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Polychlorinated biphenyls and breast cancer risk by combined estrogen and progesterone receptor status

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Abstract. Studies have suggested that breast cancer risk factor profiles may vary according to joint estrogen receptor (ER) and progesterone receptor (PR) tumor status. Most of the published literature to date which has investigated the association between exposure to organochlorine compounds and breast cancer has reported null or weak associations. If, indeed, the classification by hormonal receptor status identifies different forms of breast cancer, then assessing the risk of exposure to polychlorinated biphenyls (PCBs) on breast cancer as one disease or stratifying based on ER or PR status alone may obscure the association between PCBs and breast cancer. A hospital-based case-control study of 266 cases and 347 benign breast disease controls was conducted to examine the association of blood serum and adipose tissue concentrations of PCBs with breast cancer by joint ER/PR status. Total PCBs were measured in blood serum, and the following PCB congeners were measured in breast adipose tissue: 74, 118, 138, 153, 156, 170, 180, 183, 187. We did not detect any clear relationship or change in breast cancer risk based on joint ER/PR tumor status for body burden of PCBs, whether measured in blood serum or breast adipose tissue, by total PCBs or for specific congeners. These results confirm previous findings in the literature of no positive association between environmental exposure to PCBs and risk of breast cancer.

Key words: Breast cancer, Estrogen receptor, Polychlorinated biphenyls, Progesterone receptor

Introduction

Most established risk factors for breast cancer are thought to be hormone-related, suggesting the possibility that hormone receptor status in breast tissue may be associated with tumor development or progression [1]. It has been hypothesized that breast cancer is not simply one disease, but rather four distinct subtypes of cancer, which depend on combined estrogen receptor (ER) and progesterone receptor (PR) status [2]. The ER and PR status of breast cancer has been used to predict a patient's response to hormonal manipulation, predict clinical treatment, and as a selection criteria for therapy with tamoxifen [2–4]. Predictive power is enhanced when ER and PR status are considered jointly [2]. Tumors that are both ER^+ and PR^+ show the best response to endocrine therapy, whereas tumors which are ER^-PR^- show the poorest response [4]. A few studies have investigated risk factors for breast cancer, stratified by joint ER/PR distribution [2, 5–7, 49] and have found variation in risk among the four tumor subtypes for certain endogenous estrogen-related risk factors.

In the last decade, environmental exposure to certain organochlorine compounds has been suggested as a risk factor for female breast cancer [8–16]. It is conceivable that exposure to these environmental contaminants may increase breast cancer risk because some have been found to be estrogenically active and to induce of cytochrome P-450 mixed-function oxidase enzymes, which are involved in metabolism of steroid hormones and xenobiotic compounds [17].

Studies linking polychlorinated biphenyls (PCBs) and breast cancer, however, have not provided convincing evidence of an association [9–12, 14–16, 18–34]. Most of these studies treated breast cancer as a single disease. Several studies of PCBs and breast cancer stratified the data by either ER or PR status but did not take into account joint ER/PR status [19, 22, 24–27, 29, 30, 34]. The results of these studies were mixed. To date, only one study investigating PCBs and breast cancer has stratified cases by joint ER/PR status (32), but this was carried out only for the combined total of the four most frequently occurring PCBs. No previous studies have investigated the effects of individual PCB congeners on breast cancer risk stratified by joint ER/PR status. If, indeed, the classification by joint hormonal receptor status identifies different forms of breast cancer, then assessing the risk of

exposure to PCBs on breast cancer as one disease or stratifying based on ER or PR status alone may obscure the association between PCBs and breast cancer.

Although PCB uses as lubricants, protective sealants and industrial solvents were discontinued in 1977, they are highly persistent, are still released into the environment from hazardous waste sites containing PCBs, and are detected in biologic samples world-wide [35]. Given that there is widespread exposure of the general population to PCBs [36, 37], that most established breast cancer risk factors are those with hormone-related activity [38], and that those risk factors have been found to vary by joint ER/PR status [2, 5, 6, 39], we decided to further evaluate the association between environmental exposure to PCBs and breast cancer risk by joint distribution of ER/PR status. We analyzed the data from a case-control study in Connecticut for which total PCBs were measured in serum and adipose tissue, nine individual PCB congeners were measured in adipose tissue, and joint ER/PR status was assessed for cases.

