

Adipose Organochlorine Concentrations and Risk of Breast Cancer Among Postmenopausal Danish Women

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Abstract

Objective: Exposure to environmental organochlorines has been examined as a potential risk factor for human breast cancer with mixed results. Our purpose was to examine associations between organochlorines and the development of breast cancer in a large prospective study using stored adipose tissue.

Methods: We conducted a nested case-control study of 409 postmenopausal women who developed breast cancer and 409 controls selected from the 29,875 women enrolled in the Danish Diet, Cancer, and Health cohort between 1993 and 1997. We measured concentrations of 14 pesticides and 18 polychlorinated biphenyls in adipose tissue, collected upon enrollment, and estimated relative risk (RR) of breast cancer using conditional logistic regression.

Results: The results showed no higher risk of breast cancer among women with higher levels of any pesticides or polychlorinated biphenyls; the RR associated with the

upper quartile of 1,1-dichloro-2, 2-bis(*p*-chlorophenyl)ethylene concentration was 0.7 [95% confidence interval (95% CI), 0.5-1.2] contrasting the lower quartile, and for the sum of polychlorinated biphenyls the similar risk was 1.1 (95% CI, 0.7-1.7). We observed a pattern of substantially lower risk of estrogen receptor-negative breast cancer in association with higher levels of most of the pesticides and polychlorinated biphenyls; the RR for the higher quartile of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene was 0.1 (95% CI, 0.0-0.5) and for the sum of polychlorinated biphenyls it was 0.3 (95% CI, 0.1-0.9).

Conclusion: The results do not support that higher organochlorine body levels increase the risk of breast cancer in postmenopausal women. The interpretation of the inverse association for estrogen receptor-negative breast cancer is currently unclear. (Cancer Epidemiol Biomarkers Prev 2005;14(1):67-74)

Introduction

Breast cancer is the most frequent malignant disease among women in Denmark and other parts of the western world. In Denmark, the incidence rate has doubled since the 1950s (1) and about 1 in 10 women develops breast cancer in their lifetime. Estrogens influence the development of breast cancer and environmental factors play an important role (2). However, currently established breast cancer risk factors explain only a small fraction of the breast cancer cases diagnosed (1, 3).

Organochlorines present in the environment include pesticides, herbicides, insecticides, polychlorinated biphenyl (PCB), and dioxins. Most organochlorines are strongly lipophilic, resistant to biotransformation, and bioaccumulate in the food chain. Food is the major source of intake in humans. Some of the more persistent organochlorines have half-lives in human tissue of up to several decades (4-6). The accumulation in human adipose tissue and the excretion via lactation emphasizes the presence of the chemicals in the breast tissue. The IARC has classified dichlorodiphenyltrichloroethane (DDT) and other chlorinated pesticides as carcinogenic in animals (7). PCBs are multitoxic and cause tumors in animals (8, 9). The hypothesis that organochlorine compounds increase the risk of breast cancer in humans is based on the carcinogenicity and weak hormone-like effects of many organochlorines including 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE; ref. 10), dieldrin, and endosulfan (11), as well as many PCBs and in particularly their hydroxylated metabolites (8, 12, 13). Moreover, there is concordance between temporal patterns of large-scale release of these chemicals into the environment and the increase in incidence rates of breast cancer in many parts of the world.

Case-control studies of organochlorine exposure and risk of breast cancer have provided mixed results (14-25). Prospectively designed studies measured concentrations of organochlorines in blood or tissue sampled years before the time of the cancer diagnosis. Of the nine prospective studies published to date, five show no association between breast cancer and DDT/DDE and PCBs (4, 26-29), whereas four show increased risk with DDE and PCBs (30), DDE in African American women (31), dieldrin (32), and DDT and some PCB congeners (33). Six of the nine studies included fewer than 200 cases and none had access to stored adipose tissue. Although some authors find the collective of the epidemiologic evidence to be reassuring regarding no association between organochlorines and breast cancer (29, 34, 35) others claim growing evidence for higher concentrations of these persistent chemicals in breast cancer patients (19).

This is the largest prospective study to date to examine the association between PCBs and organic chlorinated pesticides and breast cancer in postmenopausal women, and the first prospective study to use stored adipose tissue in the exposure assessment.

Materials and Methods

Between December 1993 and May 1997, 79,729 women ages 50 to 64 years were invited to participate in a prospective study "Diet, Cancer and Health". To participate, women had to be born in Denmark, live in the Copenhagen or Aarhus areas, and

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be without a cancer diagnosis registered in the Danish Cancer Registry at the time of invitation. A total of 29,875 women, corresponding to about 37% of the women invited, were enrolled in the cohort, representing 7% of the entire Danish population in this age group. Upon enrollment, each participant completed self-administered questionnaires that included questions on dietary habits, health status, family history of cancer, social factors, reproductive factors, and lifestyle habits. Participation was based on written informed consent. Staff members in the study clinics obtained anthropometric measurements, including height and weight, and took an adipose tissue biopsy from the buttock of each participant using a luer-lock system (Terumo, Terumo Co., Tokyo, Japan) consisting of a needle, a venoject multisampler luer adaptor, and an evacuated blood tube, according to the method of Beynen and Katan (36), yielding an average of 40 mg (range, 1-108 mg) tissue. Within 2 hours of collection, all samples were frozen at -20°C and within 8 hours put in liquid nitrogen vapor (max, -150°C) for long-term storage.

