

Polychlorinated Biphenyls, Cytochrome P450 1A1, and Breast Cancer Risk in the Nurses' Health Study¹

Francine Laden,² Naoko Ishibe, Susan E. Hankinson, Mary S. Wolff, Dorota M. Gertig, David J. Hunter, and Karl T. Kelsey

Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115 [F. L., S. E. H., D. J. H.]; Departments of Environmental Health [F. L., K. T. K.], Epidemiology [S. E. H., D. J. H.], and Cancer Cell Biology [K. T. K.], Harvard School of Public Health, Boston, Massachusetts; Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland [N. I.]; Mount Sinai Medical School, New York New York [M. S. W.]; Centre for Genetic Epidemiology, School of Population Health, University of Melbourne, Carlton, Australia [D. M. G.]; and Harvard Center for Cancer Prevention, Harvard School of Public Health, Boston Massachusetts 02115 [D. J. H., K. T. K.]

Abstract

There is concern that exposures to the environmental chemicals polychlorinated biphenyls (PCBs) may contribute to breast cancer risk. An individual's susceptibility to the effects of PCBs may be partially determined by polymorphisms in the gene encoding the biotransformation enzyme cytochrome P450 1A1 (CYP1A1). PCB exposure induces CYP1A1 activity, and PCBs themselves or other xenobiotics can be metabolized to carcinogenic intermediates in the presence of the variant genotype. A previous case-control study provided evidence of an interaction between high exposures to PCBs and the *CYP1A1-exon 7* polymorphism (the A to G transition at nucleotide 4889), leading to a significant increase in postmenopausal breast cancer risk. We examined the interaction of PCBs with the *CYP1A1-MspI* (the T to C transition at nucleotide 6235) and *exon 7* polymorphisms among 367 breast cancer case-control pairs (293 postmenopausal pairs) in the Nurses' Health Study. Although there was no independent association of either the *CYP1A1* variants or PCBs with breast cancer risk, the relative risk among the postmenopausal women with plasma PCB levels in the highest third of the distribution in the control group and at least one *exon 7* variant allele compared with women who were homozygous for the wild-type allele and who had PCB levels in the lowest third was 2.78 (95% confidence interval, 0.99–7.82). The majority of studies have

concluded that exposure to PCBs is unlikely to be a major cause of breast cancer, but these findings indicate that further studies of genetically susceptible populations are warranted.

Introduction

Despite considerable research during the past decades, the etiology of breast cancer remains elusive. Important risk factors have been identified, *e.g.*, family history, age at menarche and menopause, parity, weight gain, and postmenopausal hormone use (1), but these explain only a modest proportion of all breast cancer cases. There is concern that exposures to environmental carcinogens may play a role and that an individual woman's susceptibility may be determined by genetic polymorphisms of biotransformation genes.

The environmental compounds PCBs³ have been suggested as potential causes of breast cancer (2, 3). There is evidence that they adversely affect reproduction and sex determination in wildlife (4, 5). Some PCBs have weak estrogenic properties *in vitro* (6) and are both tumor promoting (7) and able to induce metabolic enzymes (8). However, most recent studies have not observed an association of PCB exposure with breast cancer, and a recent combined analysis of five studies, including 1400 cases, concluded that it is unlikely that such an association exists across the general population (9). These studies did not analyze the possibility that a genetic subset of the population may be susceptible to PCBs.

A leading genetic candidate that may influence susceptibility to PCBs is CYP1A1. This biotransformation enzyme metabolizes carcinogenic exogenous substances such as PAHs found in cigarette smoke into their genotoxic intermediates (10, 11). CYP1A1 also transforms endogenous steroid hormones such as estradiol (12, 13). Some PCB congeners have been observed in animal and *in vitro* experiments to induce CYP1A1 (14–17), and PCB exposure may lead to formation of DNA adducts through a pathway involving CYP1A1 (18, 19).

The human *CYP1A1* gene is polymorphic; at least seven different variants in this gene have been identified (20). Although the functional significance of these genotypic variants remains uncertain, differences in their metabolic activity have been observed (10, 20–23). Thus, because of CYP1A1's role in metabolism of exogenous chemicals, the different variants may modify the risk of developing environmentally based disease. Previous studies have explored the risks of lung and breast cancer associated with the *exon 7* (A to G transition at nucleotide 4889, altering the amino acid isoleucine to valine) and *MspI* (T to C transition at nucleotide 6235) mutation of *CYP1A1*. Some have observed increased risks among popula-

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² To whom requests for reprints should be addressed, at Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115. Phone: (617) 525-2711; Fax: (617) 731-1541; E-mail: francine.laden@channing.harvard.edu.