Methods

Study subjects

All procedures were performed in accordance with a protocol approved by the Yale Human Investigations Committee. A detailed description of the study population and methods is presented elsewhere [40]. Briefly, women between the ages of 40 and 80 years who had breast-related surgery at Yale-New Haven Hospital (YNHH) between 1994 and 1997 were recruited into this study. A total of 326 incident breast cancer cases and 347 benign breast disease controls were able to provide 10 ml of blood serum. Of those, 304 cases and 186 controls were able to provide at least 0.4 g of residual breast adipose tissue (i.e., not needed for diagnostic purposes).

The study pathologist classified the potential participants as either cases or controls. Cases were histologically confirmed, incident breast cancer patients (International Classification of Diseases for Oncology (ICDO) codes 174.0–174.9). Controls were patients who had had breast-related surgery and were either [1] histologically diagnosed with benign breast disease, excluding atypical hyperplasia, or [2] had a diagnosis of normal breast tissue. Eligible subjects had no previous diagnosis of cancer, with the exception of non-melanoma skin cancer, and were alive at the time of interview.

Efforts were made to frequency match the cases and controls (1:1) by age within 5-year intervals (e.g., 40–44, 45–49, 50–54, …), by adjusting the number of controls randomly selected in each age stratum every few months.

Interview

After approval by each subject's physician, potential participants were interviewed by a trained interviewer, using a standardized, structured questionnaire to obtain information on major known or suspected confounding factors, including menstrual and reproductive history, lactation history, family cancer history, and demographic factors.

Blood serum/adipose tissue collection and chemical analysis

Following the interview, the participant provided a blood sample, collected by venipuncture by the study interviewers. Total PCBs measured in blood serum were calculated as the sum of the following nine measured PCB congeners: 74, 118, 138, 153, 156, 170, 180, 183 and 187. Analytical methods for determining PCB levels in 10 ml of serum have been described elsewhere [24]. Briefly, the method involved denaturation of protein by methanol, extraction of the compounds of interest in ethyl ether: hexane (1:1, v/v), gravimetric lipid determination, purification of the sample using Florisil chromatography, and identification and quantification of the compound by gas chromatography. Serum residue results of total PCBs are reported as parts per billion (ppb) on a lipid adjusted basis. More than 70% of the samples were above the detection limit [24].

Breast adipose tissue not needed for diagnostic purposes was collected by personnel from the Tissue Retrieval Facility at the Yale Cancer Center, coded and sent in batches to the study laboratory at Colorado State University, where it remained frozen until analysis. Since samples were batched and coded at Yale, laboratory personnel in Colorado were blind to the case-control status of the samples being analyzed [40]. The same nine PCB congeners measured in serum were also measured in breast adipose tissue. The laboratory method used for analyzing these compounds in breast adipose tissue has been described elsewhere [41]. Briefly, the method involved the following: extraction in hexane; separation of organochlorine pesticides from polychlorinated biphenyls and purification of the sample using Florisil chromatography, and identification and quantification of the compounds using gas chromatography. Adipose tissue levels were reported as parts per billion (ppb), which is equivalent to nanograms of compound per gram of lipid (ng/g) [40]. About 98.1% of the subjects who participated in the study had quantified levels of PCBs [25].

All analyses were conducted under an established quality control/quality assessment program including method spikes, reagent blanks, and quality control windows.

ER-alpha and PR levels were measured immunohistochemically at the Pathology Department of

YNHH. Both ER and PR status were considered positive when their H-score was higher than 75, as described by McCarty et al. [42].

Data Analysis

Breast cancer cases were stratified by the joint classification of ER and PR status (i.e., ER^+PR^+, ER^-PR^-, ER^+PR^-, or ER^-PR^+). If a case had 'unknown' ER or PR status, she was excluded from the analyses.