Of the initial 29,875 women, 326 women were diagnosed with cancer before their visit to one of the study clinics and were excluded from the study. In addition, eight women were excluded because they did not complete the lifestyle questionnaire, nine women because they reported a lifetime history of no menstruation, and 37 women due to missing information on use of hormone replacement therapy. As we aimed at evaluating postmenopausal women, we further excluded 4,798 women who reported at least one menstruation, within the 12 months before entry to the cohort and no use of hormone replacement therapy. The remaining 24,697 women were classified as either "known postmenopausal" ($n = 17,351$) or "probably postmenopausal" ($n = 7,346$). The criteria for being assigned to the "known postmenopausal" group were surgically removed ovaries, reported age of menopause or no natural menstruation during the last 12 months.

Using a unique identification number allocated to every Danish citizen, we linked all 24,697 postmenopausal cohort members to The Central Population Registry to obtain information on vital status and immigration. Follow-up for breast cancer started on date of visit to one of the study clinics (baseline) and continued until date of diagnosis of any cancer (except nonmelanoma skin cancer), date of death, date of emigration, or December 31, 2000, whichever came first. The median follow-up time was 4.8 years. Cancers diagnosed during follow-up were identified by linkage to the population-based Danish Cancer Registry using the personal identification number (37). Six breast cancer cases were not included because of the diagnosis of another previous cancer. During the follow-up period, 434 postmenopausal women were diagnosed with incident breast cancer. Information on estrogen receptor status, tumor size and lymph node involvement of the breast cancers was obtained by linkage to the files of The Danish Breast Cancer Cooperative Group (38). Information on estrogen receptor status is based on a standardized immunohistologic method and the criterion used to determine positive receptor status was $\geq 10\%$ positive cells.

We applied an individually matched case-control design nested within the cohort. For each of the cases, a control was randomly selected among those in the entire cohort who were cancer-free at the exact age at diagnosis of the case, stratified on certainty of postmenopausal status (known/probably postmenopausal), use of hormone replacement therapy at baseline (current/former/never), and age at baseline (half-year intervals). Of the 434 matched case-control pairs, 24 pairs were excluded because no adipose tissue was available for organochlorine analysis for either the case or for the matched control, and one pair was excluded due to errors in the laboratory analysis, leaving 409 cases and 409 matched controls included in the study.

Organochlorine Analyses. The organochlorines measured were 18 PCB congeners (International Union of Pure and Applied Chemistry nos. 28, 52, 54, 99, 101, 104, 105, 118, 128, 138, 153, 155, 156, 170, 180, 183, 187, and 201), *p,p'*-DDT, *p,p'*-DDE, β -hexachlorocyclohexane, α -chlordane, γ -chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor, aldrin, dieldrin, endrin, heptachlorepoxyde, hexachlorobenzene, and mirex. Samples were analyzed at Le Centre de Toxicologie, Institut national de sant e publique du Qu ebec. Laboratory personnel were blind to the case-control status. The laboratory is accredited under ISO 17025 by the Standards Council of Canada and participates in many national and international quality control programs including Environment Canada's Arctic Environment Strategy's QA/QC Program, the External Quality Assessment Scheme, QUASIMEME <http://www.quasimeme.marlab.ac.uk/>) as well as the German External Quality Assessment Scheme for Biological Monitoring in Occupational and Environmental Medicine.

PCBs and organochlorinated pesticides were extracted from adipose tissue using dichloromethane (39, 40). A fraction of this extract was used to determine the lipid content of the sample. The other fraction was used for determination of PCBs and pesticides: first it was treated by gel permeation chromatography to remove fatty residues and subsequently it was further cleaned on a Florisil column before high resolution gas chromatography-mass spectrometry analysis. Analyses were done on a gas chromatography-mass spectrometry instrument from Agilent Technologies (Hewlett-Packard; Palo Alto, CA) model 6890/5973 using a DB-XLB capillary column.

The total lipid content was determined on the designated extract using a gravimetric method (41). Two hundred microliters were precisely weighed on an analytic balance and the solvent evaporated at room temperature in a dessicator. The resulting lipid weight was adjusted to the initial sample weight and the percentage of lipid content was calculated. The organochlorine concentrations were expressed in microgram per kilogram of lipids.

For each of the analytes, the detection limit was determined by first estimating the concentration equivalent to a signal to noise ratio of 3. We then measured 10 replicates of a sample with the analytes at a concentration from 4 to 10 times the estimated detection limit. The calculated detection limit became the value equivalent to thrice the SD of those 10 replicates. The organochlorines could be divided in three groups according to detection limit. Group I included PCB congeners nos. 52, 54, 104, and 155; group II included PCB congeners nos. 28, 99, 101, and 105, *p,p'*-DDE, *p,p'*-DDT, β -hexachlorocyclohexane, oxychlordane, aldrin, dieldrin, heptachloroepoxyde, and mirex; and group III included all other compounds. For each sample, the detection limit was adjusted regarding the weight of the sample and the lipid content, providing different detection limits for the different samples. The median (and 5th, 95th percentiles) detection limits for all samples were 28.4 (14.9, 139.2) $\mu\text{g}/\text{kg}$ lipids for group I, 8.5 (4.5, 41.8) $\mu\text{g}/\text{kg}$ lipids for group II, and 2.8 (1.5, 13.9) $\mu\text{g}/\text{kg}$ lipids for group III.