³ The abbreviations used are: PCB, polychlorinated biphenyl; CYP1A1, cytochrome P450 1A1; PAH, polycyclic hydrocarbon; IUPAC, International Union of Pure and Applied Chemistry; RR, relative risk; CI, confidence interval.

tions with at least one variant allele (24–27), whereas others have not (28, 29). A previous case-control study of breast cancer provided evidence of an interaction between body burden of PCBs and the *CYP1A1-exon 7* polymorphism in generating a significant increase in breast cancer risk (30).

In this study, we examined the joint association of PCBs and the *exon 7* and *MspI CYP1A1* polymorphisms with breast cancer risk in the Nurses' Health Study.

Materials and Methods

Study Population. The Nurses' Health Study is an ongoing prospective cohort study established in 1976 when 121,700 female registered nurses completed a mailed questionnaire on risk factors for breast cancer and other diseases. At enrollment, the participants were between the ages of 30 and 55 years. Every 2 years, participants completed follow-up questionnaires to update information and to report the occurrence of breast cancer and other illnesses.

Diagnoses of breast cancer were reported on the biennial follow-up questionnaire. Nonrespondents were contacted by telephone, and deaths were identified through next of kin or searches of the National Death Index. Each diagnosis was confirmed by review of medical records and pathology reports.

From 1989 to 1990, 32,826 of these women provided a blood sample. The completeness of follow-up as a proportion of potential person-years through 1994 is 98% for this subcohort of the Nurses' Health Study. Women who sent a blood sample were very similar to other women in the cohort with respect to reproductive risk factors for breast cancer such as age at menarche, parity, and age at the birth of their first child. Women who gave a blood specimen were slightly more likely to have a history of benign breast disease or a family history of breast cancer. These differences should not influence the internal validity of comparisons between cases and controls in the subcohort of women who gave a blood specimen. We defined cases as women who did not have a diagnosis of cancer (other than nonmelanoma skin cancer) when they sent in the blood specimen and in whom breast cancer was subsequently diagnosed before June 1, 1994. For each case, we selected a control woman who had not reported a diagnosis of cancer, matched on year of birth, menopausal status, month and time of blood collection, fasting status at blood draw, and postmenopausal hormone use. PCB levels were measured for 378 cases (339 invasive and 39 carcinoma *in situ* breast cancers) and their controls.

Assessment of Covariates. Information on known and suspected breast cancer risk factors was obtained from the biennial questionnaires, and the questionnaire was completed by each participant at the time of blood collection. We included the following established risk factors for breast cancer in the multivariate models: age at menopause, age at menarche, parity, age at first full term pregnancy, body mass index [weight (kg)/height (m)²] at blood collection, history of benign breast disease, and history of breast cancer in a mother or sister. Duration of lactation was also included because it is a major route of excretion for PCBs and, therefore, may confound the association with breast cancer. Menopausal status, one of the matching factors, was defined by a woman's response to the question whether her menstrual periods had ceased permanently. Women who had had a hysterectomy with one or both ovaries left intact were classified as premenopausal until the age at which 10% of the cohort had undergone natural menopause (age 46 for smokers and 48 for nonsmokers) and as postmenopausal at the age at which 90% of the cohort had

undergone natural menopause (age 54 for smokers and 56 for nonsmokers). In the intervening years, these women were classified as being of uncertain menopausal status and excluded from menopause-specific analyses.

Laboratory Analyses. The laboratory methods have been described in detail elsewhere (3, 31). Briefly, a polar extract of plasma lipids was additionally enriched by chromatographic cleanup and then analyzed by gas chromatography with electron-capture detection. Results were obtained in parts/billion (ppb, *i.e.*, nanograms/milliliter plasma) of 21 individual PCB congeners. For this analysis, we calculated a sum of the 16 more chlorinated PCB congeners (pentachlorobiphenyls, hexachlorobiphenyls, and heptachlorobiphenyls). This total is the equivalent of the sum of PCBs assessed in our previous publications (32, 33). IUPAC congeners 118 (a pentachlorinated isomer), 138 and 153 (hexachlorinated isomers), and 180 (a heptachlorinated isomer) accounted for an average of 64% of the total sum. Therefore, if one of these congeners was not detected, the sample was considered to be unreliable and was excluded from statistical analyses of total PCBs. When a minor congener was not detected (usually because of potential contamination), its predicted value was calculated from the available congeners using regression analysis (32). The limits of detection were <1 ppb for total PCBs on the basis of a value that was three times the standard (34) of 24 determinations over the course of sample analyses of a quality control plasma pool with ~1 ppb of total PCBs. The limit of detection for the individual congeners was 0.07 ppb. Values below the method detection limit were reported when quantifiable to provide the best estimate of the organochlorine level. PCBs are stable in frozen blood; organochlorine levels in serum frozen at -20°C were unchanged over a period of 1 year (35).

