

Serum organochlorine pesticides and PCBs and breast cancer risk: results from a prospective analysis (USA)

Joanne F. Dorgan^{1,*}, John W. Brock², Nathaniel Rothman¹, Larry L. Needham², Rosetta Miller³, Hugh E. Stephenson Jr.⁴, Nicki Schussler⁵, Philip R. Taylor⁶

¹Division of Cancer Epidemiology and Genetics, National Cancer Institute, Executive Plaza North, Room 443, 6130 Executive Boulevard, Bethesda, MD 20892–7374, USA; ²Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA; ³Ellis Fischel Cancer Center, Columbia, MO, USA; ⁴Growdon Distinguished Professor of Surgery Emeritus, University of Missouri Health Sciences Center, Columbia, MO, USA; ⁵Information Management Services, Inc., Silver Spring, MD, USA; ⁶Division of Clinical Sciences, National Cancer Institute, Bethesda, MD, USA (*Author for correspondence)

Received 11 March 1998; accepted in revised form 16 July 1998

Key words: breast neoplasms, epidemiology, organochlorine pesticides, polychlorinated biphenyls.

Abstract

Objective: To prospectively evaluate relationships of organochlorine pesticides and polychlorinated biphenyls (PCBs) with breast cancer, we conducted a case-control study nested in a cohort using the Columbia, Missouri Breast Cancer Serum Bank.

Methods: Women donated blood in 1977–87, and during up to 9.5 years follow-up, 105 donors who met the inclusion criteria for the current study were diagnosed with breast cancer. For each case, two controls matched on age and date of blood collection were selected. Five DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane] analogs, 13 other organochlorine pesticides, and 27 PCBs were measured in serum.

Results: Women in the upper three quartiles of hexachlorobenzene were at twice the risk of breast cancer compared to those in the lowest quartile. However, there was no evidence for a dose-response relationship, and the association was limited to women whose blood was collected close to the time of diagnosis. Women with higher serum levels of other organochlorine pesticides and PCBs showed no increased risk of breast cancer overall, although positive associations were suggested for PCB-118 and PCB-138 when blood was collected close to the time of diagnosis.

Conclusions: Results of this study do not support a role for organochlorine pesticides and PCBs in breast cancer etiology.

Introduction

Although manufacture of chlorinated pesticides and polychlorinated biphenyls (PCBs) was discontinued in the US in the 1970s, these compounds persist in humans, animals, and the environment [1]. Many organochlorine pesticides and PCBs exhibit sex hormone activity and have potential for influencing risk of hormone dependent cancers including breast cancer. Because of their structural similarity to estradiol, some of these compounds and their metabolites bind to the estrogen receptor and exhibit estrogenic activity [2]. Conversely, others either bind to the aryl hydrocarbon receptor and act as antiestrogens or bind to the

androgen receptor. Several organochlorines also induce cytochrome P450 enzymes involved in the metabolism of estrogens [3]. Because the cytochrome P450s also metabolize xenobiotics and chemical carcinogens [4], their induction by organochlorine compounds could also affect breast cancer risk through non-hormonal mechanisms. Modulation of signal transduction and cross-talk between growth regulatory pathways and oxidative damage to DNA are other non-hormonal mechanisms through which organochlorine pesticides and PCBs could potentially influence breast cancer risk [5, 6].

Relationships of serum concentrations of DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene], a stable

metabolite of the organochlorine pesticide DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane], and PCBs to breast cancer have been evaluated in three prospective epidemiologic studies. Women with high serum levels of DDE or PCBs were at an increased risk of breast cancer in one study [7]. In a second study, high serum levels of DDE were associated with an increased risk of breast cancer in Caucasians and Blacks but not in Asians [8]. However, in a recent analysis from the Nurses' Health Study [9], serum levels of DDE and PCBs were not associated with breast cancer risk. Two recent case-control studies also did not report associations of serum organochlorine pesticides and PCBs for all women [10, 11].

The Columbia Missouri Breast Cancer Serum Bank was established by the National Cancer Institute as part of its Biological Markers Project to identify serum markers for breast cancer. Most serum samples for this serum bank were collected in the late 1970s and early 1980s, shortly after manufacture of organochlorine pesticides and PCBs was banned in the US. We conducted a nested case-control study using this serum bank to prospectively evaluate, in a recently exposed population, associations of serum levels of organochlorine pesticides and PCBs with breast cancer risk.

Materials and methods

Women who donated serum to the Columbia Missouri Breast Cancer Serum Bank were volunteers identified primarily through the Breast Cancer Detection and Demonstration Project in Columbia. A total of 7,224 women who initially were free of breast cancer donated blood to the bank on one or more occasions between 1977 and 1987. Over 90% of the women first gave blood in 1980 or earlier. Active follow-up by mail continued until 1989, but 70% of the cohort were last contacted in 1982–83, at least partly because of funding changes. At the time of last contact, 91% of the total cohort were alive and free of breast cancer, two percent had been diagnosed with breast cancer, and five percent were dead from a cause other than breast cancer. Pathology reports were obtained for all women who reported a malignant breast biopsy or mastectomy on follow-up.