Routine checks of the accuracy and precision of the organochlorine measurements were done using reference materials from the National Institute of Standards and Technology (Gaithersburg, MD) and by participation in an external proficiency testing program, showing the coefficient of variation to be 5% to 7% for the PCB congeners and 5% to 15% for the pesticides. Based on spiked levels (5 $\mu\text{g}/\text{kg}$ in corn oil, $n = 3$) recovery was between 72% and 96% for the different organochlorines.

Statistical Methods. In accordance with the sampling design, the breast cancer rate ratios were estimated using a conditional logistic regression analysis. Tests and two-sided 95% confidence interval (95% CI) were based on Wald's test on the log scale for the rate ratios. The analyses were done using the SAS statistical software (SAS Institute, Cary, NC).

We calculated sum of PCB concentrations including only PCB congeners with >50% of the samples above the detection limit. Measurements that were below the detection limit contributed zero to the PCB sum.

The women in our study were categorized to quartiles based on the distribution of organochlorines among controls with detectable levels, and risk was estimated contrasting each of the upper quartiles with the lowest quartile. Women with organochlorine levels below the detection limit were assigned to the lowest exposure category if the detection limit for the actual sample was below the 25th percentile among controls (i.e., the cutoff point between the two lower exposure categories) because only these of the samples below the detection limit belonged for certain to the lowest exposure category. Samples below the detection limit and with the detection limit above this cutoff point were excluded from the analysis to avoid misclassification between exposure categories. In the linear trend analyses, based on log-transformed concentrations and disregarding exposure groupings, all samples with values below the detection limit were excluded to avoid misclassification of the exposure estimate.

In addition to analyses of all breast cancers combined, data was analyzed stratified according to estrogen receptor status of the cancer to address the hypothesis that weak hormone-like properties of several of the organochlorines are involved in the breast cancer etiology.

Specific PCB congeners have shown different types of biological effects; grouping of PCBs according to these effects has been suggested (42). We present our results with PCB congeners ordered in suggested groupings to facilitate comparisons within and across the groups.

All risk estimates were adjusted for the effect of potential confounders: previous benign breast tumor (yes/no), level of education (<8, 8-10, >10 years), body mass index (kg/m², linear), alcohol consumption (g/d, linear), nulliparous (yes/no), number of deliveries (linear), age at first delivery (years, linear), duration of hormone replacement therapy use (years, linear), and lifetime duration of lactation (months, linear).

We repeated the risk analyses following a case-cohort approach where the individual matched pairs were broken, and all controls within a match stratum were considered as

reference for cases within the same stratum (43). This approach is expected to increase the statistical power.

Results

Table 1 shows baseline characteristics of cases and controls. On the average, cases had higher education, consumed more alcohol, had a higher proportion of nulliparous women, a lower number of deliveries, higher age at first delivery, a shorter lifetime duration of lactation, and a higher proportion of previous benign breast tumor.

Organochlorine concentrations were quantified in almost all adipose samples for *p,p'*-DDE, β -hexachlorocyclohexane, *trans*-nonachlor, hexachlorobenzene, and PCB congener nos. 118, 138, 153, 156, 170, 180, 183, 187, and 201 (Table 2). Pesticides α -chlordane and γ -chlordane, aldrin, endrin and mirex, and PCB congeners 52, 54, 101, 104, and 155 had <2% of measurements above the detection limit. Only organochlorine compounds with >50% of samples above the detection limit were considered in the further analyses. A tendency of slightly higher mean and median levels among controls than cases was evident for most pesticides and to a lesser extent for PCB congeners. Among the pesticides, the differences were 10% to 12% or less except for mirex with only few measurements above the detection limit. For the PCB congeners, the differences between cases and controls exceeded 5% only for congeners, with few samples above the detection limit. The proportion of samples above the detection limit was about 5% higher among controls for most pesticides, but for PCB congeners this proportion was almost identical for cases and controls (Table 2). Women with estrogen receptor-positive breast cancer had higher (mostly about 5-10%) mean adipose tissue concentrations than women with estrogen receptor-negative breast cancer for all organochlorine compounds except for *cis*-nonachlor and dieldrin (data not shown).

Table 3 shows a pattern of lower risk for all breast cancers in association with higher concentrations for all chlorinated pesticides except *cis*-nonachlor and dieldrin. Inverse associations and trends were statistically significant for β -hexachlorocyclohexane, oxychlordane, *trans*-nonachlor, and hexachlorobenzene. Relative risks for the upper quartile ranged between 0.5 and 0.7 contrasting the lowest quartile. A similar pattern was seen in estrogen receptor-positive breast cancer, but no significant trend was present. For estrogen receptor-negative breast cancer, a statistically significant and substantially lower risk in association with higher exposure was seen for the same four pesticides and for *p,p'*-DDE. The pattern was weaker,

Table 1. Characteristics of the 409 cases and the 409 control women at adipose tissue donation