Plasma sample sets were constructed to contain matched pairs of cases and controls (with the order of samples randomized) and were analyzed in batches of 12 pairs. In addition, each batch included two blinded split samples from pooled plasma from premenopausal or postmenopausal women. For each batch, we calculated the coefficient of variation. On the basis of a possible 35 batches, the median coefficient of variation was 12.0% for the sum of the 16 PCB congeners. Samples were assayed separately for total cholesterol and triglyceride levels. We calculated lipid-adjusted values using the formula described by Phillips *et al.* (36) and present the data in units of $\mu\text{g/g}$ (micrograms of organochlorine/gram of lipid). PCBs values were missing for one member of seven case-control pairs, and lipids were missing for one member of four pairs either because the samples were lost during analysis or at least one of the four main PCB congeners was not measured because of chromatographic interference.

To assess associations between *CYP1A1* polymorphisms and breast cancer risk, DNA was obtained from buffy coat either by use of Chelex solution as described by Walsh *et al.* (37) or by use of a DNA extraction kit (Qiagen, Inc., Chatsworth CA). As with the organochlorine analysis, laboratory personnel were blinded to case status. For analysis of the *CYP1A1-MspI* RFLP, a modified version of the original method (21), which was described previously (29), was used. For the *exon 7* polymorphism, a modified version of the PCR/restriction digestion assay was used as described previously (29, 38). The modification of the assay involved changes in buffer conditions and in the restriction enzyme used for RFLP analysis. The buffer concentrations used were as follows: 100 mM KCl,

Table 1 Risk of breast cancer by *CYP1A1-exon7* polymorphism and plasma levels of lipid-adjusted PCBs among all women and postmenopausal women only

CYP1A1 genotype	Tertile PCBs ^a (cut-offs $\mu\text{g/g}$)	Cases	Controls	RR (95% CI)	Multivariate RR ^b (95% CI)
Postmenopausal women (293 case/control pairs)					
WT/WT	1 (0.13–0.47)	84	81	1.00	1.00
WT/WT	2 (0.47–0.67)	82	87	0.91 (0.59–1.42)	1.00 (0.63–1.60)
WT/WT	3 (0.67–1.99)	84	90	0.90 (0.55–1.48)	0.97 (0.57–1.67)
Variants ^c	1 (0.13–0.47)	9	16	0.53 (0.21–1.31)	0.52 (0.20–1.36)
Variants	2 (0.47–0.67)	15	12	1.19 (0.51–2.78)	1.29 (0.51–3.21)
Variants	3 (0.67–1.99)	19	7	2.76 (1.04–7.35)	2.78 (0.99–7.82)
Test for interaction ^d				0.03	0.05
All women (367 case/control pairs)					
WT/WT	1 (0.13–0.46)	113	101	1.00	1.00
WT/WT	2 (0.46–0.65)	100	103	0.86 (0.58–1.28)	0.93 (0.60–1.43)
WT/WT	3 (0.65–1.99)	103	107	0.84 (0.54–1.32)	0.89 (0.55–1.45)
Variants ^b	1 (0.13–0.46)	12	21	0.49 (0.23–1.07)	0.54 (0.24–1.22)
Variants	2 (0.46–0.65)	18	21	0.72 (0.36–1.46)	0.76 (0.35–1.63)
Variants	3 (0.65–1.99)	21	14	1.33 (0.62–2.83)	1.36 (0.60–3.12)
Test for interaction				0.10	0.19

^a Cut-offs for tertiles based on the appropriate control distribution, all women or postmenopausal only depending on the analysis.

