Serum Organochlorine Levels and Breast Cancer: A Nested Case-Control Study of Norwegian Women[1]

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Abstract

This study investigated the potential association between organochlorine exposure and breast cancer using stored sera collected from 1973 through 1991 from the Janus Serum Bank in Norway. Breast cancer cases were ascertained prospectively from among 25,431 female serum bank donors. A total of 150 controls were matched to cases by birth dates and dates of sample collection. One g of serum per subject was analyzed for a total of 71 organochlorine compounds. For 6 pesticides [*B*-hexachlorocyclohexane, heptachlor epoxide, oxychlordane, *trans*-nonachlor, *p, p′*-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene, and *p, p′*-2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane] and 26 individual polychlorinated biphenyl (PCB) congeners there were >90% of samples over the limit of detection. There was no evidence for higher mean serum levels among cases for any of these compounds, nor any trend of increasing risk associated with higher quartiles of exposure. The remaining compounds (including dieldrin) were analyzed with respect to the proportion of cancer cases and controls having detectable levels; no positive associations were noted in these analyses. Our study did not confirm the recent findings of a Danish study of increased concentrations of dieldrin in the serum of breast cancer cases. The evidence to date on the association between serum organochlorines is not entirely consistent, but there is accumulating evidence that serum levels of *p, p′*-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene and total PCBs are not important predictors for breast cancer in the general population. Studies to date have not been able to evaluate whether exposure to highly estrogenic, short-lived PCB congeners increases breast cancer risk, nor have they fully evaluated the risk associated with organochlorine exposure in susceptible subgroups or at levels above general population exposure, including women with occupational exposure.

Introduction

In 1993, the first study reporting an association between organochlorine levels in human serum and breast cancer was published (1). In this nested case-control study, concentrations of PCBs[3] and DDE in blood sera were compared between women who developed breast cancer within 6 months of donating a blood specimen and matched controls. Breast cancer was strongly associated with serum DDE concentration and had a positive, but nonsignificant, association with PCBs. Elevations in DDE concentrations in breast tissue obtained from women with breast cancer compared with those with benign breast disease had been reported previously by one study (2). The findings of these two studies were of substantial interest because the incidence of breast cancer has been rising worldwide concurrent with environmental pollution, particularly organochlorine residues in the food chain (3, 4). The current study was one of several that were initiated to investigate further the potential association between serum organochlorine levels and breast cancer (5–15). Using the unique resources of the Janus Serum Bank in Norway, it was designed to measure a large number (71) of organochlorine compounds in relation to female breast cancer risk (16).

Materials and Methods

This is a case-control study of serum organochlorine levels in relation to breast cancer risk. The Janus Serum Bank contains more than 400,000 serum samples from almost 300,000 individuals. Samples were collected from the Red Cross Blood Center in Oslo and from persons undergoing routine health examinations in various counties of Norway (16). The serum bank was established in 1973, and blood was collected to the end of 1991. The purpose of the serum bank is to search in these premorbidity sera for chemicals or for biochemical, immunological, or other changes that might indicate cancer development at an early stage (16). Cases were selected from among

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[3] The abbreviations used are: PCB, polychlorinated biphenyl; *B*-HCCH, *B*-hexachlorocyclohexane; DDE, *p,p′*-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; QA, quality assurance; LD, limit(s) of detection; ER, estrogen receptor; PR, progesterone receptor; OR, odds ratio; BMI, body mass index; PUFA, polyunsaturated fatty acid; CI, confidence interval.

25,431 female Janus Serum Bank donors who were working outside the home or were resident on farms as of the 1970 or 1980 census. The selection of women who had worked outside the home or were resident on farms was done to enrich the sample in women with potential occupational or environmental exposure to organochlorines. A total of 272 individuals who developed breast cancer were identified in this cohort by matching them with records of the Norwegian Cancer Registry through December 31, 1993. Because available funds permitted analysis of only 300 samples, 150 individuals who had a blood sample taken 2 or more years before diagnosis were randomly selected from the 272 potentially eligible cases. An equal number of controls were matched to cases by date of sample collection (+/− 2 years) and date of birth (+/− 4 years). Controls were also required to be alive and free of cancer, except for basal cell carcinoma of the skin, as of the date of case diagnosis.

One g of serum from each case and control were provided to the Toxicology Branch, Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control for analysis. Serum samples were stored by the Janus Serum Bank at −25°C, were hand carried from the serum bank to the laboratory, and were verified to be frozen upon arrival. Samples were blind-coded. Analysis was conducted for a total of 71 compounds: 22 dioxins, furans, and non-*ortho* co-planar PCBs; 36 other PCB congeners; and 13 pesticides or their metabolites. Chemical structures and a summary of the metabolism of the pesticides and other organochlorines were presented in Ahlborg *et al.* (4). The analytical technique and quality control procedures have been described previously (17–19). Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls, chlorinated pesticides, and selected metabolites were measured in serum by high resolution gas chromatography/high resolution isotope dilution mass spectrometry. The analytes of interest were isolated from serum using a C_{18} solid-phase extraction procedure and then by a multicolumn automated cleanup procedure. The analytes were separated on a DB-5 ms capillary column and quantified using selected ion monitoring high resolution (10,000 resolving power) mass spectrometry. Total cholesterol and triglyceride measurements were made using standard enzymatic procedures on an Abbott Spectrum CCx Series II analyzer (Abbott Laboratories, Diagnostics Division, Abbott Park, IL). The cholesterol procedure is based on the hydrolysis of cholesterol esters in serum to free cholesterol by cholesterol esterase. The cholesterol produced is oxidized by cholesterol oxidase in a reaction that results in the formation of hydrogen peroxide. Hydrogen peroxide reacts with 4-aminoantipyrine and phenol in the presence of horseradish peroxidase to yield a quinoneimine dye that absorbs at 500 nm. In the triglycerides procedure, serum triglycerides are converted to glycerol and free fatty acids by lipoprotein lipase. The glycerol is then converted to glycerol-3-phosphate by glycerol kinase in the presence of ATP. The glycerol-3-phosphate is reacted with glycerol-3-phosphate oxidase in the presence of oxygen to form hydrogen peroxide. This in turn forms a colored complex with aminoantipyrine and chlorophenol in the presence of peroxidase. The concentration of each analyte is calculated from its individual standard linear calibration. Results were reported on both a whole-weight and lipid-adjusted basis. Total lipids were calculated according to the following formula: (2.27 × total cholesterol + triglycerides + 62.3) (20). Detection limits, on a whole-weight and lipid-adjusted basis, were reported for each sample and corrected for sample weight and analyte recovery (21, 22). A total of eight samples for which total lipids could not be determined were assigned a value of 600 mg/dl. If the results for an analyte could not be reported because of an interference, or if QA parameters did not meet specified QA criteria, this was indicated, and a missing value was reported.