Women included in the current study were restricted to those who had at least four mL of serum remaining in the bank and who, at the time of blood collection, had no history of cancer other than non-melanoma skin cancer. Of the 6,426 women who met these criteria, 105 subsequently were diagnosed with histologically confirmed breast cancer. For each of these

cases, two controls were selected from among the eligible women using incidence density sampling. Controls were alive and free of cancer (except non-melanoma skin cancer) at the age of the case's diagnosis and were matched to the case on year of age (± 1), month (± 2), and year (± 1) of blood draw, history of benign breast disease at the time of enrollment, and for women who donated blood on more than one occasion, sequence number of blood draw. For nine cases, only one control who met these criteria could be identified, and for eight cases, no controls could be found. For these 17 cases, matching criteria were relaxed to identify 25 controls as follows: (i) age ± 2 years ($n = 6$) or ± 3 years ($n = 1$); (ii) blood draw ± 3 –4 months ($n = 11$) or ± 2 years ($n = 1$); (iii) any benign breast disease status ($n = 4$); (iv) any blood draw sequence number ($n = 1$); and (v) age ± 5 years, blood draw ± 3 years ($n = 1$). Five women were included in the study more than once, as the control for two cases ($n = 2$), as a case and as a control for another case ($n = 2$), and as a case and as a control for two other cases ($n = 1$). Serum samples from two controls were not analyzable. Consequently, 105 cases and 208 controls were included in analyses.

After obtaining informed consent, serum specimens were collected and clinical data including age, height, weight, menstrual and reproductive histories, smoking, medication (including hormone) use, and family history of breast cancer, were obtained by self-report or medical record review. Approximately 10 mL of serum were collected from each woman using standard procedures. Serum was aliquoted into glass vials within 2 hours of collection and subsequently stored at -70 °C. The median time from serum collection to measurement of pesticides and PCBs was 16.7 years for both cases and controls.

All assays were performed by the National Center for Environmental Health, Centers for Disease Control by personnel masked to case status. Organochlorine pesticides and PCBs were measured by electron capture detection after solid-phase extraction and cleanup followed by dual-column gas chromatography [12]. Measured values were corrected for recovery using analyte-specific average recoveries for the laboratory. Results were obtained for: five DDT analogs (*o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, and *p,p'*-DDD), hexachlorobenzene, lindane (γ -hexachlorocyclohexane) and its constituent β -hexachlorocyclohexane, heptachlor and its metabolite heptachlor epoxide, chlordane (α - and γ -isomers) and its metabolite oxychlordane, *trans*-nonachlor (a component of technical grade chlordane and heptachlor), aldrin, dieldrin, endrin, mirex, and 27 PCB congeners. Serum cholesterol and triglycerides were measured on a Kodak Ektachem 250 Dry Chemistry

Analyzer. Lipid corrected levels of pesticides and PCBs were calculated by dividing their serum concentrations by total serum lipids calculated by the formula $\text{total lipids} = 0.623 + 2.27 \times \text{total cholesterol} + \text{triglycerides}$ [13].

Thirty-five masked quality control samples made by pooling serum from blood donors to the serum bank who did not fulfill the criteria for inclusion in the current study were analyzed with study samples. Coefficients of variation (CV) for total DDT and total PCBs were 20% and 30%, respectively. CVs for the individual pesticides and PCBs reported in detail in this manuscript were: *p,p'*-DDE, 21% *p,p'*-DDT, 60% β -hexachlorocyclohexane, 48% hexachlorobenzene, 33% dieldrin, 82% PCB-118, 16% and PCB-138, 24%. The high CVs for some analytes were probably due in part to the low concentrations of pesticides and PCBs in the samples. Quality control sample variances expressed as a percent of the population variance estimated from our study controls were less than five percent for *p,p'*-DDT, *p,p'*-DDE, β -hexachlorocyclohexane, dieldrin, and PCB118; 12% for PCB138; and 35% for hexachlorobenzene.

Demographic characteristics of cases and controls were compared using conditional logistic regression [14]. Continuous variables were transformed to the log scale prior to analysis and descriptive statistics are reported as geometric means and standard deviations. Relationships of serum levels of organochlorine pesticides and PCBs to breast cancer risk for the matched sets were also evaluated using conditional logistic regression. Because of the extremely low levels of many organochlorine pesticides and PCBs in serum samples, initial analyses compared percentages of cases and controls above the assay limit of detection (LOD). Tests of significance were performed using conditional logistic regression after categorizing individuals. Total serum lipids were included in these models as a covariate to adjust for their influence on serum pesticide and PCB levels [13]. Exposure hazard ratios were calculated as estimates of relative risk (RR) for pesticides and PCBs for which more than 50% of participants had serum levels above the assay LOD. For these analyses, pesticides and PCBs were expressed per gram of serum lipids to remove the effect of lipids on serum concentrations before establishing quartiles. Women were stratified into quartiles based on their levels relative to the distribution in controls and a set of categorical (dummy) variables was included in models. Because of the smaller number of participants, tertiles were used in some subgroup analyses. Models also were fit using continuous data to test for trends. Established breast cancer risk factors and personal characteristics related to risk in our data were evaluated as potential con-

founders; included were height, weight, body mass index (BMI) ($\text{weight [kg]}/\text{height [m}^2\text{]}$), parity, age at menarche, menopausal status, exogenous estrogen use, history of breast cancer among first degree relatives, education, and number of packs of cigarettes smoked per day. Individual characteristics related to pesticide or PCB levels were included in preliminary models, but none confounded associations with breast cancer. Final models included only serum pesticide or PCB levels expressed per gram of lipid. Interactions of pesticides and PCBs with age (a matching criterion), menopausal status, date of blood collection, and time from blood collection to diagnosis were tested by including cross products terms in models. All analyses were performed using SAS Statistical Software [15].