^b RR adjusted for history of breast cancer in mother or a sister, a history of benign breast disease, age at menarche (<12 years, 12 years, >12 years), body mass index (<25, 25–29, 30+ kg/m²), number of children, and age at birth of first child (nulliparous, 1–2 children and age \leq 24 at first birth, 1–2 children and age >24 at first birth, \geq 3 children and age \leq 24 at first birth, and \geq 3 children and age >24 at first birth), and duration of lactation (0, 1–6 months, and >6 months).

^c Variants are all women who are either heterozygous or homozygous for the variant allele.

^d Test for interaction, likelihood ratio test comparing model with cross-classified variables with model with main effects only.

100 mM (NH₄)₂SO₄, 200 mM Tris (pH 8.8), 70 mM MgSO₄, and 1% Triton X-100. The PCR product was digested overnight at 37°C with *Nco*I (New England Biolabs, Inc., Beverly, MA).

Statistical Analysis. We defined high, medium, and low levels of plasma PCBs based on tertiles of the appropriate control distributions (all women or postmenopausal only). Genotype status was defined as wild-type (those homozygous for the wild-type allele) or variant (those with at least one variant allele). We assessed the presence of interactions between each of the *CYP1A1* polymorphisms and total PCBs. In conditional logistic regression models, we compared the risk of breast cancer for subjects in each category of joint exposure to that of subjects who were homozygous wild type for the *CYP1A1* polymorphism and who had the lowest exposure level of total PCBs. The interaction was evaluated using the log likelihood ratio test, comparing these models with the model containing indicator variables for the cross-classified variables only.

Results

The distribution of breast cancer risk factors in the cases and controls has been described in previous publications focusing on the association of breast cancer risk with PCBs (32, 33) and with the *CYP1A1* variants (29). In this subpopulation for which information on both PCBs and *CYP1A1* are available and when restricting to postmenopausal case-control pairs, the relationships are not substantially different. In the whole study sample, the median age of the subjects was 60 years (range, 43–69 years). Among the postmenopausal women, the median age of menopause was 49 for both the cases and controls. Maternal history of breast cancer, history of breast cancer in a sister, and history of benign breast disease were more common in the cases than in the controls ($P \leq 0.05$); differences for other breast cancer risk factors, including lactation, were not statistically significant.

The lipid-adjusted levels of total PCBs ranged from 0.18 to 1.61 $\mu\text{g/g}$ for the cases (median = 0.54) and 0.13 to 1.99 $\mu\text{g/g}$ (median = 0.54) for the controls. These levels are quite low but are comparable with those found in other United States

populations when age and region of residence of the participants are taken into account (9), including the postmenopausal women in New York state studied by Moysich *et al.* (0.08–1.90 $\mu\text{g/g}$; Ref. 30).

As described in previously published studies, there was no independent association of either the *CYP1A1* variants (29) or PCBs (32, 33) with breast cancer risk (see citations for more details). However, we observed a borderline statistically significant elevated risk of postmenopausal breast cancer among women with serum PCB levels in the third tertile and at least one variant allele of the *CYP1A1-exon 7* genotype compared with women homozygous for the wild-type allele and with the lowest levels of PCBs (multivariate RR, 2.78; 95% CI, 0.99–7.82; Table 1). P for the overall test of interaction was 0.05. The equivalent RR for all women combined (including pre-, post-, and uncertain menopausal status) was only modestly elevated (multivariate RR, 1.36; 95% CI, 0.60–3.12, P for interaction = 0.19). Among postmenopausal women with both the variant allele and high PCB exposure, there was a statistically significant elevated risk of breast cancer compared with the women with the variant allele and low PCB exposures, and there was a positive linear trend within this group ($P = 0.03$). Furthermore, among the women with PCB levels in the upper third of the distribution, the association of the *CYP1A1-exon7* variant with breast cancer risk was elevated compared with the wild-type (RR, 2.89; 95% CI, 1.14–7.29). There was no evidence of an interaction of plasma levels of PCBs with the *CYP1A1-Msp*I polymorphism (Table 2).

Because of the role of *CYP1A1* in metabolism of chemicals (PAHs) associated with cigarette smoke, we also evaluated the possibility of effect modification by smoking status. Breast cancer risk was statistically significantly increased among the 22 women (19 cases and 3 controls) with PCB levels above the median and the variant *CYP1A1* polymorphism who had ever smoked (age adjusted RR, 5.48; 95% CI, 1.53–19.67) but not among those who had never smoked (RR, 1.44; 95% CI, 0.57–3.66). Admittedly, these numbers are too small to adequately assess this additional interaction.