The distribution of both reported data and LD for each analyte was examined before linking the data with case-control status to determine how nondetected samples would be treated in the analysis. For those analytes that had >90% of samples above the LD, the value of the LD divided by the square root of 2 was used to estimate the value of samples below the LD for analyses comparing serum organochlorine concentrations in cancer cases and controls. This procedure is recommended when the data are not highly skewed (23). For analytes with >90% of sample values above the LD, paired *t* tests were used to compare the whole-weight and lipid-adjusted serum concentrations of each analyte between each matched case and control. In addition, the distribution of each analyte (or analyte group) was divided into quartiles, based on the distribution in controls, and an OR was calculated for each quartile compared with the lowest. Conditional logistic regression was used to examine the relationship between each analyte and breast cancer, taking into account the potential confounders and the effect modifiers (occupational category, age at first birth, number of births prior to donation of blood sample, region of residence, and region of birth).

For those analytes which had >0 and <90% of samples with detectable values, a high degree of overlap was observed between the range of organochlorine concentrations in detected samples and the LD for nondetected samples. This precluded substitution of a meaningful estimate for sample values below the LD. Therefore, for these analytes, a more limited analysis was carried out that compared the odds of having a sample above the LD for cancer cases and controls. ORs and confidence intervals were calculated using conditional logistic regression (24).

PCB congeners were also analyzed in groups on the basis of predicted estrogenicity and enzyme induction, as proposed by Wolff *et al.* (25, 26). These groups are as follows: group 1 (potentially estrogenic); group 1A (weak phenobarbitol inducers, estrogenic, not persistent); group 1B (weak phenobarbitol inducers, persistent); group 2 (potentially antiestrogenic and immunotoxic, dioxinlike); group 2A, non-*ortho* and mono-*ortho* (moderately persistent); group 2B, di-*ortho* substituted (limited dioxin activity, persistent); and group 3 (phenobarbitol, CYP1A and CYP2B inducers, biologically persistent).

For all study participants, data on age at birth of first child, number of children as of shortly before the specimen was taken, county of birth, county of residence at blood sample, and occupation as of 1960, 1970, and 1980 were provided by the Janus Serum Bank and the Norwegian Cancer Registry. Neither BMI nor menopausal status are available for the women in the sample. Diagnosis date, histology, stage, and number of children before diagnosis of cancer were provided for breast cancer cases. ER and PR concentrations were abstracted from original medical records. Parity, occupation, and residence data were categorized into 4–5 strata (Table 1). For individuals whose occupations were identified in more than one census, the most recent occupation was used in the analysis. Stratified analyses for DDE and total PCBs were conducted by age at breast cancer diagnosis (<50 years and ≥50 years), by interval between blood sample and diagnosis, and by ER and PR concentration. To examine trends in organochlorine concentrations by date of blood collection, mean organochlorine levels were calculated for each of four time strata; cut points were defined to have

Table 1 Selected characteristics of breast cancer cases and controls

	Total study range	Controls		Cases		*P*
		Mean	SD (in years)	Mean	SD (in years)	
Year blood collected (mo/yr)	3/73–1/90	8/79	5.4	7/79	5.5	0.16[a]
Age at blood collection (yr)	18.4–60.0	41.2	6.9	41.1	6.7	0.39[a]
Age at first birth[b] (yr)	16–40	23.2	4.0	24.1	4.7	0.02[a]
No. of births[b]	0–7	2.2	1.4	2.1	1.5	0.70[a]
Serum triglycerides (mg/dl)	48–287	136.8	48.8	132.5	48.3	0.53[a]
Total cholesterol (mg/dl)	88–485	206.2	54.6	199.0	50.2	0.17[a]
Total lipids (mg/dl)[c]	355–1311	665.5	135.4	644.9	128.9	0.16[a]
Date of diagnosis of breast cancer (mo/yr)	2/76–12/93	n.a.[d]		5/88	4.5	n.a.
Interval between blood sample and diagnosis (yr)	2.0–18.2	n.a.		8.8	4.8	n.a.
	No.	%	No.	%		*P*
Occupational category						
Professional, technical, sales, clerical	48	32.2	56	37.6		0.81[e]
Manufacturing, labor, craft, repair	11	7.4	8	5.4		
Farm, fishery	24	16.1	24	16.1		
Service	60	40.3	61	40.9		
Unknown	6	4.0				
Region of birth[f]						
Southeast	78	52.3	70	47.0		0.17[e]
Southwest	29	19.5	33	22.1		
Oslo	4	2.7	14	9.4		
North	23	15.5	21	14.1		
Other	12	8.0	11	7.4		
Region of residence at time of blood collection[f]						
Southeast	88	59.0	89	59.7		0.30[e]
Southwest	22	14.8	33	22.1		
Oslo	14	9.4	12	8.0		
North	22	14.8	15	10.1		

[a] *P* from paired *t* test.
[b] In calculating numbers of births and age at first birth, only births before the date of the blood specimen were included.
[c] Total lipids = (2.27 × cholesterol + triglycerides + 62.3).
[d] n.a., not applicable.
[e] *P* from χ^2 test.
[f] Southeast region includes the counties of Ostfold, Akershus, Hedmark, Oppland, Buskerud, Vestfold, Telemark, Aust-Agder, Vest Agder; Southwest region includes the counties of Rogaland, Hordaland, Sogn og Fjordane, More og Romsdal; North region includes the counties of Sor-Trondelag, Nord-Trondelag, Nordland, Troms, and Finnmark.

approximately equal numbers of samples/strata. All data analyses were conducted using the Statistical Analysis System (27).

