95% CI)				P for trend ^c
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
CB 105	Age-adjusted	1.0	1.4 (0.7–2.6)	1.2 (0.6–2.4)	1.2 (0.6–2.3)	0.69
	Multivariate	1.0	1.2 (0.6–2.3)	1.0 (0.5–1.8)	0.8 (0.4–1.6)	0.42
CB 118	Age-adjusted	1.0	1.7 (0.9–3.3)	1.6 (0.8–3.1)	1.9 (1.0–3.7)	0.11
	Multivariate	1.0	0.6 (0.8–3.0)	1.2 (0.6–2.4)	1.4 (0.7–2.8)	0.58
CB 138	Age-adjusted	1.0	0.9 (0.5–1.8)	1.2 (0.7–2.2)	1.0 (0.6–1.9)	0.74
	Multivariate	1.0	0.8 (0.4–1.6)	1.2 (0.6–2.2)	0.9 (0.5–1.7)	0.95
CB 153	Age-adjusted	1.0	0.9 (0.5–1.7)	1.1 (0.6–2.0)	0.8 (0.4–1.4)	0.58
	Multivariate	1.0	0.9 (0.5–1.7)	1.2 (0.6–2.2)	0.9 (0.5–1.7)	0.94
CB 156	Age-adjusted	1.0	1.4 (0.8–2.5)	1.1 (0.6–2.0)	0.7 (0.4–1.3)	0.18
	Multivariate	1.0	1.6 (0.8–2.9)	1.4 (0.7–2.6)	1.0 (0.5–2.0)	0.90
CB 167	Age-adjusted	1.0	2.2 (1.1–4.2)	1.6 (0.8–3.2)	2.0 (1.0–4.1)	0.19
	Multivariate	1.0	2.0 (1.0–3.9)	1.4 (0.7–2.9)	1.9 (0.9–3.9)	0.24
CB 180	Age-adjusted	1.0	0.9 (0.5–1.6)	0.8 (0.5–1.5)	0.7 (0.4–1.4)	0.32
	Multivariate	1.0	1.1 (0.6–2.0)	1.1 (0.6–2.2)	1.2 (0.6–2.2)	0.67

^a Median values for quartile 1 (cases/controls); quartile 4 (cases/controls), in ng/g lipid: CB 105, 1/1; 11.5/10.7; CB 118, 25.2/20.3; 73.4/70.9; CB 138, 67/59.1; 154/165; CB 153, 157/143; 342.5/351; CB 156, 12.9/12; 28.3/27.5; CB 167, 5/4.6; 14.7/15; CB 180, 108.5/103; 224.5/227.

^b Multivariate adjusted for age and BMI.

^c Tests for trend were performed by assigning consecutive integers to levels of categorized variables.

Table 5 ORs with 95% CIs of developing endometrial cancer according to quartiles of levels of compound groups (nmol/g lipid)^a