Results

Characteristics of cases and controls at the time of blood collection are summarized in Table 1. All but one case and one control were White. The mean age of both groups was 57.4 (± 9.9) years and the majority of both cases and controls were postmenopausal. Although cases were somewhat more likely to be nulliparous than controls, cases and controls did not differ significantly on any of the reproductive or menstrual characteristics investigated. A history of breast cancer among mothers and sisters was reported more frequently by cases than controls, but this difference also was not significant. Controls, however, were significantly ($P < 0.01$) more likely to smoke cigarettes compared to cases.

At the time of diagnosis of breast cancer, cases' mean age was 59.7 (± 10.3) years and 85.7% were postmenopausal. The majority of cancers (71%) were infiltrating ductal carcinomas. The median time from blood collection to diagnosis was 2.7 years with a range of 27 days to 9.5 years.

Serum levels of many of the pesticides and PCBs measured were extremely low and frequently below the assay limit of detection (LOD). Percentages of cases and controls with levels above the LOD are shown in Table 2 for each analyte. Adjusted for total serum lipids, the probability of having measurable serum levels above the LOD did not differ by case status for any of the pesticides or PCBs assayed.

More detailed analyses were performed for individual pesticides and PCBs with at least 50% of values above the assay LOD and for total DDT and total PCBs. As shown in Table 3, there was no evidence from our data of an increased risk of breast cancer among women with elevated serum levels of DDT or PCBs. Hexachlorobenzene was the only pesticide for

Table 1. Columbia, Missouri Breast Cancer Serum Bank. Characteristics of cases and controls at blood collection unless otherwise specified

	Cases (<i>n</i> = 105)	Controls (<i>n</i> = 208) ^a
Continuous variables	Mean (±SD) ^b	Mean (±SD) ^b
Age (yrs)	57.4 (±10.1)	57.4 (±9.9)
Height (cm)	162.4 (±5.1)	162.4 (±5.1)
Weight (kg)	68.0 (±11.4)	69.4 (±12.0)
Body mass index (kg/m ²)	25.8 (±4.0)	26.6 (±4.4)
Parity ^c	2.5 (±1.2)	2.5 (±1.3)
Age at first pregnancy (yrs) ^c	23.1 (±3.3)	22.6 (±3.9)
Age at menarche (yrs)	12.8 (±1.4)	12.8 (±1.5)
Age at menopause (yrs) ^d	48.9 (±4.1)	48.4 (±5.9)
Categorical variables	Percentage	Percentage
Education (yrs) ^e		
< 12	12.9	17.1
12	44.7	43.9
13–15	25.9	25.0
16+	16.5	14.0
Postmenopausal	79.0	82.7
Uses exogenous estrogens	21.0	17.8
Nulliparous	18.1	14.4
Smokes cigarettes	9.5	21.2
Benign breast disease diagnosis in prior 2 years	10.5	8.6
Positive history of breast cancer in first degree relative	16.2	10.1

^a Five participants included in the study more than once, as a case and a control or as a control for more than one case, counted multiple times.

^b Geometric mean and standard deviation of geometric mean.

^c Parous women only (86 cases, 178 controls).

^d Postmenopausal women only (83 cases, 172 controls).

^e Data available for 85 cases and 164 controls.

which there was any evidence of a positive association with breast cancer. Women in the upper three quartiles were at twice the risk of breast cancer compared to those in the lowest quartile, and for those in the second and fourth (highest) quartiles, the RR was significant ($P < 0.05$). However, there was no evidence for a dose-response relationship. Women with the highest levels of *p,p'*-DDT were at a significantly ($P = 0.04$) reduced risk of breast cancer compared to those with the lowest levels, but there was no gradient in risk across quartiles. A tendency towards a lower risk of breast cancer for women with elevated levels of β -hexachlorocyclohexane also was apparent.

Tests for interaction did not suggest effect modification of organochlorine pesticide and PCB-breast cancer associations by menopausal status at blood collection. Additionally, restriction of analysis to cases who were postmenopausal at diagnosis did not yield markedly different results from those shown in Table 3 for pre- and postmenopausal cases combined, except that the test for trend was no longer significant for *p,p'*-DDT ($P = 0.12$). There also was no evidence for effect modification by date of blood collection. Time from blood collection to diagnosis, however, appeared to

modify associations of some organochlorine pesticides and PCBs with breast cancer. Tests for interaction based on continuous variables were statistically significant ($P \leq 0.03$) for hexachlorobenzene, PCB-118, and PCB-138. Table 4 presents RRs for these analytes separately for women diagnosed within 2.7 years (the midpoint) and for women diagnosed later in relation to blood collection. Hexachlorobenzene exhibited a significant positive association with breast cancer among women diagnosed close in time to blood collection, but not among women diagnosed later. Marginally significant positive associations with breast cancer risk also were observed for PCB-118 and PCB-138 for women diagnosed soon after blood collection. Conversely, a significant inverse association with PCB-138 was observed for women diagnosed later.