	Case		Controls	
	Missing (n)	Mean (SD) or fraction (%)	Missing (n)	Mean (SD) or fraction (%)
Age (y)	0	57.5 (4.0)	0	57.5 (4.0)
Education (y)	0		1	
<8		30.6%		34.1%
8-10		47.9%		47.3%
>10		21.5%		18.6%
Body mass index (kg/m ²)	0	25.6 (4.4)	1	25.5 (4.1)
Alcohol consumption (g/d)	0	16.0 (16.5)	1	14.1 (15.2)
Nulliparous	0	13.0%	0	12.2%
No. deliveries	0	1.85 (1.1)	0	1.95 (1.2)
Age at first delivery (y)	3	25.3 (5.5)	9	24.8 (5.5)
Lifetime lactation (mos)	1	7.4 (7.6)	6	7.9 (9.2)
Hormone replacement therapy use (y)	5	4.6 (6.1)	19	4.6 (6.7)
Benign tumor previously	0	20.5%	4	12.6%
Receptor status of tumor	32			
Positive		77.5%		
Negative		22.5%		

Table 2. Concentrations ($\mu\text{g}/\text{kg}$ lipids) of 32 organochlorine compounds in adipose tissue from 409 cases and 409 controls

Compound	Above detection limit		Mean		Median	
	Cases, n (%)	Controls, n (%)	Cases	Controls	Cases	Controls
<i>p,p'</i> -DDE	407 (100)	408 (100)	639.0	686.3	476.7	507.1
<i>p,p'</i> -DDT	266 (65)	293 (72)	22.7	25.3	20.8	20.0
β -Hexachlorocyclohexane	394 (96)	401 (98)	72.0	79.6	64.3	71.8
α -Chlordane	0 (0)	0 (0)				
γ -Chlordane	0 (0)	0 (0)				
Oxychlordane	341 (83)	368 (90)	27.7	30.5	25.9	26.9
<i>cis</i> -Nonachlor	247 (60)	272 (67)	5.8	5.9	5.1	5.4
<i>trans</i> -Nonachlor	398 (97)	405 (99)	36.8	40.7	33.5	35.7
Aldrin	0 (0)	0 (0)				
Dieldrin	264 (65)	290 (71)	19.5	20.4	16.4	14.8
Endrin	0 (0)	1 (0)		9.0		9.0
Heptachlorepoxide	128 (31)	154 (38)	10.0	10.7	9.0	9.5
Hexachlorobenzene	404 (99)	408 (100)	71.5	78.5	69.7	74.1
Mirex	2 (0)	4 (1)	8.0	24.5	8.0	8.4
PCB 28	92 (22)	80 (20)	18.8	17.4	11.0	11.4
PCB 52	2 (0)	2 (0)	112.4	93.2	112.4	93.2
PCB 54	0 (0)	1 (0)		891.4		891.4
PCB 99	310 (76)	324 (79)	21.9	22.1	20.5	19.6
PCB 101	8 (2)	3 (1)	8.5	11.8	6.4	9.2
PCB 104	0 (0)	0 (0)				
PCB 105	126 (31)	129 (32)	10.3	10.3	9.1	9.5
PCB 118	399 (98)	405 (99)	38.8	39.2	35.9	35.1
PCB 128	77 (19)	65 (16)	3.3	3.3	2.8	2.9
PCB 138	407 (100)	408 (100)	137.6	140.4	133.0	130.3
PCB 153	408 (100)	409 (100)	273.0	278.9	265.9	266.6
PCB 55	0 (0)	0 (0)				
PCB 156	402 (98)	405 (99)	35.8	37.5	34.4	35.4
PCB 170	406 (99)	407 (100)	83.1	86.2	81.9	82.5
PCB 180	408 (100)	409 (100)	193.2	201.1	188.1	193.2
PCB 183	388 (95)	393 (96)	20.0	20.1	18.7	18.6
PCB 187	405 (99)	407 (100)	51.0	51.8	49.1	49.3
PCB 201	396 (97)	400 (98)	19.7	20.2	18.9	18.7

insignificant, or absent for *p,p'*-DDT, *cis*-nonachlor, and dieldrin (i.e., the three pesticides with the lowest proportion of samples above the detection limit).

No risk patterns or statistically significant results were found for the sum of PCBs or any of the PCB congeners in relation to either all breast cancers or estrogen receptor-positive breast cancer (Table 4). The upper quartile of the sum of PCBs had a relative risk (RR) for all breast cancers of 1.1 (95% CI, 0.7-1.7) when compared with the lower quartile. For estrogen receptor-negative breast cancer, however, a consistent risk pattern was evident showing lower risk in association with higher concentrations with a statistically significant inverse trend for the majority of the PCBs. As for the pesticides, the risk reduction in association with higher PCB concentrations was substantial for estrogen receptor-negative breast cancer. The risk in the 4th quartile was typically 60% to 90% lower than in the 1st quartile. The risk pattern was consistent across all PCB congeners and all functional PCB groups.

The adjustment for potential confounding factors had only a minor influence on the risk estimates in Tables 3 to 4 (unadjusted results are not shown). When data was analyzed following a case-cohort approach breaking the individual matched case-control pairs, all statistically significant negative trends in Tables 3 to 4 persisted, with lower *P*s that those obtained with the standard conditional logistic regression analyses.

We found the most marked differences in risk between estrogen receptor-positive and -negative breast cancer for DDE and PCBs. In a post hoc analysis, we stratified DDE and PCB concentrations by two markers of tumor progression (i.e., tumor size and lymph node involvement). Table 5 shows higher mean concentrations of DDE and PCBs in adipose tissue from women with smaller tumors and tumors without lymph node involvement.