Group of compounds	Model ^b	OR (95% CI)				P for trend ^c
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
All PCBs ^d	Age-adjusted	1.0	1.2 (0.6–2.3)	1.4 (0.7–2.6)	1.5 (0.8–2.8)	0.23
	Multivariate	1.0	1.1 (0.6–2.2)	1.1 (0.6–2.2)	1.2 (0.6–2.2)	0.72
Estrogenic compounds, not including CB 153 ^e	Age-adjusted	1.0	1.4 (0.7–2.7)	1.6 (0.8–3.2)	1.7 (0.8–3.4)	0.15
	Multivariate	1.0	1.2 (0.6–2.4)	1.2 (0.6–2.4)	1.2 (0.6–2.4)	0.76
Estrogenic compounds, including CB 153 ^f	Age-adjusted	1.0	1.3 (0.7–2.4)	1.0 (0.5–2.0)	1.2 (0.6–2.4)	0.74
	Multivariate	1.0	1.1 (0.6–2.1)	0.9 (0.4–1.7)	1.1 (0.6–2.2)	0.90
Anti-estrogenic compounds ^g	Age-adjusted	1.0	1.8 (0.9–3.4)	1.7 (0.9–3.4)	1.7 (0.8–3.3)	0.22
	Multivariate	1.0	1.7 (0.9–3.3)	1.4 (0.7–2.8)	1.5 (0.7–3.0)	0.48
Compounds with no known hormonal effect, including <i>p,p'</i> -DDE ^h	Age-adjusted	1.0	1.2 (0.6–2.3)	1.6 (0.9–3.0)	1.6 (0.9–3.1)	0.08
	Multivariate	1.0	1.2 (0.6–2.2)	1.4 (0.7–2.7)	1.3 (0.7–2.4)	0.39
Compounds with no known hormonal effect, excluding <i>p,p'</i> -DDE ⁱ	Age-adjusted	1.0	1.8 (1.0–3.4)	1.4 (0.7–2.7)	1.3 (0.6–2.5)	0.83
	Multivariate	1.0	1.9 (1.0–3.6)	1.6 (0.8–3.2)	1.5 (0.7–3.0)	0.47

^a Including women with levels below the quantification limit (half of the quantification limit was taken as an estimated value).

^b Multivariate adjusted for age and BMI.

^c Tests for trend were performed by assigning consecutive integers to levels of categorized variables.

^d CB 28, CB 52, CB 101, CB 105, CB 118, CB 138, CB 153, CB 156, CB 167, and CB 180.

^e *o,p'*-DDT; *p,p'*-DDT; *p,p'*-DDD; β -HCH; γ -HCH; *trans*-nonachlor; oxychlorodane; CB 28; CB 52; and CB 101.

^f *o,p'*-DDT; *p,p'*-DDT; *p,p'*-DDD; β -HCH; γ -HCH; *trans*-nonachlor; oxychlorodane; CB 28; CB 52; CB 101; and CB 153.

^g CB 105, CB 118, CB 156, and CB 167.

^h α -HCH, CB 138, CB 180, HCB, and *p,p'*-DDE.

ⁱ α -HCH, CB 138, CB 180, and HCB.

ever, after adjustment there was no substantial increase in risk associated with high concentrations of any of the congeners evaluated, and there were no significant trends in risk (Table 4). Likewise, no differences were seen for PCB congeners CB 28, CB 52, or CB 101, which we considered in two categories using undetectable levels as reference. Finally, in the analyses of the 10 different PCB measurements in continuous form, there was no significant association between any of the congeners and endometrial cancer risk (data not shown).

No significant associations or trends were observed when we compared quartiles of exposure for different groups of compounds (Table 5). The high concentration of CB 153 has a large influence on the results of the estrogenic group. Therefore, we also considered the estrogenic group after exclusion of CB153 (Table 5). In analyses stratified by BMI (above or below mean value among controls, *i.e.*, 25.39 kg/m²), breast-feeding history (ever *versus* never), parity (nulliparous *versus* parous),

use of topical estriol (ever *versus* never), menopausal status (pre- *versus* postmenopausal), and smoking (ever or never smoked regularly), there were no indications of associations or dose-response relationships with endometrial cancer, and risk estimates in all subgroups were nonsignificant (data not shown).

Discussion

In this case-control study, we found no association between endometrial cancer risk and serum concentrations of organochlorine pesticides, pesticide metabolites, or PCB congeners. The negative findings were found both in analysis of individual compounds and groups of substances with different putative hormonal activity, and the lack of association persisted in all subgroups analyzed. According to our *a priori* hypothesis, substances with an estrogen-like effect would be expected to

increase the risk of endometrial cancer in a fashion similar to unopposed estrogens used as hormone replacement. Similarly, those classified as antiestrogenic would be expected to lower risk.