Table 2 Risk of breast cancer by *CYP1A1-MspI* polymorphism and plasma levels of lipid-adjusted PCBs among all women and postmenopausal women only

CYP1A1 genotype	Tertile PCBs ^a (cut-offs $\mu\text{g/g}$)	Cases	Controls	RR (95% CI)	Multivariate RR ^b (95% CI)
Postmenopausal women (293 case/control pairs)					
WT/WT	1 (0.13–0.47)	79	78	1.00	1.00
WT/WT	2 (0.47–0.67)	73	79	0.94 (0.61–1.46)	1.00 (0.62–1.60)
WT/WT	3 (0.67–1.99)	85	82	1.08 (0.67–1.75)	1.18 (0.69–2.01)
Variants ^c	1 (0.13–0.47)	14	19	0.70 (0.32–1.54)	0.53 (0.27–1.23)
Variants	2 (0.47–0.67)	24	20	1.22 (0.63–2.38)	1.37 (0.67–2.79)
Variants	3 (0.67–1.99)	18	15	1.27 (0.59–2.76)	1.08 (0.47–2.48)
Test for interaction ^d				0.47	0.22
All women (367 case/control pairs)					
WT/WT	1 (0.13–0.46)	106	97	1.00	1.00
WT/WT	2 (0.46–0.65)	86	99	0.81 (0.54–1.21)	0.84 (0.54–1.30)
WT/WT	3 (0.65–1.99)	102	103	0.93 (0.60–1.45)	1.00 (0.62–1.63)
Variants ^b	1 (0.13–0.46)	19	25	0.70 (0.36–1.35)	0.63 (0.31–1.28)
Variants	2 (0.46–0.65)	32	25	1.16 (0.65–2.09)	1.24 (0.66–2.33)
Variants	3 (0.65–1.99)	22	18	1.16 (0.58–2.34)	0.94 (0.44–2.01)
Test for interaction				0.24	0.21

^a Cut-offs for tertiles based on the appropriate control distribution, all women or postmenopausal only depending on the analysis.

^b RR adjusted for history of breast cancer in mother or a sister, a history of benign breast disease, age at menarche (<12 years, 12 years, >12 years), body mass index (<25, 25–29, 30+ kg/m²), number of children, and age at birth of first child (nulliparous, 1–2 children and age \leq 24 at first birth, 1–2 children and age >24 at first birth, \geq 3 children and age \leq 24 at first birth, and \geq 3 children and age >24 at first birth), and duration of lactation (0, 1–6 months, and >6 months).

^c Variants are all women who are either heterozygous or homozygous for the variant allele.

^d Test for interaction, likelihood ratio test comparing model with cross-classified variables with model with main effects only.

Discussion

Among 293 postmenopausal case-control pairs, there was no evidence of an association of either the variant *CYP1A1* genotype or exposure to PCBs with breast cancer risk (29, 32). There was no association between PCBs and risk for women *CYP1A1* homozygous wild type. However, the risk for women with high plasma PCB levels and at least one variant of the *CYP1A1-exon 7* allele was elevated when compared with women with both low PCBs and the wild-type allele (RR, 2.78; 95% CI, 0.99–7.82, *P* for interaction = 0.03). The CIs were wide as the numbers are small; however, the trends in sign and the magnitude of these ORs are quite similar to those observed by Moysich *et al.* (30). Furthermore, there was no indication of an interaction of PCBs with the *CYP1A1-MspI* allele. Variations in risk of breast cancer with different genotypic variants of the *CYP1A1* gene have been suggested because of the role of *CYP1A1* in metabolism of both potentially genotoxic chemicals and endogenous hormones and the evidence of differences in activity for the different polymorphisms (10, 20–23). Although no overall increase in breast cancer was observed in the Nurses' Health Study for either the *MspI* or *exon 7* variant genotype, there was a suggestion of an interaction with early onset of smoking with both polymorphisms (29). In a case-control study of 216 cases and 282 controls, Ambrosone *et al.* (27) observed a significant increase in risk among postmenopausal women with the variant *exon 7* allele. The risk was most evident for light smokers (<30 pack years; Ref. 27). Taioli *et al.* (26) reported an increase in breast cancer risk among African-American women (20 cases, 81 controls) but not among Caucasian women (29 cases, 175 controls) who had the *MspI* variant genotype. They hypothesized that the polymorphism may be a marker for altered estradiol metabolism leading to increased susceptibility to estrogen-related breast cancer in African Americans (26). Rebbeck *et al.* (28) and Bailey *et al.* (39) did not observe an association of *CYP1A1* polymorphisms with breast cancer or any interaction with cigarette smoking or race.