Discussion

We found no indication of higher breast cancer risk in association with higher adipose tissue concentrations of any of the chlorinated pesticides or PCBs. In contrast, the results showed inverse associations between the risk of breast cancer and concentrations of β -hexachlorocyclohexane, oxychlordane, *trans*-nonachlor, and hexachlorobenzene. Furthermore, we found a consistent pattern of substantially lower risk of estrogen receptor-negative breast cancer in association with higher concentrations of most investigated organochlorines, whereas no significant trend was observed for estrogen receptor-positive breast cancer.

Inverse associations between organochlorine concentrations and breast cancer risk have been reported previously. Laden et al. (29) mostly found lower breast cancer risk in the upper quartiles of DDE and four PCB congeners, but none of these associations reached statistical significance. Ward et al. (28) found indications of lower risk of breast cancer in the upper quartiles of β -hexachlorocyclohexane and DDT, and a very consistent pattern of lower risk in the upper quartile of most of the included PCB congeners. In contrast, Aronson et al. (15) found a higher risk of breast cancer for higher concentrations of most included PCB congeners, but a decreased risk for higher levels of *cis*-nonachlor, *trans*-nonachlor, oxychlordane, hexachlorobenzene, and β -hexachlorocyclohexane in postmenopausal women. This is consistent with our results for the four last-mentioned pesticides. Inverse associations observed in this study were mostly restricted to estrogen receptor-negative breast cancer, for which the risk reductions in association with highest exposure were substantial, mostly significant, and consistent across most compounds. In Helzlsouer et al. (26), this pattern was evident among women who donated blood in 1989, which is close to the period of enrollment in the present study, but not in those who donated blood in 1974. Gammon

Table 3. Adjusted RR for breast cancer and 95% CI in association with adipose concentrations of organochlorine pesticides

Compound	Concentration (µg/kg lipids)	All breast cancers [RR (95% CI)]	Estrogen receptor positive breast cancer [RR (95% CI)]	Estrogen receptor negative breast cancer [RR (95% CI)]
<i>p,p'</i> -DDE	15-282	1.0	1.0	1.0
	283-507	1.0 (0.7-1.5)	1.2 (0.7-1.9)	0.6 (0.2-1.8)
	508-903	0.9 (0.6-1.4)	0.9 (0.6-1.6)	0.6 (0.2-1.6)
	904-6,693	0.7 (0.5-1.2)	1.1 (0.6-1.8)	0.1 (0.0-0.5)
	<i>P</i> for trend*	0.29	0.82	0.005
<i>p,p'</i> -DDT	<i>n</i> †	363	260	74
	6-14	1.0	1.0	1.0
	14-20	0.8 (0.5-1.3)	1.0 (0.5-1.9)	0.1 (0.0-0.6)
	20-31	1.4 (0.9-2.3)	1.4 (0.8-2.5)	1.7 (0.4-6.9)
	31-159	0.6 (0.3-1.0)	0.6 (0.3-1.1)	0.5 (0.1-2.1)
<i>P</i> for trend	0.19	0.18	0.85	
β-Hexachlorocyclohexane	<i>n</i>	173	125	36
	7-55	1.0	1.0	1.0
	55-72	0.8 (0.5-1.2)	0.9 (0.5-1.5)	0.5 (0.1-2.0)
	72-92	0.6 (0.4-0.9)	0.6 (0.3-1.0)	0.6 (0.2-1.7)
	92-754	0.5 (0.3-0.9)	0.6 (0.3-1.0)	0.2 (0.1-0.8)
<i>P</i> for trend	0.007	0.08	0.02	
Oxychlorane	<i>n</i>	343	244	71
	6-21	1.0	1.0	1.0
	21-27	0.6 (0.4-1.0)	0.6 (0.4-1.2)	0.4 (0.1-1.4)
	27-37	0.8 (0.5-1.3)	0.8 (0.5-1.5)	0.7 (0.2-2.8)
	37-142	0.5 (0.3-0.9)	0.6 (0.3-1.1)	0.1 (0.0-0.7)
<i>P</i> for trend	0.03	0.09	0.04	
<i>cis</i> -Nonachlor	<i>n</i>	274	193	59
	1.6-3.7	1.0	1.0	1.0
	3.7-5.4	1.6 (0.9-2.9)	2.4 (1.1-5.2)	0.9 (0.1-5.9)
	5.4-6.8	0.9 (0.5-1.6)	0.8 (0.4-1.7)	0.8 (0.1-5.3)
	6.8-28.5	1.5 (0.8-2.7)	1.5 (0.7-3.0)	1.4 (0.2-9.9)
<i>P</i> for trend	0.82	0.94	0.29	
<i>trans</i> -Nonachlor	<i>n</i>	150	112	33
	3-26	1.0	1.0	1.0
	26-36	0.7 (0.5-1.1)	0.8 (0.5-1.4)	0.4 (0.1-1.4)
	36-50	0.7 (0.4-1.1)	0.8 (0.4-1.3)	0.3 (0.1-1.1)
	50-172	0.7 (0.5-1.2)	0.9 (0.5-1.5)	0.2 (0.1-0.9)
<i>P</i> for trend	0.05	0.29	0.02	
Dieldrin	<i>n</i>	351	250	74
	4-12	1.0	1.0	1.0
	12-15	1.0 (0.6-1.8)	1.2 (0.6-2.5)	0.3 (0.0-2.3)
	15-23	1.4 (0.8-2.5)	1.4 (1.8-2.7)	0.6 (0.1-3.5)
	23-221	0.9 (0.5-1.6)	0.9 (0.5-2.0)	0.7 (0.1-5.7)
<i>P</i> for trend	0.99	0.55	0.42	
Hexachlorobenzene	<i>n</i>	166	119	37
	8-58	1.0	1.0	1.0
	58-74	0.6 (0.4-1.0)	0.7 (0.4-1.1)	0.6 (0.2-1.7)
	74-91	0.7 (0.4-1.1)	0.7 (0.4-1.1)	0.5 (0.2-1.6)
	91-704	0.5 (0.3-0.9)	0.6 (0.4-1.1)	0.2 (0.0-0.6)
<i>P</i> for trend	0.002	0.08	0.004	
<i>n</i>	360	257	74	