Recognizing that a large number of statistical comparisons would be made in the analysis of these data, the literature review was updated in September 1998 to develop an *a priori* hypotheses of the likely direction of effect for the analytes with ≥90% of values above the LD. These *a priori* hypotheses are summarized below.

Among the pesticides and their metabolites that had >90% of sample values above the LD, our *a priori* hypothesis was that serum concentrations of *B*-HCCH, *p,p′*-DDE, and *p,p′*-DDT would be positively associated with breast cancer risk, and that there would be no association between levels of hepatachlor epoxide, oxychlordane, or *trans*-nonachlor and breast cancer risk. Among the 26 PCB congeners with >90% detectable samples, none were in the category of "estrogenic, nonpersistent, weak phenobarbitol inducers" [Wolff *et al.*'s (25, 26) Group 1A] likely to be associated with increased breast cancer risk through an estrogenic mechanism. We hypothesized *a priori* that we would see the following associations with breast cancer: (*a*) no association with the sum of PCB congeners; (*b*) positive association with Congener 99, based on one previous study (28); and (*c*) a negative association with the dioxin-like non-*ortho* and mono-*ortho* coplanar compounds (Wolff Group 2A). We made no hypothesis *a priori* about the Wolff group 1B and three congeners because it was unclear to what extent the congeners measured in these groups are estrogenic.

Results

A total of 150 breast cancer cases and 150 matched controls were selected for the study; one matched pair was deleted from the analysis after it was determined that the case did not meet the criteria of having the blood sample drawn at least 2 years before diagnosis. Stages at diagnosis were: 43.3% stage 1; 42.7% stage 2; 8.0% stage 3; 5.3% stage 4; and 0.7% having inadequate information to be staged accurately. All but 6 of the 147 tumors with known histology were adenocarcinomas. A total of 90 cancer cases (60.4%) were diagnosed before age 50 (presumed to be premenopausal), and 59 (39.6%) of cancer cases were diagnosed at age 50 or older (presumed to be peri- or postmenopausal). The blood samples were drawn between 1973 and 1990, with an average date of blood collection of August 1979 for the controls and July 1979 for the breast cancer cases (Table 1). Age at blood collection ranged from 18 to 60 years, and averaged 41.2 years for controls and 41.1 years for cancer cases (Table 1). Although cancer cases and controls did not differ with respect to the number of children born before the date of the blood sample, cancer cases were significantly older than controls at the time their first child was born (Table 1). Breast cancer cases and controls were quite similar with respect

Table 2 Levels of pesticides or their metabolites and PCB congeners measured among breast cancer cases and controls[a]

Pesticide or pesticide metabolite/ PCB congener (Ballschmiter no. Wolff group[b])	n[c]	Mean concentration in serum (ng/g serum)		P[d]	Mean lipid-adjusted concentration (ng/g lipid)		P[d]	OR by Q,[e] relative to lowest Q		
		Controls	Cases		Controls	Cases		Q2	Q3	Q4
B-HCCH	144	0.414	0.381	0.34	63.4	60.0	0.45	1.0	0.7	0.7
Heptachlor epoxide	144	0.055	0.045	0.07	8.5	7.1	0.10	1.5	1.8	1.0
Oxychlordane	143	0.072	0.064	0.16	10.9	10.0	0.23	1.0	1.0	0.9
Trans-nonachlor	145	0.076	0.065	0.04	11.7	10.4	0.10	1.0	0.9	1.0
p-p′-DDE	144	8.23	7.93	0.76	1260	1230	0.84	0.7	1.0	1.2
p,p′-DDT	146	0.898	0.76	0.20	137.7	119.5	0.27	0.2	0.5	0.3
3,3′,4,4′,5-penta (126-2A)	142	1.1×10^{-3}	1.1×10^{-3}	0.70	0.164	0.166	0.86	1.1	1.3	1.1
3,3′,4,4′,5,5-hexa (169-2A)	144	0.5×10^{-3}	0.5×10^{-3}	0.14	0.084	0.080	0.30	0.9	1.1	0.6
2,4,4′,5-tetra (74-2A)	134	0.183	0.168	0.11	28.7	27.0	0.30	0.9	0.6	0.6
2,2′,4,4′,5-penta (99-3)	142	0.189	0.163	0.06	28.8	25.7	0.12	0.5	0.6	0.6
2,3′4,4′5-penta (118-2A)	141	0.342	0.322	0.28	52.8	50.7	0.46	0.8	0.4	0.6
2,3,3′,4,4′-penta (105-2A)	143	0.073	0.071	0.67	11.2	11.1	0.96	0.9	0.5	1.0
2,2′,3,4′,5,5′-hexa (146-3*)	141	0.151	0.137	0.08	23.4	21.8	0.23	0.8	0.6	0.6
2,2′,4,4′,5,5′-hexa (153-3)	143	1.26	1.15	0.06	195.6	183.4	0.21	0.9	0.7	0.6
Several (138-158-not classified)	143	0.907	0.835	0.11	140.0	132.8	0.32	0.5	0.7	0.6
2,3′,4,4′,5,5′-hexa (167-2A)	139	0.043	0.040	0.16	6.7	6.4	0.45	0.8	0.6	0.7
2,3,3′,4,4′5-hexa (156-2A)	142	0.107	0.097	0.07	16.6	15.5	0.23	0.8	0.8	0.5
2,3,3′,4,4′,5′-hexa (157-2A*)	135	0.024	0.023	0.67	3.7	3.8	0.90	0.8	0.9	0.6
2,2′,3,3′,5,5′,6-hepta (178-3)	134	0.054	0.053	0.82	8.5	8.4	0.86	0.7	0.7	0.5
2,2′,3,4′,5,5′,6-hepta (187-1B)	142	0.247	0.237	0.62	38.2	37.2	0.73	0.7	0.8	0.5
2,2′,3,4,4′,5′,6-hepta (183-3)	142	0.107	0.103	0.71	16.4	16.2	0.87	0.7	0.7	0.4
2,2′,3,3′,4,5,6-hepta (177-1B)	138	0.08	0.07	0.43	12.4	12.1	0.80	1.0	0.8	0.6
2,2′,3,3′,4,5,5′-hepta (172-3*)	135	0.044	0.040	0.09	6.93	6.41	0.22	0.6	0.9	0.5
2,2′,3,4,4′,5,5′-hepta (180-3)	142	0.742	0.669	0.04	115.8	107.7	0.18	0.6	0.9	0.5
2,2′,3,3′,4,4′,5-hepta (170-2B)	137	0.335	0.306	0.07	52.0	49.2	0.32	0.4	1.0	0.5
2,3,3′,4,4′,5,5′-hepta (189-2A*)	134	0.017	0.018	0.28	2.79	3.02	0.18	0.4	0.8	1.0
2,2′3,3′,4,5,5′,6′-octa (201-1B)	138	0.094	0.088	0.24	14.7	14.0	0.48	0.6	0.9	0.5
Several (196-203-not classified)	141	0.111	0.102	0.11	17.3	16.3	0.28	0.6	0.9	0.5
2,2′,3,4,4′,5,6-octa (195-3*)	129	0.035	0.030	0.07	5.43	4.84	0.17	1.2	0.9	0.6
2,2′,3,3′,4,4′,5,5′-octa (194-3*)	141	0.116	0.107	0.11	18.3	17.2	0.28	0.8	1.3	0.6
2,2′,3,3′,4,4′,5,5′,6-nona (206-3*)	129	0.036	0.033	0.20	5.61	5.20	0.31	1.1	0.7	0.8
2,2′,3,3′,4,4′,5,5′,6,6′-deca (209-3*)	132	0.029	0.025	0.04	4.52	3.97	0.06	0.7	0.5	0.5
PCB group 1B	143	0.419	0.396	0.40	65.0	62.6	0.56	0.6	0.6	0.5
PCB group 2A	147	0.77	0.73	0.26	120.0	116.3	0.5	0.8	0.6	0.6
PCB group 2B	137	0.335	0.306	0.07	52.0	49.2	0.32	0.4	1.0	0.5
Total PCB group 2	147	1.09	1.03	0.27	169.8	165.2	0.59	0.9	0.8	0.8
PCB group 3	143	2.64	2.39	0.04	427.1	398.7	0.18	0.7	0.8	0.6
Total PCBs	147	5.09	4.76	0.18	806.6	776.1	0.47	0.6	0.8	0.5