Our study has several strengths, including its population-based design, relatively large sample size, restriction of the analysis to women who never used hormone replacement therapy (which could mask possible hormone-like effects of organochlorine compounds), and the availability of detailed questionnaire information, which allowed us to adjust for potential confounding effects. Furthermore we conducted analyses of 20 specific compounds rather than of *p,p'*-DDE and/or total PCB only as in most previous studies. Laboratory analyses were carried out under strict quality control and enabled us to study the cancer risk caused by exposure to single PCB congeners as well as to groups of PCB with different hormonal activities. To our knowledge, the only other study that has tried to classify compounds according to potential hormonal activity only did so for different PCB congeners (15). We also included several chlorinated pesticides in our grouping.

Samples from cases were collected immediately after diagnosis and before surgery or any other cancer therapy. Therefore, organochlorine concentrations could not have been influenced by the cancer treatment.

If organochlorine concentrations were affected by the disease itself (*e.g.*, because of weight loss, which is very rare in early disease phases), we probably would have observed higher organochlorine concentrations among cases, and thus would have a biased overestimation of the relative risks. However, no clear differences in organochlorine concentrations between case patients and control subjects were observed. Moreover, in Sweden most endometrial cancers are diagnosed in early stages (34) when no changes in weight due to disease are observed.

Selection bias would have occurred only if nonparticipation, which was substantial in our study, was related differently to organochlorine concentrations among eligible cases and controls. Among cases, the main reason for nonparticipation was the failure of the hospital staff to collect blood samples before surgery. Therefore, nonparticipation probably reflects mostly characteristics of the medical personnel, and not patients' characteristics. Differential participation according to organochlorine concentration among control subjects also seems unlikely because we did not find any association between organochlorine levels and characteristics possibly associated with nonparticipation, such as educational level, smoking habits, diet, and use of exogenous hormones (data not shown). We measured both original organochlorine products and major metabolites such as *p,p'*-DDE and oxychlordane. However, it is possible that we overlooked other meaningful exposures. Some nonpersistent estrogenic DDT and PCB compounds cannot be detected in humans decades after exposure (35), and we did not measure a variety of other persistent compounds such as hydroxylated PCBs, polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and non-*o*-PCBs. In addition, our grouping of substances is uncertain because there are relatively few animal or human data on hormonal activity of organochlorines.

The study laboratory participated in the fourth round of the "World Health Organization's interlaboratory quality assessment study on human milk and blood," which included analyses of PCBs in blood plasma. The results obtained were in good agreement with the consensus values, which ranged from 2 to 697 pg/g serum (17). The lower quantification limit in this analysis was due to the larger serum volume used in the WHO study.

It is difficult to compare the organochlorine concentrations

found in our study with results from previous cancer studies because of differences in analytical procedures. Our LODs are in the lower range of those reported in other recent cancer studies (8, 12, 15, 36). Our method permitted low LODs partly because we used dual capillary columns instead of single packed columns (4, 5, 8, 9). Dual capillary columns decrease the interference and increase the selectivity and sensitivity in the analysis. Moreover, earlier studies used lower serum volumes in the analysis (0.5–2 ml) than we did (~4 ml; Refs. 8, 12, 15, 36). The LODs decrease with increasing serum volumes. Some of the studies did not lipid-adjust their results (4, 5, 9), although the concentration of organochlorines in serum or plasma is dependent on the lipid content. Moreover, in some of the studies, single packed columns were used in the analysis (4, 5, 8, 9). We used dual capillary columns, which increases the sensitivity and selectivity and decreases the interference in the analysis. In older methods for PCB analysis, technical PCB mixtures were used as standards in the analysis (2–5, 9, 18). As in other more recent cancer studies (7, 12, 36), we performed congener-specific analysis of PCB, which makes comparison with the older studies difficult. Finally, different biological materials have been used (*e.g.*, adipose tissue, serum), which further complicates comparisons. However, after standardizing for lipid content in different tissues, the mean concentrations of organochlorines in our study were in the lower range of those reported previously for controls in North American and European breast cancer studies, where sampling occurred in the late 1980s and early 1990s (average concentrations, 1020–2200 ng/g lipid for *p,p'*-DDE and 350–1300 ng/g lipid for total PCBs; Refs. 3–6, 11, 12, 18). The somewhat lower average exposure in our study (sampling 1996–1997) at least partially reflects the continuous decline in exposure in Europe and North America after the banning of these compounds (18, 37–40). It could be that exposure in our population was too low to cause biological effects, although endometrial cancer is known to be the most estrogen-sensitive malignancy in women. In addition, all compounds analyzed in our study had a substantial range of variation, one to two orders of magnitude. However, no evidence of trend emerged in categorized or continuous analyses. We observed irregular patterns of increased risk for certain compounds (*e.g.*, for compounds with no known hormonal effect, excluding *p,p'*-DDE; Table 5).

