AD_____

AWARD NUMBER DAMD17-94-J-4108

TITLE: The Molecular Epidemiology of Breast Cancer: Risk From Environmental Exposures and Genetic Susceptibility

PRINCIPAL INVESTIGATOR: Kirsten Moysich, Ph.D.

CONTRACTING ORGANIZATION: Research Foundation of State University
                          Of New York at Buffalo
                          Amherst, New York 14228-2567

REPORT DATE: September 1998

TYPE OF REPORT: Final

PREPARED FOR: Commanding General
                U.S. Army Medical Research and Materiel Command
                Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
                        Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

19990811 111

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE September 1998	3. REPORT TYPE AND DATES COVERED Final (1 Sep 94 - 31 Aug 98)

4. TITLE AND SUBTITLE
The Molecular Epidemiology of Breast Cancer: Risk from Environmental Exposures and Genetic Susceptibility

5. FUNDING NUMBERS
DAMD17-94-J-4108

6. AUTHOR(S)
Kirsten B. Moysich, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Research Foundation of State University
 Of New York at Buffalo
Amherst, New York 14228-2567

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

This postdoctoral research fellowship included a thorough investigation of genetic and environmental determinants of breast cancer risk. Major findings from this research include the following: 1) smoking may be an important risk factor for breast cancer among postmenopausal women with genetically-determined slow acetylation phenotype; 2) the null *GSTM1* genotype may be associated with breast cancer risk among premenopausal smokers, but is not an overall risk factor for breast cancer; 3) the *CYP1A1* variant genotype is not a strong risk factor for breast cancer, but may be associated with greater risk among smokers; 4) consumption of processed meats, high sources of known mammary mutagens, may increase risk for premenopausal breast cancer, particularly among women with rapid *NAT2* genotype; 5) the CYP2E1 variant genotype is not associated with risk, but may be a risk factor for premenopausal smokers; 6) higher serum organochlorine levels were not associated with greater risk of postmenopausal breast cancer; 7) higher serum levels of PCBs were associated with increased risk of breast cancer among parous women who never lactated; and 8) the *CYP1A1* variant genotype was a risk factor for women with high PCB body burden. Methodological issues concerning the investigation of organochlorine compounds in epidemiologic studies were explored.

14. SUBJECT TERMS
Breast Cancer, Molecular Epidemiology, Environmental Factors, Genetic Polymorphisms

15. NUMBER OF PAGES
80

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

_____ Where copyrighted material is quoted, permission has been obtained to use such material.

_____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

_____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

_____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

_____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

_____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

_____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

_____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature   Date   11/01/98

TABLE OF CONTENTS

PART ONE - GENETIC SUSCEPTIBILITY AND BREAST CANCER RISK

This postdoctoral fellowship intially funded the research conducted by Dr. Christine Ambrosone, whose work was primarily concerned with the investigation of the effect of polymorphisms in genes involved in actication or detoxification of carcinogenes on the association between environmental exposures and breast cancer risk.

Introduction

Of women living in urban areas, particularly in the northeast, with similar exposures to environmental contaminants, only a portion develop breast cancer. Breast cancer etiology most probably results from a complex interaction of factors including individual hormonal milieu, dietary factors, exposure to environmental contaminants as well as individual genetic susceptibility.

Of all risk factors associated with breast cancer, a positive family history of breast cancer has been found consistently to significantly elevate breast cancer risk. Women with one affected first-degree relative have a risk that is two to three times greater than the risk those without a family history (1). Considerable attention has focused on identification of a tumor suppressor gene (BRCA 1) which greatly increases breast cancer risk (2). However, it may be that more commonly occurring genetic differences in carcinogen metabolism and detoxification may explain part of this increased risk.