[a] Only compounds with >90% of samples above LD are included.
[b] PCB congeners were grouped according to a classification system developed by Wolff *et al.* (1997), as described in "Materials and Methods." For those compounds that had not been classified previously by this scheme in the published literature, the group was inferred by the authors and designated with an *.
[c] No. of pairs in which both members had valid analytical results, including results below the limit of detection.
[d] P calculated from paired t test.
[e] Q, quartile; Q1 OR = 1.00.

to occupational categories (Table 1). Approximately 59% of study subjects lived in the Southeast, similar to the proportion (about 50%) of the total Norwegian population that lives in this region. In analyses of region of birth and region of residence, cancer cases were more likely than controls to have been born in Oslo, and less likely to have lived in the Northern region at the time the blood sample was taken. Among breast cancer cases and controls combined, region of residence was related to the time interval when samples were taken ($\chi^2 = 145$; $P = 0.001$); however, there was no evidence that matched cancer cases and controls were more likely to be concordant on region of residence than would be predicted by chance alone (κ statistic = 0.1). Cancer cases tended to have lower serum cholesterol and total lipids than controls, although neither difference was statistically significant (Table 1).

Table 2 presents mean organochlorine levels in both whole-weight and lipid-adjusted units for each pesticide or metabolite and PCB congener measures, and for groups of PCB congeners. Analyses presented in Table 2 include only those organochlorine compounds for which ≥90% of samples were above the LD. ORs by quartile, based on the lipid-adjusted data, are also shown. None of the six pesticides/metabolites or 26 individual PCB congeners measured showed a positive association with breast cancer risk; in fact, all of the observed differences in means were in a slightly negative direction. ORs by quartile generally showed an inverse trend with breast cancer risk, although a few compounds showed ORs >1 in the higher quartiles. PCB congener 99, for which a positive association was hypothesized *a priori* based on one previous study, had no association with breast cancer in the current study. All of the PCB congener groups seemed to have an inverse relationship with breast cancer risk; there was no evidence that the compounds in group 2A, thought to be antiestrogenic, had a stronger inverse relationship than compounds in other groups. The

Table 3 Mean serum levels of DDE and PCBs, stratified by various covariates

Covariate	Mean lipid-adjusted level of DDE (ng/g lipid)				Mean lipid-adjusted level of PCBs (ng/g lipid)			
	n^a	Controls	Cases	P^b	n^a	Controls	Cases	P^b
Age <50	89	1067	989	0.57	89	749.8	692.0	0.20
Age ≥50	55	1571	1620	0.88	58	893.9	905.1	0.89
Yr between blood sample and diagnosis								
<10	90	1112	935	0.21	91	772.0	709.3	0.21
≥10	54	1506	1721	0.50	56	862.9	884.6	0.77
ER concentration[c]								
<10	40	1054	1086	0.84	39	778.6	693.9	0.23
≥10, <20	14	922.3	758.4	0.37	14	778.5	623.0	0.09
≥20	29	1592	1196	0.40	31	740.6	791.7	0.51
PR concentration[c]								
<10	24	957.0	899.6	0.72	23	805.7	714.9	0.43
≥10, <100	28	1058	1071	0.94	29	784.1	678.6	0.09
≥100	19	1163	1066	0.64	20	718.6	782.6	0.48

[a] No. of case-control pairs in stratum.
[b] *P* calculated from paired *t* test.
[c] PMOL/g protein; P, pico.

combined effect of high DDE and high total PCBs was explored by examining the matched OR for being in the top quartile for both organochlorines. A total of 15 breast cancer cases and 19 controls fell in the top quartile for both; the matched OR was 0.75. Conditional logistic regression models examining the relationship between the individual pesticides or metabolites, PCB congeners, and PCB groups and breast cancer risk, controlling for the various covariates both singly and in combination, found that none of the covariates changed either the magnitude or direction of the unadjusted estimate. Therefore, only the unadjusted data are presented.