It is reassuring that the previously published study on organochlorines and endometrial cancer (15) also did not reveal any associations, although median concentrations were higher than ours. That study (15) included 90 endometrial cancer cases and 90 controls, and the substances analyzed were *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, β -HCB, dieldrin, HCB, oxychlordane, *trans*-nonachlor, heptachlor epoxide, and 27 PCBs (grouped as "Total PCB," estrogenic PCBs, antiestrogenic PCBs, and enzyme-inducing PCBs).

We studied women with no substantial use of menopause hormones among whom the effects of weakly estrogenic substances should be most apparent. Because we found no associations, we conclude that the studied environmental contaminants do not cause endometrial cancer at the concentrations found in our population. These reassuring results are likely generalizable to other populations where similar levels of these contaminants are present in the environment.

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References

- Safe, S. H. Xenoestrogens and breast cancer. *N. Engl. J. Med.*, 337: 1303–1304, 1997.
- Wassermann, M., Nogueira, D. P., Tomatis, L., Mirra, A. P., Shibata, H., Arie, G., Cucos, S., and Wassermann, D. Organochlorine compounds in neoplastic and adjacent apparently normal breast tissue. *Bull. Environ. Contam. Toxicol.*, 15: 478–484, 1976.
- Mussalo-Rauhamaa, H., Hasanen, E., Pyysalo, H., Antervo, K., Kauppila, R., and Pantzar, P. Occurrence of β -hexachlorocyclohexane in breast cancer patients. *Cancer (Phila.)*, 66: 2124–2128, 1990.
- Falck, F., Jr., Ricci, A., Jr., Wolff, M. S., Godbold, J., and Deckers, P. Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch. Environ. Health*, 47: 143–146, 1992.
- Wolff, M. S., Toniolo, P. G., Lee, E. W., Rivera, M., and Dubin, N. Blood levels of organochlorine residues and risk of breast cancer. *J. Natl. Cancer Inst.*, 85: 648–652, 1993.
- Dewailly, E., Dodin, S., Verreault, R., Ayotte, P., Sauve, L., Morin, J., and Brisson, J. High organochlorine body burden in women with estrogen receptor positive breast cancer. *J. Natl. Cancer Inst.*, 86: 232–234, 1994.
- Guttes, S., Failing, K., Neumann, K., Kleinstein, J., Georgii, S., and Brun, H. Chlororganic pesticides and polychlorinated biphenyls in breast tissue of women with benign and malignant breast disease. *Arch. Environ. Contam. Toxicol.*, 35: 140–147, 1998.
- Hunter, D. J., Hankinson, S. E., Laden, F., Colditz, G. A., Manson, J. E., Willet, W. C., Speizer, F. E., and Wolff, M. S. Plasma organochlorine levels and the risk of breast cancer. *N. Engl. J. Med.*, 337: 1253–1258, 1997.
- Krieger, N., Wolff, M. S., Hiatt, R. A., Rivera, M., Vogelman, J., and Orentreich, N. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. *J. Natl. Cancer Inst.*, 86: 589–599, 1994.
- Lopez-Carrillo, L., Blair, A., Lopez-Cervantes, M., Cebrian, M., Rueda, C., Reyes, R., Mohar, A., and Bravo, J. Dichlorodiphenyltrichloroethane serum levels and breast cancer risk: a case-control study from Mexico. *Cancer Res.*, 57: 3728–3732, 1997.