Multiple linear logistic regression was carried out to assess the association between PCBs (total PCBs and each of the nine congeners) and breast cancer risk, stratified by joint ER/PR status and controlling for potential confounders. Analyses were carried out for blood serum (total PCBs) and for breast adipose tissue (congeners 74, 118, 138, 153, 156, 170, 180, 183 and 187 and total PCBs). The covariates we included in the final models were the following continuous variables: age, age at menarche, lifetime months of breastfeeding, body mass index, and parity and the following categorical variables: age at first birth (≤30, >30, nulliparous) and family history of breast cancer in a first degree relative (yes vs. no). Adjusted odds ratios, their 95% confidence intervals (CIs), and tests for trend for each subtype of breast cancer based on joint ER/PR status were computed for each compound. We carried out the same analyses, limiting the study population to only post-menopausal women. All odds ratios in this study were calculated using maximum likelihood estimation methods and were carried out using the SAS program LOGISTIC [43].

To obtain a test whether the effects of each congener are identical, within each subtype, we compared the likelihood ratio test for a model with total PCBs to one with all nine individual congeners entered. This test of homogeneity within each tumor subtype allowed for the estimation of the effect of each congener in the presence of others, which validated our investigation of total PCBs. To carry out this analysis, we fitted a model that included all of the individual congeners at the same time, and we compared this model to one with total PCB congeners (the sum of the nine congeners) as the independent variable.

Results

Baseline characteristics of the four tumor subtypes of cases who provided blood serum (which includes all cases which additionally provided breast tissue), are compared in Table 1 via chi-squared analyses. The four subtypes of cases did not differ significantly with respect to most baseline factors, with the exception of age and age at menopause.

A total of 613 subjects (266 cases with known joint ER/PR status; 347 controls) were included in the blood serum analysis. Among the cases, 85 (32%) were ER^+PR^+, 89 (33%) were ER^-PR^-, 55 (21%) were ER^+PR^-, and 37 (14%) were ER^-PR^+. The covariate adjusted ORs for each of the four subgroups of breast cancer cases according to joint ER/PR status for total PCBs measured in blood serum are presented in Table 2. We found no clear associations between any of the tumor subtypes and total PCBs. Correspondingly, when we limited the population to post-menopausal women, we found no association for any tumor subtype, with increasing concentrations in blood serum (data not shown).

A total of 430 subjects (244 cases with known joint ER/PR status; 186 controls) were included in the breast adipose tissue analysis. Among the cases, 84 (30%) were ER^+PR^+, 79 (32%) were ER^-PR^-, 49 (20%) were ER^+PR^-, and 32 (13%) were ER^-PR^+. When we tested whether the effects of each congener are identical, within each subtype, by comparing the likelihood ratio test for a model with total PCBs to one with all nine individual congeners entered simultaneously, we found, that for all four tumor subtypes, there were no significant differences between the two models tested. Therefore, we have strong evidence that there is equality of effect for each individual congener.

The covariate adjusted ORs for each of the four subtypes of breast cancer cases according to joint ER/PR status for each PCB congener are shown in Table 3. Congeners are grouped by the structural and biological activity groups proposed by Wolff et al. [44] – potentially estrogenic, potentially antiestrogenic, and phenobarbital CYP1A/2B inducer. We found no clear patterns of association between any of the four tumor subtypes and tertiles of individual PCB congeners or total PCBs. Although select estimates for ER^+PR^- tumors were slightly elevated for PCB congeners in the CYP1A, CYP2B inducer group (Group 2), these estimates were not significant, nor were their tests for trend. Non-significant inverse associations were found for $ER{-}PR^+$ tumors with increasing concentrations of most congeners, however, there was no evidence of a linear trend, and these estimates were based on small numbers. When we limited the population to post-menopausal cases (n = 174) and controls (n = 111), an association emerged which we had not detected when all study subjects (pre- and post-menopausal) were analyzed as one group: an increased risk of ER^+PR^+ tumors with increasing tertiles of BZ-183 (2nd tertile: OR: 1.3; 95%CI: 0.5–3.3; 3rd tertile: OR: 2.4; 95%CI: 1.0–6.0); test for trend was significant ($p = 0.03$). Overall, however, we found no evidence of variation in risk among the four tumor subtypes (data not shown).