Table 3 presents data for DDE and total PCBs stratified by age at diagnosis, interval between blood sample and diagnosis, and ER and PR concentration. Unlike the analyses in the total group, in the stratified analyses, mean concentrations among cancer cases were not uniformly lower than means among controls. For DDE, higher levels among cancer cases compared with controls were observed for women age 50 years and older at diagnosis and for cancer cases with ≥10 years between blood sample and diagnosis. For total PCBs, slightly higher concentrations were found in cancer cases than in controls for women age 50 years and older at diagnosis, and for the highest tertiles of ER and PR concentration. However, none of the observed differences were statistically significant. No significant differences were noted in stratified analyses for the other pesticides or their metabolites and PCB groups.

Table 4 summarizes results for those organochlorines with ≥1 but <90% of samples above the LD. There were no samples above the LD for six dioxins and dibenzofurans, including 2,3,7,8-TCDD, for which the LD ranged from 0.22 to 1.7 fg/g serum. The data in Table 4 should be interpreted with caution, especially for those organochlorines with <50% of samples above the LD. Among the chemicals listed, dieldrin was of particular interest because a study published in January 1998 found elevated serum concentrations of dieldrin in Danish breast cancer cases compared with controls (7). In the Danish study, in which serum samples were collected in 1976, 78% of all samples had detectable levels of dieldrin, and the median concentration was 24.42 ng/g lipid. In our study, 67.9% of samples had detectable values, and the median concentration was 16.1 ng/g lipid. The matched OR for having a detectable concentration was 1.00. The mean concentration of dieldrin in 112 case-control pairs where both had detectable values was 23.3 pg/g lipid among cancer cases and 26.1 pg/g lipid among controls. None of the other organochlorines reported in Table 4 showed a statistically significant positive association with breast cancer.

Because no significant associations were found between organochlorine levels in serum and breast cancer risk, data from the two groups were combined for a descriptive analysis of trends in organochlorine concentration by date of blood sample. Some compounds showed rather dramatic decreases over time, *e.g.*, *p,p*-DDT, whereas others, such as the highly persistent group 3 PCB congeners, showed only a slight trend (Table 5). Temporal trends observed in this study are consistent with data about uses of organochlorine pesticides and PCBs in Norway, *e.g.*, the use of DDT was severely restricted in 1970 and a ban was imposed in 1980; the general use of PCBs was restricted in 1971, and in 1979 their use was restricted to closed systems only (29). There was no evidence for differences between occupational categories with respect to any of the six pesticides or metabolites, total PCBs, or PCB groups. For all of these individual compounds or groups, the distributions of occupational categories among women in the highest decile of organochlorine level was similar to that in the remainder of the population, and there were no differences in mean organochlorine levels by occupational category, controlling for specimen date (data not shown).

Discussion

The current study did not find any evidence for an association between organochlorine levels in serum and breast cancer. For two of the organochlorines studied (*p,p′*-DDE and total PCBs), there have been a number of large and well-conducted studies with which our results can be compared. For other pesticides or metabolites and individual PCB congeners, data are considerably more limited. An unexpected finding in our study was the consistent pattern of slightly lower organochlorine levels in cancer cases compared with controls.

Table 6 summarizes data from eight previous studies examining the relationship between *p,p′*-DDE levels in serum and breast cancer. In comparing serum organochlorine levels between studies, it is important to note that previous studies have reported concentrations in a number of units (ppb, ng/ml, and ng/g). The levels measured in different studies are, however,

Table 4 Organochlorines with >0 and <90% detectable values: odds of having a concentration above the LD among breast cancer cases compared with controls

Organochlorine measured[a]	LD range[b]	No. detected	Discordant pairs		OR	95% CI[c]
			Case detected/ Control not detected	Case not detected/ Control detected		
Hexachlorobenzene	0.22–5.1	105	13	21	0.6	0.3–1.3
g-HCCH	0.08–0.86	7	3	4	0.7	0.1–4.0
Aldrin	0.01–0.08	3	1	2	0.5	0.0–6.5
Dieldrin	0.05–0.47	176	11	11	1.0	0.4–2.6
Endrin	0.10–0.81	6	0	6	0.1	0.0–0.7
o,p′-DDT	0.05–1.11	175	16	15	1.1	0.5–2.4
Mirex	0.02–0.18	13	3	9	0.3	0.1–1.2
1,2,3,7,8-penta D	0.11–0.51	15	6	6	1.0	0.3–3.7
1,2,3,4,6,7,8-hepta D	0.52–1.98	136	24	29	0.8	0.4–1.5
1,2,3,4,6,7,9-hepta D	0.11–0.41	39	19	14	1.4	0.6–3.1
OCDD	2.88–23.5	225	16	19	0.8	0.4–1.8
2,3,7,8-tetra F	0.07–0.62	12	4	8	0.5	0.1–1.8
2,3,4,7,8-penta F	0.11–1.01	170	16	23	0.7	0.3–1.4
1,2,3,4,7,8-hexa F	0.07–0.31	52	10	16	0.6	0.3–1.5
1,2,3,6,7,8-hexa F	0.07–0.38	44	13	14	0.9	0.4–2.2
2,3,4,6,7,8-hexa F	0.07–0.41	19	9	6	1.3	0.4–4.1
1,2,3,4,6,7,8-hepta F	0.14–1.05	195	17	14	1.2	0.5–2.8
1,2,3,4,7,8-hexa D	0.07–0.54	1	0	1	0.1	0.0–10.3
OCDF	1.21–17.1	2	0	2	0.1	0.0–2.7
3,3′,4,4′-tetra P	2.45–15.2	15	5	6	0.8	0.2–3.2
3,3′,4,5-tetra P	0.15–1.38	43	14	16	0.9	0.4–2.0
2,2′,5-tri P	0.07–0.27	60	25	22	1.1	0.6–2.2
2,4,4′-tri P	0.12–1.21	134	33	30	1.1	0.6–2.0
2,2′,5,5′-tetra P	0.08–0.44	21	7	11	0.6	0.2–1.8
2,2′,4,5′-tetra P	0.03–0.19	38	11	13	0.8	0.3–2.1
2,2′,3,5′-tetra P	0.05–0.26	42	13	13	1.0	0.4–2.4
2,3′,4,4′-tetra P	0.02–0.07	203	15	13	1.2	0.5–2.7
2,2′,4,5,5′-penta P	0.11–0.30	13	5	8	0.6	0.2–2.1
2,2′,3,4,5′-penta P	0.02–0.07	44	15	14	1.1	0.5–2.5
2,3,3′,4,4′,6-hexa P	0.06–0.15	8	4	4	1.0	0.2–5.0
2,2′,3,5,5′,6-hexa P	0.04–1.11	40	15	15	1.0	0.4–2.3
2,2′,3,4′,5′,6-hexa P	0.10–0.29	3	1	2	0.5	0.0–6.6
2,2′,3,3′,4,4′-hexa P	0.01–0.03	198	22	26	0.8	0.4–1.6
2,3,3′,4,4′,5-hexa P	0.01–0.03	255	9	14	0.6	0.2–1.6
2,3,3′,4,4′,5,5′-hepta P	0.01–0.10	144	24	36	0.6	0.3–1.1
2,2′,3,3′,4,4′,5,6-octa P	0.01–0.07	253	4	13	0.3	0.1–0.9