- van't Veer, P., Lobbezoo, I. E., Martin-Moreno, J. M., Guallar, E., Gomez-Aracena, J., Kardinaal, A. F., Kohlmeier, L., Martin, B. C., Strain, J. J., Thamm, M., van Zoonen, P., Baumann, B. A., Huttunen, J. K., and Kok F. J. DDT (dicophane), and postmenopausal breast cancer in Europe: case-control study. *Br. Med. J.*, 315: 81–85, 1997.
- Moysich, K. B., Ambrosone, C. B., Vena, J. E., Shields, P. G., Mendola, P., Kostyniak, P., Greizerstein, H., Graham, S., Marshall, J. R., Schisterman, E. F., and Freudenheim, J. L. Environmental organochlorine exposure and postmenopausal breast cancer risk. *Cancer Epidemiol. Biomark. Prev.*, 7: 181–188, 1998.
- Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. *Lancet*, 350: 1047–1059, 1997.
- Grady, D., Gebretsadik, T., Kerlikowske, K., Ernster, V., and Petitti, D. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. *Obstet. Gynecol.*, 85: 304–313, 1995.
- Sturgeon, S. R., Brock, J. W., Potischman, N., Needham, L. L., Rothman, N., Brinton, L. A., and Hoover, R. N. Serum concentrations of organochlorine compounds and endometrial cancer risk (United States). *Cancer Causes Control*, 9: 417–424, 1998.
- The National Board of Health and Welfare. Centre for Epidemiology. Statistics–Health and Diseases. Cancer Incidence in Sweden 1995, 107 pp. Stockholm, Sweden: National Board of Health and Welfare, 1998.
- Atuma, S. S., and Aune, M. Method for the determination of PCB congeners and chlorinated pesticides in human serum. *Bull. Environ. Contam. Toxicol.*, 62: 8–15, 1999.
- Hunter, D. J., Laden, F., and Wolff, M. S. Reply in correspondence. *N. Engl. J. Med.*, 338: 991, 1998.
- Ahlborg, U. G., Lipworth, L., Titus-Ernstoff, L., Hsieh, C.-C., Hanberg, A., Baron, J., Trichopoulos, D., and Adami, H.-O. Organochlorine compounds in relation to breast cancer, endometrial cancer and endometriosis: an assessment of the biological and epidemiological evidence. *Crit. Rev. Toxicol.*, 25: 463–531, 1995.
- Shekar, P. V. M., Werdell, J., and Basrur, V. S. Environmental estrogen stimulation of growth and estrogen receptor function in preneoplastic and cancerous human breast cell lines. *J. Natl. Cancer Inst.*, 89: 1774–1782, 1997.
- Loeber, J. G., and van Helsen, F. L. Uterotrophic effects of the β -HCH, a food-chain contaminant. *Food Addit. Contam.*, 1: 63–66, 1984.
- Biggsby, R. M., Caperell-Grant, A., and Madhukar, B. V. Xenobiotics released from fat during fasting produce estrogenic effects in ovariectomized mice. *Cancer Res.*, 57: 865–869, 1997.
- Cooper, R. L., Chadwick, R. W., Rehnberg, G. L., Goldman, J. M., Booth, K. C., Hein, J. F., and McElroy, W. K. Effect of lindane on hormonal control of reproductive function in the female rat. *Toxicol. Appl. Pharmacol.*, 99: 384–394, 1989.
- Wedig, H., and Vernon, L. G. Can increased hepatic estrogen metabolism interfere with ovulation in the rat? Effects of chronic phenobarbital or chlordane exposure. *Proc. Soc. Exp. Biol. Med.*, 144: 796–801, 1973.