Serum concentrations of PCB-118 and PCB-138 were highly correlated in controls (Spearman's $r = 0.8$). When the relationships of these PCBs with breast cancer among women diagnosed more than 2.7 years after blood collection were evaluated simultaneously, the inverse relationship of PCB-138 with breast cancer was still apparent, but the trend was no longer significant ($P = 0.11$). Among all women, a significant inverse

Table 2. Columbia, Missouri Breast Cancer Serum Bank. Percent of participants with pesticide and PCB levels at or above the assay limit of detection (LOD)

Analyte	Assay LOD ^a (ppb)	% greater than or equal to LOD		P-value ^c
		Cases (n = 105)	Controls (n = 208) ^b	
Total DDT ^f				
<i>o,p'</i> -DDT	1.28	4.8	4.3	0.85
<i>p,p'</i> -DDT ^d	0.88	84.8	87.4	0.60
<i>o,p'</i> -DDE ^e	0.83	1.0	1.9	0.53
<i>p,p'</i> -DDE ^d	0.87	98.1	99.5	0.26
<i>p,p'</i> -DDD ^e	0.78	16.4	19.8	0.56
Chlordane and heptachlor ^d				
α-chlordane	0.33	0	0	
γ-chlordane ^d	0.32	0	0	
Oxychlordane ^d	0.29	16.2	13.0	0.55
Heptachlor	0.33	20.0	19.2	0.90
Heptachlor epoxide	0.29	0	0	
<i>trans</i> -nonachlor	0.39	49.5	42.8	0.22
Lindane				
β-hexachlorocyclohexane	0.32	79.0	85.6	0.12
γ-hexachlorocyclohexane	0.41	3.8	2.4	0.46
Aldrin and dieldrin ^d				
Aldrin	0.37	0	0	
Dieldrin ^d	0.29	56.2	61.8	0.26
Hexachlorobenzene	0.13	98.1	95.2	0.16
Endrin ^e	0.58	0	0	
Mirex ^e	0.50	0	0.5	0.99
Total PCBs ^f				
PCB-28	0.36	9.5	5.3	0.16
PCB-52	0.36	1.9	0	0.99
PCB-56 ^d	0.41	0	0	
PCB-66	0.29	5.7	5.3	0.87
PCB-74 ^d	0.26	19.0	17.9	0.77
PCB-99 ^e	0.70	0	1.0	0.99
PCB-101	0.32	1.0	0	0.99
PCB-105	0.34	2.9	2.9	0.99
PCB-110	0.25	0	0	
PCB-118 ^e	0.38	77.9	72.5	0.25
PCB-138 ^e	0.35	91.4	90.3	0.74
PCB-146	0.32	1.0	0.5	0.64
PCB-153 ^d	0.55	46.7	42.0	0.39
PCB-156 ^e	0.35	5.8	6.8	0.74
PCB-170	0.39	24.8	25.5	0.87
PCB-172	0.39	0	0	
PCB-178 ^e	0.61	0	0.5	0.99
PCB-180	0.37	19.0	22.1	0.50
PCB-183	0.41	0	0.5	0.99
PCB-187	0.33	3.8	2.9	0.66
PCB-189 ^d	0.50	0	0	
PCB-193 ^d	0.33	1.0	0	0.99
PCB-194	0.46	0	0.5	0.99
PCB-195 ^d	0.33	1.0	0.5	0.63
PCB-201 ^d	0.97	0	1.0	0.99
PCB-203	0.40	1.9	1.0	0.48
PCB-206	0.47	1.0	0	0.99

^a Recovery corrected.

^b Five participants included in the study more than once, as a case and a control or as a control for more than one case, counted multiple times.

^c Matched on age, benign breast disease diagnosis during prior 2 years, month and year of blood collection, and adjusted for total serum lipids.

^d One control with a missing value.

^e One case and one control with missing values.

^f One case and two controls with missing values.

association with breast cancer was observed for *p,p'*-DDT. Adjustment for *p,p'*-DDT did not materially change results for other pesticides and PCBs shown in Table 3.

Because of concerns about incomplete follow-up through the end of the study in 1989, we reanalyzed serum pesticide and PCB-breast cancer associations after truncating the follow-up period to 1982–83 when

Table 3. Columbia, Missouri Breast Cancer Serum Bank. Risk ratios (RR) and 95% confidence intervals (CI) for breast cancer by quartile of serum pesticides and PCBs (ng/g lipid)

Analyte	No. cases	No. controls ^a	RR ^b	95% CI	P-value ^c
Total DDT					0.65
204–1,646	31	50	1.0		
1,647–2,804	37	53	1.1	0.6–1.9	
2,805–4,020	10	53	0.3	0.1–0.7	
4,021–21,077	26	50	0.8	0.4–1.6	
<i>p,p'</i> -DDT					0.05
0–180	29	50	1.0		
181–292	29	53	1.0	0.5–2.0	
293–467	33	53	1.1	0.6–2.1	
468–1,724	14	51	0.4	0.2–1.0	
<i>p,p'</i> -DDE					0.77
31–1,377	33	50	1.0		
1,378–2,355	32	52	0.9	0.5–1.7	
2,356–3,500	14	54	0.4	0.2–0.8	
3,501–20,667	26	51	0.8	0.4–1.5	
β -hexachlorocyclohexane					0.65
0–75	39	51	1.0		
76–126	21	55	0.5	0.2–0.9	
127–176	18	50	0.5	0.2–0.9	
177–1,600	27	52	0.6	0.3–1.3	
Dieldrin					0.44
0–23	33	52	1.0		
24–51	21	51	0.7	0.3–1.3	
52–103	28	52	0.8	0.4–1.6	
104–921	23	52	0.7	0.3–1.3	
Hexachlorobenzene					0.38
0–62	13	50	1.0		
63–83	36	54	2.5	1.2–5.3	
84–105	26	53	1.9	0.9–4.3	
106–406	30	51	2.3	1.0–5.0	
Total PCBs					0.79
17–257	29	51	1.0		
258–369	21	53	0.7	0.3–1.4	
370–563	33	51	1.1	0.6–2.2	
564–2,682	21	51	0.7	0.3–1.5	
PCB-118					0.77
0–49	22	50	1.0		
50–74	25	52	1.1	0.6–2.3	
75–109	34	52	1.6	0.8–3.2	
110–533	23	53	1.0	0.5–2.2	
PCB-138					0.82
0–69	23	50	1.0		
70–93	29	52	1.3	0.6–2.5	
94–124	26	53	1.2	0.6–2.3	
125–359	26	52	1.2	0.6–2.4	