NOTE: Adjusted for education, BMI, alcohol intake, number of childbirths, age at first delivery, duration of lactation, years of use of HRT, and history of benign breast disease.

The exposure categories correspond to the four quartiles among controls; the lower quartile is the reference.

*Based on log-transformed concentrations analysed as a continuous variable in a linear model.

†Number of matched case-control pairs used in the analysis. Persons with missing value in any of the included variables could not be used; neither could the other person in the matched case-control pair.

et al. (25) observed a protective effect of PCBs on breast cancer being both estrogen receptor and progesterone receptor negative. Also similar to our results, other studies reported lower concentrations of organochlorine compounds in women with estrogen receptor-negative breast cancer than in women with estrogen receptor-positive breast cancer (14, 21, 22, 44).

The use of PCBs and many of the chlorinated pesticides was first restricted (1960s) and later banned (1970s and 1980s) in many parts of the western world; therefore, the large-scale release to the environment and related highest exposures of humans probably occurred several decades ago (33, 45). In accordance with the decrease in exposure over the last decades, organochlorine concentrations in humans have been found to be lower in more recent samples and to be higher in samples from older individuals (45, 46). We also observed higher organochlorine levels in older women in this study (data not shown). We would, thus, expect the concentrations we measured in adipose tissue sampled in

the mid-1990s to reflect not only the exposure and body burden accumulated decades ago, but also the individual ability to metabolize and eliminate these compounds. Bearing this in mind, the finding in the present study of lower risk of breast cancer among women with higher concentrations of several organochlorine compounds can be interpreted in different ways.

We could hypothesize that organochlorines in the measured concentrations have a direct protective effect against breast cancer (e.g., via weak anti-estrogenic activity or via other mechanisms exerted by some of the measured organochlorines or by unmeasured but possibly correlated compounds such as dioxins). In fact, an experimental study suggested a cancer-preventive effect of low concentrations of DDT (9). Alternatively, it may be that low organochlorine concentrations measured in adipose tissue taken during the 1990s reflect a genetically determined high individual ability to metabolize these compounds. This in turn could lead to higher

Table 4. Adjusted RR for breast cancer and 95% CI in association with adipose PCB concentrations