[a] D, dibenzodioxin; F, dibenzofuran; P, polychlorinated biphenyl.
[b] In ng/g serum for pesticides, metabolites, and PCB congeners and fg/g serum for dioxins and furans.
[c] ORs and CIs were calculated as weighted averages over all strata (with each matched pair as its own strata) in which the weights were the inverse variance of the log ORs (24).

directly comparable because 1 ppb is equal to 1 ng/g and 1 ml serum weighs ~1.026 g. DDE concentrations measured among controls in these studies ranged from 2.9 ppb in samples collected in Columbia in 1998 (8) to 43.1 ppb in samples collected in the United States in the 1960s (Ref. 5; Table 7); the average concentration measured among controls in our study was 8.2 ng/g serum (Table 2). Two previous studies reported positive findings (1, 8), and a third (5) reported nonsignificant positive trends in ORs by tertile for whites and blacks, but not for Asians. Recent studies have found an association between serum levels of *p,p′*-DDT and breast cancer (13) and higher levels of *p,p′*-DDE in the plasma of women with large breast tumors and lymph node involvement (14). Among five relatively small studies measuring DDE in breast tissue (2, 28, 30–32), Falck *et al.* (2) reported higher concentrations of DDE in breast fat samples from unselected breast cancer cases compared with controls with nonmalignant breast disease, whereas Dewailly *et al.* (28) found increased DDE concentrations only in ER-positive cancer cases compared with controls with nonmalignant breast disease. One large case-control study of breast cancer in Europe, which examined DDE concentrations in adipose tissue collected by aspiration from the buttocks, found decreased DDE concentrations in cancer cases compared with controls, a difference that was more pronounced after adjustment for BMI, age at first birth, and current alcohol drinking (33). Two large recent studies, conducted in Germany and Canada, found no significant difference between DDE concentrations of breast cancer cases and controls (34, 35). *p,p′*-DDE, *o,p*-DDT, and *p,p′*-DDT are considered estrogenic and give positive results in the E-Screen assay (36), although *o,p*-DDT, which was not detectable in our study, appears to be the most highly estrogenic of these compounds (4, 36). Although there have been conflicting results in human studies, our study results and accumulating evidence from several recent studies do not support the hypothesis that breast cancer is associated with environmental exposure to DDT or DDE.

Table 7 summarizes data from seven previous studies examining the relationship between PCB levels in serum and breast cancer. PCB concentrations measured among controls in these studies ranged from 2.2 ng/ml in samples collected in the United States in 1989 (9) to 8.7 ng/ml in samples collected in Denmark in 1976 (Ref. 7; Table 7); the average concentration

Table 5 Temporal trends in organochlorine levels measured in serum samples of Norwegian women, 1973–1990

Pesticide or pesticide metabolite, or PCB congeners by Wolff group	Mean lipid-adjusted concentration (ng/g lipid) by yr of sample[a]				*P* for trend test
	1973–1975 (*n* = 83)	1976 (*n* = 70)	1977–1982[b] (*n* = 60)	1985–1990 (*n* = 85)	
B-HCCH	81.3	80.1	71.4	19.3	0.001
Heptachlor epoxide	10.1	9.4	8.0	4.2	0.0002
Oxychlordane	13.7	10.9	11.5	6.1	0.0002
Trans-nonachlor	13.9	10.2	10.4	9.5	0.0002
p-p′-DDE	1890	1632	1031	444.4	0.04
p,p-DDT	225.3	163.8	103.4	19.8	0.004
Group 1	70.3	67.7	67.3	53.4	0.001
Group 2A	146.6	127.9	132.4	74.5	0.001
Group 2B	55.1	55.4	52.0	43.7	0.0007
Group 2 (total)	199.6	180.1	183.6	117.1	0.002
Group 3	455.2	446.6	426.0	345.4	0.006
Total PCBs	890.2	848.4	839.0	622.7	0.010

[a] Cut points were selected to yield approximately equal numbers of samples in each interval.
[b] There were no blood samples collected in 1983 and 1984.

measured among controls in our study was 5.1 ng/g serum (Table 7). None of the previous studies evaluating the relationship between total PCBs in serum and breast cancer has reported a strongly positive relationship (Table 7). Wolff *et al.* (1) found a higher concentration of PCBs in the serum of breast cancer cases compared with controls, but this association was reduced after control for levels of DDE (Table 7). Krieger *et al.* (5) found a nonsignificant negative association for white and Asian women, but a slightly (nonsignificantly) increasing trend in OR by tertile for black women. In the remaining serum studies, there were no significant associations between total serum PCBs and breast cancer. One previous case-control study of breast cancer restricted to postmenopausal women has measured individual PCB congeners but reported results for congeners grouped by degree of chlorination (10). Findings in that study included a modest increase in risk for women with detectable levels of less chlorinated PCBs (OR, 1.66; 95% CI, 1.07–2.88), and among parous women who had never lactated, an increased risk associated with higher total PCBs, moderately chlorinated PCBs, and greater numbers of PCB congeners detected. A subsequent report from the same study found that the *CYP1A1* genotype modified the relationship between PCB levels and breast cancer risk (37). There is some evidence that the *CYP1A1* variant phenotype for which an association between PCB levels and breast cancer was present results in *CYP1A1* activity that is more inducible in lymphocytes (38). Moysich *et al.* (37) hypothesize that greater induction of *CYP1A1* by PCBs in individuals with the variant phenotype may lead to increased breast cancer risk, either through the mechanism of increased metabolism of estradiol to the possibly mutagenic catechol estrogens, or through increased activation of environmental carcinogens. Among studies of total PCBs in adipose tissue of breast cancer cases and controls, one found a positive association (2) and two found no association (28, 30). Three studies measured specific PCB congeners and found positive associations with some of them (31, 34, 35).