^a Five participants included in the study more than once, as a case and a control or as a control for more than one case, counted multiple times.

^b Matched on age, benign breast disease diagnosis during prior 2 years, month and year of blood collection.

^c Based on continuous variable.

follow-up was better than 90% complete. During this time, 78 cases of breast cancer were diagnosed. As can be seen in Table 5, risk of breast cancer in relation to serum pesticides and PCBs generally did not differ for this subgroup of women compared to the entire cohort shown in Table 3. One exception was an apparent positive association of PCB-138 with risk for cases

diagnosed early during follow-up. Further exploration of this association, however, suggests that it was due to many of these cases being diagnosed close in time to blood collection. Fifty-one of the 78 cases diagnosed during the truncated follow-up period donated blood within 2.7 years of diagnosis. Risk ratios for breast cancer among these women by increasing tertiles of

Table 4. Columbia, Missouri Breast Cancer Serum Bank. Risk ratios (RR) and 95% confidence intervals (CI) for breast cancer by tertile of serum hexachlorobenzene and PCBs (ng/g lipid) and duration from blood collection to diagnosis

Analyte	≤2.7 years (53 cases, 104 controls) ^a			> 2.7 years (52 cases, 104 controls)		
	RR ^b	95% CI	P-value ^c	RR ^b	95% CI	P-value ^c
Hexachlorobenzene			0.02			0.21
0–93	1.0			1.0		
94–153	1.6	0.7–3.9		1.9	0.8–4.4	
154–406	2.6	1.1–6.2		0.6	0.2–1.7	
PCB–118			0.10			0.18
0–57	1.0			1.0		
58–94	1.1	0.5–2.4		1.3	0.6–3.2	
95–533	1.4	0.6–3.2		0.9	0.4–2.4	
PCB–138			0.07			0.05
0–78	1.0			1.0		
79–112	1.7	0.7–4.2		0.8	0.4–1.9	
113–359	1.9	0.8–4.8		0.7	0.3–1.6	

^a Five participants included in the study more than once, as a case and a control or as a control for more than one case, counted multiple times.

^b Matched on age, benign breast disease diagnosis during prior 2 years, month and year of blood collection.

^c Based on continuous variables.

PCB-138 were 1.0, 1.8 (95% confidence interval [CI] = 0.7–4.5), and 2.0 (CI = 0.8–5.0) (P -trend = 0.07). For the remaining 27 cases diagnosed further in time from blood collection, comparable risk ratios were 1.0, 0.9 (CI = 0.3–2.8), and 1.1 (CI = 0.3–3.5) (P -trend = 0.29).

Discussion

Results of this prospective study do not support a positive association between serum levels of organochlorine pesticides and PCBs with breast cancer. Hexachlorobenzene was the only organochlorine pesticide measured that was positively associated with breast cancer, and this was limited to women whose blood was collected close in time to diagnosis. The two PCB congeners (PCB-118 and PCB-138) evaluated in detail in relation to breast cancer also tended to be positively associated with risk when blood was collected close in time to diagnosis but inversely or not associated with risk when the interval was longer. Serum concentrations of many of the organochlorine pesticides and PCBs measured in this study were extremely low and frequently below the assay LOD. Precise evaluation of the relationships of these pesticides and PCBs with breast cancer could not be performed.

Pesticides and PCBs have been shown to stimulate the growth of preneoplastic breast epithelial cells and estrogen responsive breast tumor cells *in vitro* [16–18]. Furthermore, some of these compounds are very stable, stored in adipose tissue (including breast adipose tissue) and are excreted in milk [19, 20].

Falck *et al.* [21] compared PCB and pesticide levels in breast adipose tissue from 20 breast cancer cases with an equal number of women who had benign breast disease. Women with breast cancer had significantly higher levels of p,p' -DDE and PCBs but levels of hexachlorobenzene, heptachlor epoxide, oxychlorane, *trans*-nonachlor, and p,p' -DDT did not differ between the two groups. Dewailly *et al.* [22], also observed higher levels of breast adipose tissue p,p' -DDE in women with estrogen receptor positive breast tumors but not in women with receptor negative tumors. Mussalo-Rauhamaa *et al.* [23], however, found no difference in breast adipose tissue levels of p,p' -DDT, p,p' -DDE, o,p' -DDD, hexachlorobenzene, heptachlor epoxide, or PCBs, but β -hexachlorocyclohexane levels were elevated in cases' adipose tissue. Similarly, no difference in PCB and DDE levels in adipose tissue from breast cancer cases and controls was reported by Unger *et al.* [24]. In a recent large European study [25], DDE levels in buttocks adipose tissue aspirates were lower in breast cancer cases compared to controls.