For lung cancer, research has indicated that some of the variability in risk among smokers may be due to polymorphisms in genes involved in carcinogen metabolism and detoxification. Studies investigating polymorphisms in genes for cytochrome P450 enzymes, CYP1A1 and CYP2D6, and glutathione-S-transferase (GST), a detoxicant, have demonstrated increased lung cancer risk with specific genotypes. It has also been

demonstrated that women with squamous cell lung cancer may be more susceptible to the effects of carcinogens in cigarette smoke than men are (3). It is plausible that genetic polymorphisms are similarly involved in breast cancer risk. There is some indication that family history of breast cancer modifies the effects of other risk factors, with disease in women with genetic susceptibility possibly resulting from different etiologic pathways. Some studies have noted that risk associated with some reproductive factors varies significantly for women with family history of the disease compared to those without a family history, although results of studies have not been consistent (1,4,5). In addition, risk associated with intake of some nutrients may vary according to family history status (unpublished observations). In any case, pharmacogenetic studies have revealed that individuals vary in metabolism of drugs from ten to two hundred fold, and this variability may also indicate response to environmental agents (6). Therefore, investigation of genetic variability in genes involved in the metabolism and detoxification of carcinogenic substances may further explain the relationship between breast cancer risk and exposure to environmental contaminants.

Methods

This case-control study of genetic and environmental determinants of breast cancer risk utilizes data collected from 1986 to 1991 in Western New York. Participants ranged in age from 40 to 80 years. Women with incident, primary, histologically confirmed breast cancer were recruited from all the major hospitals in Western New York, and were frequency-matched by age and county of residence to controls. Controls 65 and older were identified from Health Care Finance Administration lists, and those younger than 65 were from New York State Motor Vehicles registries. Cases and controls completed in-

person interviews regarding health, reproductive, and lifestyle histories. A detailed food-frequency interview was also administered. Participants were asked to provide a blood specimen, and approximately 65% consented to phlebotomy. Samples were processed and stored at -70° C.

DNA was extracted from stored blood clots by the Laboratory for Human Carcinogenesis at the National Cancer Institute. PCR and RFLP were used to amplify and evaluate polymorphisms in NAT2, CYP1A1, CYP2E1, CYP2D6 and GSTμ. Results from the toxicological research and the genetic data were merged with the questionnaire data base, and associations evaluated between breast cancer risk and a number of environmental and genetic factors.

Results and Discussion

N-Acetyltransferase (NAT2) is involved in the metabolism of aromatic and heterocyclic amines. N-Acetylation is a detoxifying process in the metabolism of aromatic amines, and three polymorphisms have been found to account for 90-95% of the slow acetylation phenotype among Caucasians. We found that neither smoking nor *NAT2* status was independently associated with breast cancer risk. When we stratified by *NAT2* status, there were no clear patterns of increased risk associated with smoking among premenopausal women. However, among postmenopausal women, *NAT2* strongly modified the association of smoking with risk. For slow acetylators, current smoking and smoking in the distant past (20 years prior to the interview), significantly increased breast cancer risk in a dose-dependent manner (odds ratios [OR] for highest quartile of cigarettes smoked 2 and 20 years prior to the interview and 95% confidence intervals, respectively, 4.4, [1.3-14.8] and 3.9, [1.4-10.8]. Among rapid acetylators, smoking was

associated with a null or inverse risk. Case-series and smoking-matched case-control analyses corroborated these findings. We concluded that these results, which indicate that smoking may be an important risk factor for breast cancer among postmenopausal women with genetically-determined slow acetylation phenotype, demonstrate heterogeneity in response to carcinogen exposures, and may explain previously inconsistent findings for cigarette smoking as a risk factor for breast cancer.

CYP1A1 and GSTμ Polycyclic aromatic hydrocarbons (PAHs), possible human breast carcinogens, are metabolized by cytochrome P4501A1 (*CYP1A1*) and glutathione S-transferase (*GSTM1*). A *CYP1A1* polymorphism (isoleucine to valine substitution in exon 7) or the null allele for *GSTM1* may affect the mutagenic potential of PAHs. DNA analyses suggested no increased breast cancer risk with the null *GSTM1* genotype (OR=1.10, CI, 0.73-1.64), although there was some indication that the null genotype was associated with risk among the youngest postmenopausal women (OR=2.44, CI, 0.89-6.64). Slightly elevated risk was associated with the *CYP1A1* polymorphism (OR=1.61, CI, 0.94-2.75) and was highest for those who smoked up to 29 pack years (OR 5.22, CI, 1.16-23.56). Statistical power to detect an effect may be limited by small numbers, and larger sample sizes would be required to corroborate these suggestive findings.