- Cassidy, R. A., Vorhees, C. V., Minnema, D. J., and Hastings, L. The effects of chlordane exposure during pre- and postnatal periods at environmentally relevant levels on sex steroid-mediated behaviors and functions in the rat. *Toxicol. Appl. Pharmacol.*, 126: 326–337, 1994.
- Ecobichon, D. J., and MacKenzie, D. O. The uterotrophic activity of commercial and isomerically-pure chlorobiphenyls in the rat. *Res. Commun. Chem. Pathol. Pharmacol.*, 9: 85–95, 1974.
- Jansen, H. T., Cooke, P. S., Porcelli, J., Liu, T. C., and Hansen, L. G. Estrogenic and antiestrogenic actions of PCBs in the female rat: *in vitro* and *in vivo* studies. *Reprod. Toxicol.*, 7: 237–248, 1993.
- Soontornchat, S., Li, M. H., Cooke, P. S., and Hansen, L. G. Toxicokinetic and toxicodynamic influences on endocrine disruption by polychlorinated biphenyls. *Environ. Health Perspect.*, 102: 568–571, 1994.
- Waller, C. L., Minor, D. L., and McKinney, J. D. Using three-dimensional quantitative structure-activity relationships to examine estrogen receptor binding affinities of polychlorinated biphenyls. *Environ. Health Perspect.*, 103: 702–707, 1995.
- Wolff, M. S., Camann, D., Gammon, M., and Stellman, S. D. Proposed PCB congener grouping for epidemiological studies. *Environ. Health Perspect.*, 105: 13–14, 1997.
- Patnode, K. A., and Curtis, L. R. 2,2',4,4',5,5'- and 3,3',4,4',5,5'-Hexachlorobiphenyl alteration of uterine progesterone and estrogen receptors coincides with embryotoxicity in mink (*Mustela vison*). *Toxicol. Appl. Pharmacol.*, 127: 9–18, 1994.
- Kelce, W. R., Stone, C. R., Laws, S. C., Gray, L. E., Kempainen, J. A., and Wilson, E. M. Persistent DDT metabolite *p,p'*-DDE is a potent androgen receptor antagonist. *Nature (Lond.)*, 375: 581–585, 1995.
- Breslow, N. E., and Day, N. E. Statistical Methods in Cancer Research. I. The Analysis of Case-Control Studies. IARC Publ. No. 32, pp. 5–338. Lyon, France: IARC, 1980.
- Stenbeck, M., Rosen, M., and Holm, L. E. Cancer survival in Sweden during three decades, 1961–1991. *Acta Oncol.*, 34: 881–891, 1995.
- vom Saal, F. S., Welshons, W. V., and Hansen, L. G. Organochlorine residues and breast cancer. *N. Engl. J. Med.*, 337: 988, 1998.
- Hoyer, A. P., Grandjean, P., Jorgensen, T., Brock, J. W., and Hartvig, H. B. Organochlorine exposure and risk of breast cancer. *Lancet*, 352: 1816–1820, 1998.
- Lundén, Å., and Norén, K. Polychlorinated naphthalenes and other organochlorine contaminants in Swedish human milk, 1972–1992. *Arch. Environ. Contam. Toxicol.*, 34: 414–423, 1998.
- Atuma, S., Hansson, L., Johnsson, H., Slorach, S., de Wit, C. A., and Lindström, G. Organochlorine pesticides, polychlorinated biphenyls and dioxins in human milk from Swedish mothers. *Food Addit. Contam.*, 15: 142–150, 1998.
- Hovinga, M. E., Sowers, M. F., and Humphrey, H. E. Historical changes in serum PCB and DDT levels in an environmentally-exposed cohort. *Arch. Environ. Contam. Toxicol.*, 22: 362–366, 1992.
- Ekbom, A., Wicklund-Glynn, A., and Adami, H.-O. DDT and testicular cancer. *Lancet*, 347: 553–554, 1996.

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