The strongest support for a role of organochlorine pesticides and PCBs in breast cancer derives from studies of serum concentrations of these compounds. Serum organochlorine pesticide and PCB levels reflect adipose tissue depots and can be used as a measure of exposure [26, 27]. Wolff *et al.* [7] first reported an increased risk of breast cancer among women with elevated serum DDE and PCB levels. Women with the highest DDE levels (90th percentile) were at a significant fourfold excess risk of being diagnosed with breast cancer compared to women with the lowest DDE levels (10th percentile). The risk for women with the highest *versus* lowest serum levels of PCBs also was elevated,

Table 5. Columbia, Missouri Breast Cancer Serum Bank. Risk ratios (RR) and 95% confidence intervals (CI) for breast cancer diagnosed in 1977–83 (78 cases, 154 controls) by quartile of serum pesticides and PCBs (ng/g lipid)

Analyte	RR ^a	95% CI	P-value ^b
Total DDT			0.99
204–1,646	1.0		
1,647–2,804	0.8	0.4–1.6	
2,805–4,020	0.2	0.1–0.6	
4,021–21,077	0.8	0.4–1.6	
<i>p,p'</i> -DDT			0.16
0–180	1.0		
181–292	1.0	0.4–2.3	
293–467	1.3	0.6–2.7	
468–1,724	0.5	0.2–1.2	
<i>p,p'</i> -DDE			0.90
31–1,377	1.0		
1,378–2,355	0.7	0.4–1.5	
2,356–3,500	0.3	0.1–0.8	
3,501–20,667	0.7	0.3–1.5	
β -hexachlorocyclohexane			0.93
0–75	1.0		
76–126	0.5	0.2–1.0	
127–176	0.4	0.2–0.9	
177–1,600	0.7	0.3–1.5	
Dieldrin			0.26
0–23	1.0		
24–51	0.5	0.2–1.1	
52–103	0.8	0.4–1.8	
104–921	0.6	0.3–1.4	
Hexachlorobenzene			0.20
0–62	1.0		
63–83	2.7	1.2–6.3	
84–105	1.9	0.8–4.9	
106–406	2.6	1.1–6.3	
Total PCBs			0.29
17–257	1.0		
258–369	0.9	0.4–2.0	
370–563	1.6	0.8–3.6	
564–2,682	1.1	0.4–2.6	
PCB-118			0.30
0–49	1.0		
50–74	0.9	0.4–2.1	
75–109	1.7	0.7–3.8	
110–533	1.2	0.5–2.7	
PCB-138			0.24
0–69	1.0		
70–93	1.6	0.7–3.6	
94–124	1.6	0.7–3.8	
125–359	1.7	0.7–4.2	

^a Matched on age, benign breast disease diagnosis during prior 2 years, month and year of blood collection.

^b Based on continuous variable.

but not significantly. Krieger *et al.* [8] subsequently reported no association of serum DDE or PCB level with breast cancer risk overall in a racially mixed cohort. As noted by Savitz [28], however, gradients of increasing risk with increasing DDE levels were apparent for White and Black women in this study. Odds ratios for Whites in the middle and upper tertiles were 1.9 (CI = 0.6–6.0) and 2.4 (CI = 0.5–10.6), respectively. For Blacks, the

respective odds ratios were 2.3 (CI = 0.6–8.4) and 3.9 (CI = 0.9–16.1). Hunter *et al.* [9] recently reported results of an analysis from the Nurses' Health Study on plasma organochlorine levels and breast cancer. In that analysis, neither plasma DDE or PCB levels were associated with risk. Serum DDE levels also were not related to breast cancer in a case-control study conducted recently in Mexico [10]. In another case-control

study, serum organochlorines were not associated with breast cancer for all women, but parous women who had never lactated and had elevated levels of PCBs and the pesticide mirex were at an increased risk [11].

A number of factors could explain the discrepancies in findings on relationships of serum pesticide and PCB concentrations with breast cancer from the studies reported to date including the current analysis. The studies were conducted at different times in different geographic locations, and there could have been substantial differences in exposures. The median serum concentration of DDE among controls in the current analysis was 16.3 ng/mL which is two-to-three times higher than the levels reported by Wolff [7] and Hunter [9], but less than half of that reported by Krieger [8]. The median total PCB serum concentration in the current analysis was 2.8 ng/mL which is about half of what was reported in the other three prospective studies [7–9].

PCB exposures may have differed qualitatively as well as quantitatively. Laboratory methods for estimating total PCBs in the current analysis were different from those used in previous prospective studies [7–9]. Furthermore, some PCBs and their metabolites are estrogenic, whereas other PCBs exhibit antiestrogenic effects [2]. Wolff *et al.* [29] recently categorized PCBs into functional groupings. Because most PCBs were below the LOD of the assay in the majority of our participants, we did not use these groupings in our analysis. However, in studies where PCB exposures are higher and/or assays employed are more sensitive, analyses of PCB-breast cancer associations by functional groupings should be more informative than analyses by total PCBs.