Table 1. Comparison of selected characteristics of female breast cancer cases,[a] stratified by joint ER/PR status

Characteristic	ER^+PR^+ Cases (N^b = 85) N (%)	ER^-PR^- Cases (N^b = 89) N (%)	ER^+PR^- Cases (N^b = 55) N (%)	ER^-PR^+ Cases (N^b = 37) N (%)	χ^2 test (*p*-value)
Age					
< 50	26 (31%)	35 (39%)	10 (18%)	13 (35%)	
≥50	59 (69%)	54 (61%)	46 (82%)	24 (65%)	0.05
Age at menarche					
≥15	7 (8%)	9 (10%)	7 (13%)	3 (8%)	
13–14	39 (46%)	43 (48%)	21 (38%)	9 (24%)	
< 13	39 (46%)	37 (42%)	28 (50%)	25 (68%)	0.19
unknown					
Number of pregnancies					
≥3	45 (56%)	51 (65%)	29 (55%)	22 (63%)	
1 or 2	36 (44%)	28 (35%)	24 (45%)	13 (37%)	0.57
Age at 1st full-term pregnancy					
< 30	66 (78%)	64 (72%)	36 (64%)	30 (81%)	
≥30	9 (11%)	12 (13%)	16 (29%)	4 (11%)	
Nulliparous	10 (12%)	13 (15%)	4 (7%)	3 (8%)	0.07
Lifetime lactation (months)					
≥12	18 (21%)	14 (16%)	10 (18%)	7 (19%)	
1–11	18 (21%)	16 (18%)	13 (23%)	9 (24%)	
Never lactated	49 (58%)	59 (66%)	33 (59%)	21 (57%)	0.91
Family breast cancer history					
No	62 (73%)	70 (79%)	40 (71%)	29 (78%)	
Yes	23 (27%)	19 (21%)	16 (29%)	8 (22%)	0.70
BMI (kg/m^2)					
< 21.0	9 (11%)	15 (17%)	9 (16%)	5 (14%)	
21.0–24.9	36 (42%)	31 (35%)	24 (43%)	12 (32%)	
≥25	40 (47%)	43 (48%)	23 (41%)	20 (54%)	0.75
Age at Menopause					
< 50	44 (52%)	32 (36%)	27 (48%)	19 (51%)	
≥50	22 (26%)	27 (30%)	22 (39%)	8 (22%)	
Pre-menopausal	19 (22%)	30 (34%)	7 (13%)	10 (27%)	0.05
Race					
White	75 (88%)	74 (83%)	51 (91%)	32 (86%)	
Black/other	10 (12%)	15 (17%)	5 (9%)	5 (14%)	0.55

[a]Cases which provided blood serum.
[b]Number of cases in each tumor subgroup.

Table 2. Risk of breast cancer for total PCBs (sum of PCB congeners 74, 118, 138, 153, 156, 170, 180, 183, 187) measured in blood serum, by joint distribution of ER/PR status

	ER^+PR^+ (N = 85/346)[a]		ER^-PR^- (N = 89/346)[a]		ER^+PR^- (N = 55/346)[a]		ER^-PR^+ (N = 37/346)[a]	
Organochlorine (ppb)	N^b	OR (95% C.I.)[c]	N^b	OR (95% C.I.)[c]	N^b	OR (95% C.I.)[c]	N^b	OR (95% C.I.)[c]
Total PCBs								
< 570.21	41	1.0	31	1.0	18	1.0	11	1.0
570.21–751.25	24	0.7 (0.4–1.2)	30	1.0 (0.6–1.8)	18	1.0 (0.5–2.1)	13	1.3 (0.5–3.0)
> 751.25	20	0.5 (0.3–1.0)	28	0.9 (0.5–1.6)	20	1.3 (0.6–2.6)	13	1.2 (0.5–2.8)
p-trend		0.29		0.49		0.35		0.73

[a]Number of cases/controls in each tumor subgroup – all subjects.
[b]Number of cases in each exposure sub-category; number of controls is constant at 62 in each tertile.
[c]Adjusted for the following continuous variables: age, age at menarche, lifetime months of breastfeeding, body mass index, and parity and the following categorical variables: age at first birth (≤30, > 30, nulliparous) and family history of breast cancer in a first degree relative (yes vs. no).