A major difficulty in interpreting the human data on PCB exposure and breast cancer is that the PCB congeners with the highest potential estrogenicity are also the most quickly metabolized (26). Exposure to these short-lived congeners may or may not be correlated with exposure to the more persistent congeners, depending on the commercial PCB products from which the contamination arose (39). Measurement of stable PCB congeners during midlife may not reflect exposure to the relatively short-lived, more estrogenic, congeners early in life, which may be a sensitive period for breast cancer induction (40). Moreover, there are many potential toxicities associated with PCB congeners, some of which are antiestrogenic, and which may vary, for example, with respect to P-450 enzyme induction and other properties (41). For these reasons, Hansen (41) has encouraged the reporting of congener-specific data in studies of the relationship between PCB exposure and health effects, and Moysich *et al.* (42) have proposed the use of multiple congener groupings in epidemiological research. In our study, the trends in ORs by quartile were uniformly negative, so it was not considered useful to analyze congeners by additional groupings. However, levels of specific congeners are reported to facilitate comparison with future studies conducting congener-specific analyses.

No associations with breast cancer risk were observed for four other organochlorines (*B*-HCCH, heptachlor epoxide, oxychlordane, and *trans*-nonachlor) evaluated in this study. *B*-HCCH has estrogenic properties (43, 44), and previous epidemiological studies have yielded conflicting results (7, 11, 13, 28, 30, 34, 35). Previous toxicological and epidemiological studies of heptachlor epoxide, oxychlordane and *trans*-nonachlor do not suggest any association with breast cancer risk (2, 11, 13, 28, 30, 35, 45, 46).

The consistency of the slightly negative associations found between specific organochlorine levels and breast cancer risk in our study warrants some discussion. Some of the possible explanations for this unexpected consistency are methodological, including systematic bias in the selection of either cancer cases or controls, overmatching, which could lead to bias toward the null, or random sampling error, which could have caused differences in the distribution of important predictors of organochlorine levels among cancer cases and controls. We evaluated these possibilities through review of the methods used for selecting cancer cases and controls, and by examining available covariate data and stratified organochlorine results. We did not find any evidence for systematic bias in the procedures used to select cancer cases and controls. We were concerned that matching by date of specimen would have inadvertently caused overmatching by region of residence, because the health surveys that collected the blood samples contributed to the Janus Serum Bank were conducted in different parts of Norway at different times. However, cancer cases and controls were not more likely to be matched on region of residence than

Table 6 Summary of previous case-control studies of DDE in serum or plasma

Reference	Country/United States region	Period samples collected	No. cases/controls	Interval between blood sample and diagnosis	% postmenopausal	Mean DDE concentration in serum[a,b]		ORs by tertile, quartile (Q1–Q4) or quintile (Q1–Q5)[a]				
						Controls	Cases	Q1	Q2	Q3	Q4	Q5
Wolff *et al.*, (1) 1993	Northeast	1985–1991	58/171	<6 mo	58.6	7.7 ppb	11.0 ppb	1.0	1.7	4.4	2.3	3.7
Krieger *et al.*, (5) 1994	Northern California	1964–1969	150/150	>6 mo; mean, 14.2 years	74.6	43.1 ppb	43.3 ppb	1.0	1.3	1.3		
Hunter *et al.*, (6) 1997	11 States	1989–1990	240/240	None required; dx. <6/1/92	68.0	6.97 ppb	6.01 ppb	1.0	0.8	0.5	0.7	0.7
Moysich *et al.*, (10) 1998	Western New York	1986–1991	154/192	Within 3 mo after diagnosis	100	10.8 ng/g	11.5 ng/g	1.0	1.0	1.3		
Høyer *et al.*, (7) 1998	Denmark	1976	240/477	None required; diagnosis <12/31/93	70.0	10.5 ng/ml	10.2 ng/ml	1.0	0.8	0.8	0.9	
Olaya-Contreras *et al.*, (8) 1998	Bogota, Columbia	1995–1996	153/153	After diagnosis	60.1	2.9 ppb	3.3 ppb	1.0	1.2	1.9		
Helzlsouer *et al.*, (9) 1999	Maryland	1974, 1979, or both	1974: 235/235	1974: 2 yr	1974: 55.3% >50	13.6 ng/ml	11.5 ng/ml	1.0	0.9	0.8	0.7	0.5
			1989: 105/105	1989: no minimum	1989: 75.2% >50	9.7 ng/ml	7.9 ng/ml	1.0	1.8	0.5		
Høyer *et al.*, (13) 2000	Denmark	1976–1978 1981–1983	155/274	Diagnosed after 2nd blood sample	68% 1st exam, 84% 2nd exam	NR[c]	NR	1.0	1.0	0.8	1.4	
Demers *et al.*, (14) 2000	Canada	1994–1997	315/219 H[e] 307 P[e]	After diagnosis	NR	NR	NR	H[e] 1.0 P[e] 1.0	0.8 0.7	0.7 1.1	1.5 0.9	1.4 1.0
Wolff *et al.*, (15) 2000	Northeast United States	1985–1990	148/295	6 mo	63.5	7.3 ng/ml	6.9 ng/ml	1.0	0.8	0.6	1.3	

[a] If the reference cited included both unadjusted and adjusted means and ORs, adjusted values were selected for the summary.
[b] Some papers report concentrations in units of ppb, others in units of ng/ml, and others in units of ng/g. These units are essentially equivalent because ppb is equivalent to ng/g, and 1 ml serum = 1.026 grams.
[c] NR, not reported.
[d] Median concentrations.
[e] H, hospital; P, population.