Dissimilarities in study designs, particularly duration of follow-up, also could have contributed to discrepancies in study results. In the study by Wolff *et al.* [7], cases were restricted to women diagnosed within six months of blood collection, in the study by Hunter *et al.* [9], cases were diagnosed within three years of blood collection; and in the study by Krieger *et al.* [8], cases included women diagnosed from six months up to 26 years after blood collection. In the current study, which included women who donated blood up to 10 years before diagnosis, tests for interaction suggested effect modification of some PCB-breast cancer associations by duration from blood collection to diagnosis. Among women who donated blood within 2.7 years of diagnosis (the midpoint), serum concentrations of PCB-118 and PCB-138 tended to be positively associated with risk similar to the finding for total PCBs previously reported by Wolff *et al.* [7]. However, among women who donated blood further in time from diagnosis, serum concentration of PCB-138 was inversely associated with risk

consistent with the report for total PCBs by Krieger *et al.* [8].

Adjustment for lactation history strengthened the relationship of serum DDE with breast cancer in the study of Wolff *et al.* [7]. Furthermore, in the recent case-control study by Moysich *et al.* [11], serum levels of total PCBs and the organochlorine pesticide mirex were positively associated with breast cancer for parous women who had never lactated, but not for all women regardless of pregnancy or lactation history. Because lactation history was not ascertained when our cohort was assembled, we were unable to consider this potentially important variable in our analysis. Although nulliparous women would have never lactated, too few of our participants were nulliparous to analyze this subgroup separately.

Because heavier women are at an increased risk of postmenopausal breast cancer and organochlorine pesticides are stored in adipose tissue [19, 30], body fatness could potentially confound pesticide-breast cancer associations. Among controls in our study, BMI was correlated positively with serum (ng/g lipid) *p,p'*-DDT and dieldrin and inversely with total PCBs and PCB-138. However, BMI was not related to risk of breast cancer and did not confound relationships of these or other organochlorines studied in detail with all breast cancers (pre- and postmenopausal) or postmenopausal cases alone.

Our cases and controls differed in the expected direction on a number of established breast cancer risk factors; cases tended to be better educated than controls and were more likely to be nulliparous and to have a first degree relative with a positive history of breast cancer. Smoking was significantly inversely associated with breast cancer in the current study. Although smoking generally is not related to a reduced risk of breast cancer, inverse associations have been reported previously [31–34]. Incomplete follow-up is a concern in the current study, although analyses did not suggest that it affected results.

Seventeen breast cancer cases could not be included in the current analysis because there was insufficient serum to perform the assays. These women did not differ significantly from the women included in the study on any of the characteristics shown in Table 1. Their ages and menopausal status at diagnosis, years of diagnosis, histological types of breast cancers, and survival status also did not differ significantly.

Because women in our study were not fasting and lipids affect serum concentrations of organochlorine pesticides and PCBs [13], we analyzed breast cancer associations with pesticides and PCBs per gram of serum lipids. In general, results were similar when we

modeled associations using: (i) serum concentrations of pesticides and PCBs and included serum lipids in the models as a covariate; or (ii) residuals from linear regressions of organochlorine pesticides and PCBs on serum lipids. The most notable disparity was that the risk of breast cancer across quartiles of *p,p'*-DDT shown in Table 3 was more erratic using the two alternate models. However, women in the highest quartile of *p,p'*-DDT were estimated to be at about half the risk of being diagnosed with breast cancer compared with those in the lowest quartile using all three methods. This unexpected finding could still have been due to uncontrolled confounding or multiple comparisons and needs to be interpreted cautiously.

In summary, results of this prospective study do not support the hypothesis that women who are exposed to organochlorine pesticides and PCBs are at an increased risk of breast cancer.

Acknowledgements

All analyses were performed by the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA. The authors wish to acknowledge the contributions of Mrs Elaine Gunther and Ms Emmalen Smith, who performed the lipid analyses. The authors also wish to acknowledge Dr Barry Graubard (Division of Cancer Epidemiology and Genetics, National Cancer Institute) for his statistical advice.