Exposure to PCBs was originally hypothesized to be associated with breast cancer risk because they have weak estro-

genic activity and are tumor-promoters (3). They also have been shown to induce *CYP1A1* activity in experiments in cell lines and various animal species. In fact, this induction is a sensitive indicator of PCB exposure (14, 17). In a recent study of pregnant women exposed to high levels of PCBs from consumption of contaminated seafood, *CYP1A1* enzyme activity and DNA adducts in the placenta were good biomarkers of exposure (40). Thus, it has been hypothesized that there may be an interaction of PCB exposure with *CYP1A1* polymorphisms in the etiology of breast cancer (30). PCBs themselves may be more likely to be metabolized to carcinogenic intermediates in the presence of the *CYP1A1* variant genotype. On the other hand, in the presence of PCB exposure, the *CYP1A1* variant may more efficiently metabolize endogenous hormones or other xenobiotics such as PAHs from cigarette smoke.

Our results are similar to those reported by Moysich *et al.* (30). In a study of 154 cases and 191 controls, they observed a RR of 2.90 (95% CI, 1.18–7.45) among postmenopausal women with serum PCB levels above the median and at least one variant allele compared with women who were homozygous for the wild-type allele and had low levels of PCBs (30). [Our observed RR for the equivalent comparison of high and low body burden of PCBs was 2.28 (95% CI, 1.03–5.04)]. Although numbers of smokers with the variant allele were small, Moysich *et al.* (30) also reported that risk was significantly increased among women with elevated PCB body burden and the *CYP1A1* polymorphism who had ever smoked cigarettes (odds ratio, 7.74; 95% CI, 1.12–53.90) but not among women who had never smoked (odds ratio, 1.43; 95% CI, 0.53–3.87). We observed similar results in our study. The numbers of smokers with the variant allele and high body burden of PCBs in both studies are too small to make any meaningful conclusions, but taken together, the results are suggestive that PCBs may enhance the metabolism of PAHs.

Although we had almost twice as many cases as the Moysich study (30), we still were limited by our small sample size as the variant allele frequency is low among Caucasians (12% among the controls in this study). Therefore, the CIs,

although statistically significant, were wide, and we could not examine the association in more detail.

Another limitation of this study may be in our definition of PCB exposure. Because of low plasma levels of individual congeners to minimize the amount of measurement error, we evaluated the risk associated with a sum of PCBs as opposed to the risk for each individual congener. We have not looked in detail at the congeners that are known to induce CYP1A1, *e.g.*, IUPAC nos. 180 and 153 (41). Levels of individual congeners tend to be correlated. Specific breast cancer analyses of the four main congeners described above (IUPAC nos. 118, 138, 153, and 180) demonstrated similar patterns to those observed for the sum of PCBs. In any case, the effect of the potential misclassification of exposure because of grouping CYP1A1 inducers with noninducers should bias the results toward the null.

Use and manufacture of PCBs in the United States was banned in 1977. Therefore, although the United States population is still exposed to PCBs from old products and persistent compounds in the environment, peak exposures occurred at least 10 years before our bloods were collected. We believe current levels are likely to be indicative of past exposures because of the long half-lives of the compounds and their resistance to metabolism, but the valuation of risks associated with low levels from a general population sample, years after primary exposure, could be biased toward the null because of nondifferential measurement error.

Although the exon 7 and MspI polymorphisms appear to be linked, the interaction was present only with the exon 7 variant. Some studies have observed an increase in susceptibility with the MspI mutation, but the evidence of increased catalytic activity and gene inducibility is stronger for exon 7 (22, 23).

In summary, neither PCBs (32, 33) nor the CYP1A1 polymorphism (29) alone was independently associated with breast cancer risk in this population. However, high levels of PCBs may be associated with breast cancer risk in the subgroup of women who have the variant CYP1A1-exon7 polymorphism. The majority of studies have concluded that PCB exposures are unlikely to be a major risk factor for breast cancer in the United States (9), but additional studies of genetically susceptible populations are warranted.

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