Table 7 Summary of previous case-control studies of total PCBs in serum

Reference	Country/United States region	Period samples collected	No. cases/control	Interval between blood sample and diagnosis	% of cases postmenopausal	Mean PCB concentration in serum[a,b]		ORs by tertile (Q1–Q3), quartile (Q1–Q4), or quintile (Q1–Q5)[c]				
						Controls	Cases	Q1	Q2	Q3	Q4	Q5
Wolff *et al.*, (1) 1993	Northeast	1985–1991	58/171	<6 mo	58.6	6.7 ppb	8.0 ppb	1.0	5.2	7.0	4.1	4.3
Krieger *et al.*, (5) 1994	Northern California	1964–1969	150/150	>6 mo Mean: 14.2 yr	74.6	4.8 ppb	4.4 ppb	1.0	1.2	0.9		
Hunter *et al.*, (6) 1997	11 States	1989–1990	240/240	None required; diagnosis <6/1/92	68.0	5.16 ppb	5.08 ppb	1.0	0.6	0.5	0.5	0.7
Moysich *et al.*, (10) 1998	Western New York	1986–1991	154/192	Within 3 mo after diagnosis	100	4.12 ng/g	4.29 ng/g	1.0	0.7	1.1		
Høyer *et al.*, (7) 1998	Denmark	1976	240/477	None required; dx. <12/31/93	70.0	8.7 ng/ml	8.6 ng/ml	1.0	0.9	0.8	1.1	
Dorgan *et al.*, (11) 1999	Missouri	1977–1987	105/208	None required; mean 2.7 yr	79.0	2.8[d] ng/ml	NR[d]	1.0	0.7	1.1	0.7	
Helzlsouer *et al.*, (9) 1999	Maryland	1974, 1979, or both	1974: 235/235 1989: 105/105	1974: 2 yr 1989: no minimum	1974: 55.3% >50 1989: 75.2% >50	4.7 ng/ml 2.2 ng/ml	4.9 ng/ml 2.1 ng/ml	1.0 1.0	1.1 0.6	0.8 0.7	0.7	0.7
Høyer *et al.*, (13) 2000	Denmark	1976–1978 1981–1983	155/274	Diagnosed after 2nd blood sample	68% 1st exam, 84% 2nd exam	NR	NR	1.0	0.8	0.8	1.6	
Wolff *et al.*, (15) 2000	Northeast United States	1985–1990	148/295	6 mo	63.5	5.0 ng/ml	5.0 ng/ml	1.0	1.5	1.2	2.0	

[a] If the reference cited included both unadjusted and adjusted means and ORs, adjusted values were selected for the summary.
[b] Some papers report concentrations in units of ppb, others in units of ng/ml, and others in units of ng/g. These units are essentially equivalent because ppb is equivalent to ng/g and 1 ml serum = 1.026 grams.
[c] Median concentrations.
[d] NR, not reported.

would have occurred by chance. Finally, although it appeared (Table 1) that there were some differences in region of residence and region of birth between cancer cases and controls, the pattern of regional differences varied by compound, and controlling for region of birth and region of residence in the analysis did not in any way alter the outcome.

Aside from methodological issues, one possible explanation for the consistently negative associations is that, in the absence of a significant etiological relationship between organochlorines and breast cancer, there may be a tendency toward a slightly negative relationship through an association of organochlorine levels with one or more unmeasured negative risk factor. For example, some studies show that higher levels of fish consumption are protective against breast cancer, possibly attributable to the n-3 PUFAs fish contain (47). Fish are also a dietary source of PCB's (48). However, a previous study conducted using samples from the Janus Serum Bank found no relationship between levels of n-3 PUFAs in serum and breast cancer risk (49). Neither fish consumption nor n-3 PUFAs were measured in the current study.

Other studies have reported negative trends in ORs with increasing levels of total PCBs and DDE (6, 9).

When our data were stratified according to age of diagnosis of breast cancer as a surrogate for menopausal status, DDE showed slightly positive relationships for women older than age 50 years and negative relationships for women younger than age 50 years, and both DDE and total PCBs showed higher concentrations in cancer case sera compared with control sera collected >10 years before diagnosis. Some breast cancer risk factors differ between pre- and postmenopausal women. For example, a prospective study of 24,329 Norwegian women found an inverse relation between serum cholesterol and risk of breast cancer that was confined to women younger than age 51 years (50). We had no data on several risk factors for breast cancer that may also be related to serum organochlorine levels, including height, BMI, and lactation history, some of which have been controlled for in the analysis of other studies. Our population differed from other populations in which the association between serum organochlorines and breast cancer has been studied by having a preponderance of women younger than age 50 years (60.4%), which may have caused organochlorine levels in our study to be more influenced by unmeasured biological correlates of premenopausal breast cancer. It is also possible that some organochlorines, particularly those which are antiestrogenic, may have a protective effect for breast cancer.

The major strengths of this study are its prospective design, which ensured that the blood samples had been collected at least 2 years before diagnosis, and that serum organochlorine levels were not affected by disease status, the number of organochlorine compounds for which measurements were available, and the availability of some data on potential confounders including age at first birth and number of children prior to blood sample donation. The major limitations include the absence of data on two important confounders: menopausal status and BMI. BMI has recently been found to be inversely correlated with PCB levels in serum (15). Because of limitations in the occupational history information available for study participants and the relatively small sample size, the occupational categories used in the analysis were quite broad. We were unable to examine association of organochlorine levels with specific occupations that are historically likely to have substantial organochlorine exposure, such as women working in the manufacture of electrical transformers and capacitors.

In summary, our study did not provide evidence that

increased breast cancer risk is associated with serum levels of the organochlorines measured. The evidence to date on the association between serum organochlorines is not consistent, but suggests that exposure to the organochlorines commonly measured (DDE and total PCBs, a measure that reflects primarily the more highly chlorinated, persistent congeners) is not an important risk factor for breast cancer in the general population, at least in the countries where studies have been conducted. Studies to date have not been able to evaluate whether exposure to the more highly estrogenic, short-lived PCB congeners increases breast cancer risk, nor have they fully evaluated the risk associated with organochlorine exposure in susceptible subgroups or at levels above general population exposure, including women with occupational exposure.

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