References

- Adami HO, Lipworth L, Titus-Ernstoff L, et al. (1995) Organochlorine compounds and estrogen-related cancers in women. *Cancer Causes Control* **6**: 551–566.
- Safe SH, Zacharewski T (1997) Organochlorine exposure and risk for breast cancer. *Prog Clin Biol Res* **396**: 133–145.
- Wolff MS, Toniolo PG (1995) Environmental organochlorine exposure as a potential etiologic factor in breast cancer. *Environ Health Perspect* **103**(Suppl 7): 141–145.
- Estabrook RW (1996) The remarkable P450s: a historical overview of these versatile hemoprotein catalysts. *FASEB J* **10**: 202–204.
- Davidson NE, Yager JD (1997) Pesticides and breast cancer: fact or fad. *J Natl Cancer Inst* **89**: 1743–1744.
- Oakley GG, Devanaboyina U, Robertson LW, Gupta RC (1996) Oxidative DNA damage induced by activation of polychlorinated biphenyls (PCBs): implications for PCB-induced oxidative stress in breast cancer. *Chem Res Toxicol* **9**: 1285–1292.
- Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N (1993) Blood levels of organochlorine residues and risk of breast cancer. *J Natl Cancer Inst* **85**: 648–652.
- Krieger N, Wolff MS, Hiatt RA, Rivera M, Vogelman J, Orentreich N (1994) Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. *J Natl Cancer Inst* **86**: 589–599.
- Hunter DJ, Hankinson SE, Laden F, et al. (1997) Plasma organochlorine levels and the risk of breast cancer. *New Engl. J Med* **337**: 1253–1258.
- Lopez-Carrillo L, Blair A, Lopez-Cervantes M, et al. (1997) Dichlorodiphenyltrichloroethane serum levels and breast cancer risk: a case-control study from Mexico. *Cancer Res* **57**: 3728–3732.
- Moysich KB, Ambrosone CB, Vena JE, et al. (1998) Environmental organochlorine exposure and postmenopausal breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **7**: 181–188.
- Brock JW, Burse VW, Ashley DL, et al. (1996) An improved analysis for chlorinated pesticides and polychlorinated biphenyls (PCBs) in human and bovine sera using solid-phase extraction. *J Anal Toxicol* **20**: 528–536.
- Phillips DL, Pirkle JL, Burse VW, Bernert JT, Henderson LO, Needham LL (1989) Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch Environ Contam Toxicol* **18**: 495–500.
- Breslow NE, Day NE (1980) *Statistical Methods in Cancer Research, Vol. I, The Analysis of Case-Control Studies*. Lyon, (France): International Agency for Research on Cancer, 248–279.
- SAS Institute, Inc. (1990) *SAS User's Guide, Version 6*. Cary (NC, USA): SAS Institute, Inc.,
- Shekhar PVM, Werdell J, Basrur VS (1997) Environmental estrogen stimulation of growth and estrogen receptor function in preneoplastic and cancerous human breast cell lines. *J Natl Cancer Inst* **89**: 1774–1782.
- Soto AM, Chung KL, Sonnenschein C (1994) The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen sensitive cells. *Environ Health Perspect* **102**: 380–383.
- Steinmetz R, Young PCM, Caperell-Grant A, et al. (1996) Novel estrogenic action of the pesticide residue β -hexachlorocyclohexane in human breast cancer cells. *Cancer Res* **56**: 5403–5409.
- Kutz FW, Wood PH, Bottimore DP (1991) Organochlorine pesticides and polychlorinated biphenyls in human adipose tissue. *Rev Environ Contam Toxicol* **120**: 1–82.
- Dewailly E, Ayotte P, Laliberte C, Weber JP, Gingras S, Nantel AJ (1996) Polychlorinated biphenyl (PCB) and dichlorodiphenyl dichloroethylene (DDE) concentrations in the breast milk of women in Quebec. *Am J Public Health* **86**: 1241–1246.
- Falck F, Ricci A, Wolff MS, Godbold J, Deckers P (1992) Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch Environ Health* **47**: 143–146.
- Dewailly E, Dodin S, Verreault R, et al. (1994) High organochlorine body burden in women with estrogen receptor-positive breast cancer. *J Natl Cancer Inst* **86**: 232–234.
- Mussalo-Rauhamaa H, Hasanen E, Pyysalo H, Kauppila AR, Pantzar P (1990) Occurrence of beta-hexachlorocyclohexane in breast cancer patients. *Cancer* **66**: 2124–2128.
- Unger M, Kiaer H, Blichert-Toft M, Olsen J, Clausen J (1984) Organochlorine compounds in human breast fat from deceased with and without breast cancer and in biopsy material from newly diagnosed patients undergoing breast surgery. *Environ Res* **34**: 24–28.
- van't Veer P, Lobbezoo IE, Martin-Moreno JM, et al. (1997) DDT (dicophane) and postmenopausal breast cancer in Europe: case-control study. *BMJ* **315**: 81–85.
- Wolff MS, Thornton J, Fischbein AS, Lilis R, Selikoff IJ (1982) Disposition of polychlorinated biphenyl congeners in occupationally exposed persons. *Toxicol Appl Pharmacol* **62**: 294–306.

26. Needham LL, Burse VW, Head SL, *et al.* (1990) Adipose tissue/serum partitioning of chlorinated hydrocarbon pesticides in humans. *Chemosphere* **20**: 975–980.
27. Savitz DA. Re: (1994) Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women [Letter]. *J Natl Cancer Inst* **86**: 1255.
28. Wolff MS, Camann D, Gammon M, Stellman SD (1997) Proposed PCB congener groupings for epidemiological studies [Letter]. *Environ Health Perspect* **105**: 13–14.
29. Kelsey JL (1993) Breast cancer epidemiology: summary and future directions. *Epidemiol Rev* **15**: 256–263.
30. Palmer JR, Rosenberg L (1993) Cigarette smoking and the risk of breast cancer. *Epidemiol Rev* **15**: 145–156.
31. O’Connell DL, Hulka BS, Chambless LE, Wilkinson WE, Deubner DC (1987) Cigarette smoking, alcohol consumption, and breast cancer risk. *J Natl Cancer Inst* **78**: 229–234.
32. Braga C, Negri E, La Vecchia C, Filiberti R, Franceschi S (1996) Cigarette smoking and the risk of breast cancer. *Eur J Cancer Prev* **5**: 159–164.
33. Brunet J, Ghadrian P, Rebbeck TR, *et al.* (1998) Effects of smoking on breast cancer in carriers mutant BRCA1 or BRCA2 genes. *J Natl Cancer Inst* **90**: 761–766.