Table 3. The risk of breast carcinoma for individual PCB congeners and total PCBs (sum of PCB congeners 74, 118, 138, 153, 156, 170, 180, 183, 187) measured in breast adipose tissue, by joint distribution of ER/PR status

	ER^+PR^+ (N = 83/186)[a]		ER^-PR^- (N = 79/186)[a]		ER^+PR^- (N = 47/186)[a]		ER^-PR^+ (N = 32/186)[a]	
PCBs (ppb)	N[b]	OR (95%C.I.)[c]	N[b]	OR (95%C.I.)[c]	N[b]	OR (95%C.I.)[c]	N[b]	OR (95%C.I.)[c]
Group 1: Potentially estrogenic and weak phenobarbital inducer								
PCB 187								
< 21.8	24	1.0	33	1.0	10	1.0	16	1.0
21.8–35.43	27	0.9 (0.4–1.8)	23	0.6 (0.3–1.2)	16	1.2 (0.5–3.3)	7	0.3 (0.1–1.0)
> 35.43	32	0.6 (0.3–1.4)	23	0.5 (0.3–1.1)	21	1.3 (0.5–3.5)	9	0.3 (0.1–0.8)
p-trend		0.55		0.67		0.46		0.18
Group 2: Potentially antiestrogenic and dioxinlike								
PCB 74								
< 21.04	30	1.0	23	1.0	16	1.0	15	1.0
21.04–39.86	27	0.9 (0.5–1.8)	26	1.1 (0.6–2.2)	15	1.1 (0.5–2.7)	8	0.5 (0.2–1.3)
> 39.86	26	0.5 (0.3–1.1)	30	1.2 (0.6–2.5)	16	0.6 (0.2–1.4)	9	0.3 (0.1–0.8)
p-trend		0.51		0.55		0.28		0.09
PCB 118								
< 28.43	31	1.0	30	1.0	19	1.0	15	1.0
28.43–59.44	24	1.1 (0.5–2.1)	26	0.9 (0.5–1.7)	16	1.3 (0.5–3.0)	8	0.5 (0.2–1.4)
> 59.44	28	0.6 (0.3–1.2)	23	0.7 (0.3–1.4)	12	0.4 (0.2–1.0)	9	0.4 (0.2–1.2)
p-trend		0.41		0.62		0.17		0.59
PCB 138								
< 63.4	26	1.0	30	1.0	15	1.0	13	1.0
63.4–102.28	25	0.8 (0.4–1.5)	28	0.9 (0.5–1.8)	16	1.0 (0.4–2.4)	6	0.5 (0.2–1.3)
> 102.28	32	0.6 (0.3–1.2)	21	0.6 (0.3–1.1)	16	0.7 (0.3–1.6)	13	0.8 (0.3–2.0)
p-trend		0.25		0.57		0.25		0.68
PCB 156								
< 15.44	30	1.0	28	1.0	16	1.0	14	1.0
15.44–26.08	23	0.6 (0.3–1.2)	33	1.0 (0.5–1.9)	18	0.9 (0.4–2.1)	8	0.8 (0.3–2.1)
> 26.08	30	0.6 (0.3–1.3)	18	0.5 (0.2–1.1)	13	0.7 (0.3–1.7)	10	0.3 (0.1–1.1)
p-trend		0.10		0.11		0.15		0.09
PCB 170								
< 26.74	34	1.0	33	1.0	17	1.0	12	1.0
26.74–39.43	14	0.4 (0.2–0.8)	23	0.6 (0.3–1.2)	14	0.9 (0.4–2.1)	13	0.8 (0.3–2.0)
> 39.43	35	0.7 (0.3–1.3)	23	0.5 (0.3–1.1)	16	0.7 (0.3–1.6)	7	0.5 (0.2–1.5)
p-trend		0.30		0.66		0.62		0.39
Group 3: Phenobarbital CYP1A and CYP2B inducers								
PCB 153								
< 100.47	26	1.0	30	1.0	11	1.0	12	1.0
100.47–152.28	25	0.8 (0.4–1.6)	29	0.9 (0.5–1.7)	17	1.4 (0.6–3.5)	10	0.4 (0.2–1.2)
> 152.28	32	0.6 (0.3–1.3)	20	0.5 (0.2–1.1)	19	1.0 (0.4–2.8)	10	0.4 (0.1–1.1)
p-trend		0.59		0.57		0.75		0.39
PCB 180								
< 75.39	25	1.0	28	1.0	9	1.0	10	1.0
75.39–111.3	22	0.6 (0.3–1.4)	23	0.8 (0.4–1.5)	17	1.8 (0.7–4.8)	12	1.0 (0.4–2.7)
> 111.3	36	0.6 (0.3–1.3)	28	0.8 (0.4–1.7)	21	1.3 (0.4–3.7)	10	0.5 (0.2–1.6)
p-trend		0.70		0.53		0.32		0.29
PCB 183								
< 9.78	24	1.0	28	1.0	12	1.0	13	1.0
9.78–14.48	22	0.7 (0.4–1.5)	29	1.0 (0.5–2.0)	13	1.1 (0.4–2.9)	9	0.7 (0.3–1.8)
> 14.48	27	1.0 (0.5–1.9)	22	0.7 (0.3–1.4)	22	1.6 (0.6–3.9)	10	0.6 (0.2–1.6)
p-trend		0.71		0.80		0.07		0.93