Compound	Concentration (µg/kg lipids)	All breast cancers [RR (95% CI)]	Estrogen receptor-positive breast cancer [RR (95% CI)]	Estrogen receptor-negative breast cancer [RR (95% CI)]
PCB 187 (group* 1B)	3-38	1.0	1.0	1.0
	38-49	0.9 (0.6-1.4)	1.1 (0.6-1.7)	0.6 (0.2-1.6)
	49-60	0.8 (0.5-1.2)	0.9 (0.5-1.5)	0.3 (0.1-0.9)
	60-322	1.2 (0.8-2.0)	1.6 (0.9-2.7)	0.4 (0.1-1.3)
	<i>P</i> for trend [†]	0.97	0.19	0.01
	<i>n</i> [‡]	360	257	74
PCB 201 (group 1B)	1-16	1.0	1.0	1.0
	16-19	0.7 (0.5-1.1)	0.7 (0.4-1.2)	0.8 (0.2-2.4)
	19-23	0.9 (0.6-1.5)	0.9 (0.5-1.6)	1.0 (0.3-3.5)
	23-114	1.1 (0.7-1.9)	1.4 (0.8-2.6)	0.4 (0.1-1.5)
	<i>P</i> for trend	0.87	0.30	0.08
	<i>n</i>	344	246	70
PCB 118 (group 2A)	3-27	1.0	1.0	1.0
	27-35	1.0 (0.7-1.6)	1.2 (0.7-2.0)	0.3 (0.1-1.0)
	35-51	1.1 (0.7-1.7)	1.0 (0.6-1.8)	1.5 (0.5-4.3)
	51-200	0.9 (0.6-1.4)	1.0 (0.6-1.7)	0.2 (0.0-0.8)
	<i>P</i> for trend	0.99	0.37	0.06
	<i>n</i>	352	252	72
PCB 156 (group 2A)	3-30	1.0	1.0	1.0
	30-35	0.8 (0.5-1.2)	0.7 (0.4-1.1)	1.0 (0.3-3.2)
	35-43	0.9 (0.6-1.4)	0.8 (0.5-1.5)	1.0 (0.3-3.0)
	43-206	0.9 (0.6-1.5)	1.0 (0.6-1.7)	0.5 (0.1-1.9)
	<i>P</i> for trend	0.26	0.95	0.05
	<i>n</i>	355	253	74
PCB 138 (group 2B)	6-97	1.0	1.0	1.0
	97-130	1.0 (0.6-1.6)	1.4 (0.8-2.3)	0.5 (0.2-1.5)
	130-170	1.0 (0.6-1.5)	1.1 (0.7-1.9)	0.4 (0.1-1.1)
	170-629	1.1 (0.7-1.7)	1.4 (0.8-2.4)	0.3 (0.1-0.9)
	<i>P</i> for trend	0.84	0.27	0.008
	<i>n</i>	363	260	74
PCB 170 (group 2B)	6-67	1.0	1.0	1.0
	67-83	0.9 (0.6-1.4)	0.9 (0.5-1.5)	1.0 (0.3-3.0)
	83-98	0.9 (0.6-1.4)	1.0 (0.6-1.7)	0.7 (0.2-2.2)
	98-502	1.1 (0.7-1.8)	1.3 (0.7-2.3)	0.4 (0.1-1.6)
	<i>P</i> for trend	0.42	0.47	0.02
	<i>n</i>	361	258	74
PCB 99 (group 3)	5.6-15.0	1.0	1.0	1.0
	15.0-19.6	1.1 (0.6-1.8)	0.9 (0.5-1.7)	1.3 (0.4-4.7)
	19.6-26.7	1.1 (0.7-1.9)	1.0 (0.6-1.8)	1.2 (0.3-4.9)
	26.7-99.4	1.1 (0.7-1.9)	1.1 (0.6-2.1)	0.3 (0.1-1.4)
	<i>P</i> for trend	0.85	0.69	0.14
	<i>n</i>	220	156	48
PCB 153 (group 3)	18-206	1.0	1.0	1.0
	206-267	1.0 (0.7-1.5)	1.3 (0.8-2.1)	0.4 (0.1-1.1)
	267-322	0.8 (0.5-1.3)	1.0 (0.6-1.7)	0.3 (0.1-1.1)
	322-1,294	1.1 (0.7-1.7)	1.4 (0.8-2.3)	0.3 (0.1-0.9)
	<i>P</i> for trend	0.66	0.41	0.008
	<i>n</i>	365	261	75
PCB 180 (group 3)	13-155	1.0	1.0	1.0
	155-193	1.1 (0.7-1.7)	1.1 (0.7-1.8)	1.3 (0.4-4.2)
	193-230	0.9 (0.6-1.4)	1.0 (0.6-1.8)	0.9 (0.3-2.6)
	230-1,084	1.1 (0.6-1.8)	1.2 (0.7-2.2)	0.3 (0.1-1.2)
	<i>P</i> for trend	0.32	0.63	0.02
	<i>n</i>	365	261	75
PCB 183 (group 3)	2-14	1.0	1.0	1.0
	14-19	1.2 (0.8-1.9)	1.6 (0.9-2.7)	0.8 (0.3-2.4)
	19-24	1.0 (0.6-1.5)	1.3 (0.8-2.3)	0.3 (0.1-0.9)
	24-106	1.3 (0.8-2.0)	1.6 (0.9-2.8)	0.4 (0.1-1.2)
	<i>P</i> for trend	0.57	0.13	0.04
	<i>n</i>	331	238	67
ΣPCBs [§]	56-671	1.0	1.0	1.0
	671-852	0.9 (0.6-1.4)	1.1 (0.6-1.8)	0.4 (0.1-1.3)
	852-1,024	0.7 (0.5-1.1)	0.8 (0.5-1.4)	0.3 (0.1-0.9)
	1,024-4,357	1.1 (0.7-1.7)	1.4 (0.8-2.5)	0.3 (0.1-0.9)
	<i>P</i> for trend	0.44	0.50	0.007
	<i>n</i>	365	261	75

NOTE: Adjusted for education, BMI, alcohol intake, number of childbirths, age at first delivery, duration of lactation, years of use of HRT, and history of benign breast disease. The exposure categories correspond to the four quartiles among controls; the lower quartile is the reference.

*Grouping as suggested by Wolff et al. (42): 1B, potentially estrogenic, phenobarbital inducers, persistent; 2A, potentially antiestrogenic and immunotoxic, dioxin-like, moderately persistent; 2B, potentially antiestrogenic and immunotoxic, limited dioxin activity, persistent; 3, phenobarbital, CYP1A, and CYP2B inducers, persistent.

[†]Based on log-transformed concentrations analyzed as a continuous variable in a linear model.

[‡]Number of matched case-control pairs used in the analysis. Persons with missing value in any of the included variables could not be used; neither could the other person in the matched case-control pair.

[§]Sum of PCB congeners included in Table 4.