Table 3. *Continued*

PCBs (ppb)	ER^+PR^+ (N = 83/186)[a] N[b]	OR (95%C.I.)	ER^-PR^- (N = 79/186)[a] N[b]	OR (95%C.I.)[c]	ER^+PR^- (N = 47/186)[a] N[b]	OR (95%C.I.)[c]	ER^-PR^+ (N = 32/186)[a] N[b]	OR (95%C.I.)[c]
Total PCBs: sum of all nine PCB congeners listed in this table								
Total PCBs								
< 394.31	29	1.0	35	1.0	14	1.0	16	1.0
394.31–558.69	21	0.6 (0.3–1.2)	20	0.5 (0.3–1.0)	17	1.0 (0.4–2.5)	4	0.2 (0.1–0.7)
> 558.69	33	0.6 (0.3–1.3)	24	0.5 (0.3–1.1)	16	0.6 (0.2–1.6)	12	0.5 (0.2–1.2)
p-trend		0.46		0.67		0.52		0.32

[a]Number of cases/controls in each tumor sub-group.
[b]Number of cases in each exposure sub-category; number of controls is constant at 62 in each tertile.
[c]Adjusted for the following continuous variables: age, age at menarche, lifetime months of breastfeeding, body mass index, and parity and the following categorical variables: age at first birth ($\leq$ 30, > 30, nulliparous) and family history of breast cancer in a first degree relative (yes vs. no).

Discussion

To our knowledge, this is the first study to analyze the association between individual PCB congeners and breast cancer risk by joint distribution of ER/PR status. We utilized both breast adipose tissue and blood to quantify lifetime exposure to nine individual PCB congeners and total PCBs. Additionally, we stratified each tumor subtype by menopausal status. We did not find any evidence that the risk for breast cancer associated with exposure to selected PCBs varies by joint ER/PR tumor status.

While earlier studies examining the effects of PCBs on breast cancer risk found positive associations, they were based on small numbers and only compared mean PCB concentration levels in serum between cases and controls [9, 18]. More recent studies with larger sample sizes have further investigated this question, [10–12, 14–16, 19, 21–33] however, many examined the association based on total PCBs. Among those studies which did investigate the relationship on a congener-specific basis, two showed an association with the specific congeners 105, 118, and 138, [14, 15] while two others found no associations [25, 26].

Some investigators have stratified cases by either ER status [19, 22, 24–27, 29, 30, 34] or PR status [27, 30] separately, however the findings have been mixed. Most studies detected no significant differences for mean and median concentrations between ER^+ and ER^- or between PR^+ and PR^- cases [19, 24, 25, 27]. However, Dewailley, et al. found that levels of PCB-118 were significantly lower in ER^- cases than controls, and levels of PCB-99 were significantly higher in ER^+ cases [34]. Additionally, the authors reported generally lower mean concentrations for each of the 10 PCBs measured among the ER^- cases and generally higher concentrations for the ER^+ cases. We also found generally lower mean and median levels of PCBs among ER^- cases (both ER^-PR^- and ER^-PR^+ cases), while generally higher levels among the ER^+ cases (both ER^+PR^+ and ER^+PR^-), though statistical significance was not reached (data not shown). It is difficult to further compare our results with those of Dewailley, et al. since their study was small (20 cases; 17 controls), and therefore, rate ratios could not be estimated for categories of exposure. Several studies which did calculate ORs for PCBs and breast cancer by ER status found no association [22, 26, 29], while one study found that PCB concentrations were associated with tumors of 'poor prognosis' (ER^- and PR^- tumors) [30]. Only one other study has investigated the association between PCBs and breast cancer, stratified by joint ER/PR status [32], for the sum of the four most frequently occurring PCB congeners among the subjects – 118, 138, 153, 180; no clear association was found for any of the four tumor subtypes. Our study supports this finding, however, on a congener specific basis.

One possible explanation for no associations found in our study and throughout much of the literature is that there may be a critical period of exposure – e.g., pre-natal, puberty – during which the effects of these compounds may be the most significant and lasting. Although serum and adipose tissues reflect a woman's lifetime body burden, we have no way of knowing the timing of exposure. Risk may also vary by genetic characteristics – metabolizing genes, tumor suppressor genes [11, 12]. There is also the consideration, however, that the estrogenicity of PCBs is orders of magnitude lower than that of endogenous estrogens [45].

A strength of our study is that we analyzed PCBs on a congener-specific basis. The rationale for doing

so has been validated by experimental studies. Some congeners have shown estrogenic activity, while others are considered weakly estrogenic or antiestrogenic [45, 46]. Additionally, PCBs have different enzyme activities. Some induce Cytochrome P450 genes, CYP-1A1 and CYP-1A2, while others induce CYP-2B1 and CYP-2B2 [45, 46]. The complex effects of PCBs may be missed by evaluating the total level of PCBs instead of individual congeners. Another strength is that our study used histologically confirmed non-cancer patients as controls. Using these women as controls reduces misclassification of disease status, since we were able to ensure that none of the controls had in situ carcinoma or atypical hyperplasia in the tissue examined by study pathologists. Considering both in situ breast cancer and atypical hyperplasia benign breast disease are relatively common among seemingly healthy women, other types of controls could be of concern. Current evidence suggests that any elevation in risk for breast cancer among women with benign breast diseases occurs mostly in women with proliferative lesions, in particular atypical hyperplasia; little or no elevation in risk is apparent when there is no evidence of proliferative disease [47]. As mentioned in the Methods section, we restricted potential controls to those women who had benign breast disease without proliferation or atypical hyperplasia. We utilized both breast adipose tissue and blood serum as media in which to measure lifetime body burden of PCBs. It has been debated recently which medium is more appropriate for such studies [41]. Since there is still not a strong consensus as to which medium is better or if the two are equivalent in assessing breast cancer risk from exposure to PCBs, it is useful to present the results obtained from both media.

A potential limitation of our study is the relatively small number of subjects within tumor subtype categorized by tertile of exposure variables. Additionally, we make multiple comparisons for each compound. However, our study is of an exploratory nature, focused more on generating a hypothesis and on detecting patterns of association for the various tumor subtypes, rather than on testing a specific hypothesis or on specific associations. This is the first study to investigate breast cancer and individual PCB congeners, by joint ER/PR tumor subtype, and we recommend that similar analyses be carried for populations of larger sample size, where the power may be greater to detect statistically significant differences.

For reasons stated above, examining PCBs on a congener-specific basis is appropriate, however, it also poses some inherent problems. Exposure to one PCB congener is likely to be highly correlated with exposure to others which were present in the same commercial product. This high correlation can lead to collinearity, which confounds the estimated associations. Holford, et al. presented methodological work to explore possible independent effects of the various congeners in the presence of collinearity using this study population [48]. Collinearity mainly affected BZ-153, which changed from being strongly protective direction to null, once the application of a ridge coefficient stabilized the parameter estimates [48].

In conclusion, we systematically investigated the risk of breast cancer associated with individual PCBs by joint ER/PR status and by menopausal status. Our case-control study investigated PCBs on a congener-specific basis, used two biological media for measurements and stratified the cases by four biologically and perhaps etiologically distinct breast tumor subtypes. Our findings were consistent with other recent studies and further establish that there is no clear association between PCB exposure and breast cancer.

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