Table 5. Mean concentrations of DDE and PCBs ($\mu\text{g}/\text{kg}$ lipids) in women with breast cancer, stratified by indicators for tumor progression

Tumor characteristics	DDE			ΣPCB		
	n	Mean	P*	n	Mean	P*
Diameter						
<18 mm (median)	189	651	0.82	190	888	0.16
≥ 18 mm	191	638		191	846	
Lymph node involvement						
No	241	669	0.20	242	885	0.07
Yes	151	595		151	831	

*Differences were tested on log-transformed organochlorine concentrations.

concentrations of more harmful organochlorine metabolites. For example, hydroxylated metabolites of DDE and several PCBs exert stronger estrogenic or anti-estrogenic properties than DDE and PCBs themselves (8, 47). Yet another interpretation could be that the induction of metabolic activity by some organochlorine compounds [ref. 8; e.g., induction of the biotransformation enzyme cytochrome P4501A1, which is involved in metabolism of, among others, endogenous steroid hormones (48)], may decrease the concentrations of harmful substances and thereby may reduce breast cancer risk. Finally, consumption of fish is expected to be an important source of human exposure to organochlorines. If consumption of fish also protects against breast cancer as suggested by experimental studies (49), the inverse associations between organochlorines and breast cancer in the present study may be due to confounding from fish consumption. However, fish consumption did not protect against breast cancer in the present study population (49) and is, therefore, not likely to be an explanation for the inverse association between organochlorines and breast cancer.

In the present study, an inverse association between concentrations of some organochlorines and the risk of breast cancer was most evident for estrogen receptor-negative breast cancer. For estrogen receptor-positive breast cancer, the results indicated such an inverse association only for a few of the pesticides, but not for other pesticides or any of the PCB congeners. It has not yet been established whether estrogen receptor-negative breast cancer is a progressed form of estrogen receptor-positive breast cancer or whether these breast cancers are two biologically different diseases with different risk factors (50). The prognosis of estrogen receptor-negative breast cancer patients is poorer than that of estrogen receptor-positive breast cancer patients, and tumors lose their estrogen dependence whereas progressing, indicating that estrogen receptor-negative breast cancer may represent a more progressed state of estrogen receptor-positive breast cancer. The estrogen receptor gene controls the expression of estrogen receptor phenotype in tumor cells and a progression from estrogen receptor-positive to -negative phenotype may be due to either loss of gene function or suppression of gene expression (50). Whatever causes these possible gene-related changes, the different effects of organochlorines on estrogen receptor-positive and -negative breast cancer, which were indicated by the results of the present study, may relate to a possible influence of organochlorines on changes in cellular phenotype. If organochlorines delayed or inhibited a progression of breast cancer tumors from estrogen receptor-positive to estrogen receptor-negative status, a deficit of estrogen receptor-negative cases would be observed among women with high organochlorine concentrations, which may explain the results of the present study. The finding in the present study of higher DDE and PCB concentrations among women

with smaller tumors and with tumors without lymph node involvement (Table 5) supports the hypothesis that these organochlorine compounds may delay tumor progression.

The data analyses involved a large number of statistical tests and, therefore, some of the individual statistically significant findings may have occurred by chance. However, due to the consistency across most of the included organochlorine compounds, the pattern of lower risk for estrogen receptor-negative breast cancer in association with higher organochlorine levels is unlikely to be a chance finding.

Adipose tissue is the principal storage medium for lipophilic organochlorine compounds in the human body (51) and we would, therefore, expect concentrations measured in adipose tissue to be a good estimate of the body burden (52). We measured organochlorine compounds in adipose tissue from the buttock although breast cancer was the end point of interest. Indirect evidence for similar organochlorine concentrations in adipose tissue from different anatomic sites stems from studies showing similar correlations between DDE concentrations measured in blood samples and adipose tissue from, respectively, breast (53, 54), buttock (55), and abdomen (56). However, uncertainty relates to the relevant timing of exposure. Whereas concentrations measured close to cancer diagnosis, as in the retrospective studies, could be relevant to identify late-stage cancer promoting effects of organochlorine compounds, prospective studies would be adequate to detect carcinogenic effects exerted years before the clinical presentation of the cancer. In the present study, tissue was sampled up to 6.5 years before the breast cancer diagnosis and the age at diagnosis ranged between 51 and 69 years. If organochlorines exert breast carcinogenic effects (e.g., *in utero* or during puberty), it is uncertain to what degree organochlorine concentrations measured in blood or tissue sampled even many years before the diagnosis would adequately reflect the body burden during the relevant time period.

The strengths of this study include prospective design, measurement of organochlorines in prediagnosis adipose tissue samples, large sample size, and ascertainment of vital status of participants based on reliable and population-based registries. Selection bias was unlikely because cases and matched controls came from the same cohort and fulfilled the same eligibility criteria. Most of the known risk factors for breast cancer were more prevalent among cases (Table 1) and the analyses were adjusted for these potential confounding factors. Because these adjustments had little effect on the risk estimates, substantial residual confounding from these factors is unlikely. We cannot, however, rule out confounding from unknown risk factors. The potential for recall bias was minimized because information on risk factors was collected before the cancer diagnoses and sampling of adipose tissue before cancer diagnosis reduced the risk of differential misclassification in the exposure assessment.

In conclusion, results from this study do not support the hypothesis that persistent organochlorine compounds increase the risk of breast cancer in postmenopausal women. On the contrary, the results showed a consistent pattern of substantially lower risk for estrogen receptor-negative breast cancer among women with higher organochlorine concentrations. Although consistent with some previous reports, the interpretation of these results is currently unclear and will require confirmation in further studies.

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