CYP2E1

Another tobacco carcinogen, N-nitrosamines, are mammary carcinogens in rodents. Because N-nitrosamines are metabolically activated by cytochrome P450IIE1 (*CYP2E1*), risk associated with the intron 6; *Dra*I restriction enzyme site was investigated. There was no association between the CYP2E1 polymorphism and breast cancer risk among pre or postmenopausal women. When women were categorized as non-smokers versus

smokers, premenopausal women with one or two C alleles who had a history of smoking were found to be at increased risk (adjusted odds ratio (OR and 95% confidence interval, 11.1, 1.5-81.4), although the number of study subjects with this genotype was small. We also evaluated the possible role of dietary heterocyclic aromatic amines (HAAs) in breast cancer risk. HAAs, formed in the cooking of meats, are activated by *N*-acetyltransferase (NAT2) and are mammary carcinogens in rodents. We investigated whether ingestion of meat, chicken and fish, sources of HAAs, may increase breast cancer risk, particularly among women with rapid *NAT2* genotype. Consumption of meats and meats cooked at high temperatures was not associated with increased breast cancer risk. Processed meats, however, including bacon, sausages, and hot dogs, increased risk for premenopausal women (4th quartile odds ratio (OR)=1.7, 95% confidence interval (CI), 1.0-2.9). Risk was greatest for women with the rapid *NAT2* genotype (4th quartile OR=18.1, 95% CI, 3.2-100.7). Beef consumption also appeared to be associated with increased risk among rapid acetylators. These associations were not observed among postmenopausal women. Although hampered by small numbers in stratified analyses, these results suggest that consumption of processed meats, high sources of known mammary mutagens, may increase risk for premenopausal breast cancer, particularly among women with rapid *NAT2* genotype.

References

1. Kelsey JL, Gammon MD. Epidemiology of breast cancer. Epidemiologic Review 1990;12:228-240.

2. Hall JM, Friedman L, Guenther C, et al. Closing in on a breast cancer gene on chromosome 17q. Am J Hum Genet 1992;50:1235-1242.

3. Ambrosone CB, Rao U, Michalek AM, et al. Lung cancer histology and family history of cancer: Analysis of histologic subtypes from 872 primary lung cancer patients. Cancer 1993;72:1192-8.

4. Sellers TA, Kushi LH, Potter JD, et al. Effect of family history, body-fat distribution, and reproductive factors on the risk of postmenopausal breast cancer. New Engl J Med 1992;326:1323-1329.

5. Brinton LA, Hoover R. Fraumeni JF Jr. Interaction of familial and hormonal risk factors for breast cancer. J Natl Cancer Inst 1982;69:817-822.

6. Omenn, GS. Future research directions in cancer ecogenetics. Mutation Res

   1991;247:283-291.

PART TWO– ORGANOCHLORINES AND BREAST CANCER

INTRODUCTION

Environmental factors have been implicated in breast cancer etiology, due to the

steady increase in incidence over the last decades (1), regional and international

differences in incidence, and observed changes in incidence rates in migrant populations

(2). One group of environmental exposures that has been examined in relation to breast

cancer are organochlorine compounds, such as 2,2-bis (4-chlorophenyl)-1,1-

dichloroethane (DDE), the major metabolite of 2,2-bis (p-chlorophenyl)-1,1,1-

trichloroethane (DDT), polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and

mirex. During the early 1940's to 1972, DDT was one of the most widely used chemicals

for controlling insect pests on agricultural crops and for controlling insects that carry

diseases such as malaria and typhus. PCBs have been manufactured commercially since

1929 for a variety of applications, including use as dielectrics in transformers and

capacitors and for cooling fluids in hydraulic systems. HCB is a widespread chlorinated

hydrocarbon originating from agricultural and industrial sources. It was originally used as

a fungicide, but currently, the major source of HCB is industrial emission as a side

product related to the manufacture of organochlorinated products. Mirex was extensively

used in the southeastern US for the control of the red fire ant and as a fire retardant

coating for various materials. Although commercial production of all of these compounds

was banned in the early 1970's, measurable levels of organochlorine residues are still found

in human tissue and blood samples. This persistence has been attributed to their slow

metabolism and high lipid solubility, leading to storage in adipose stores, including breast tissue. The primary route of excretion for these compounds for women is lactation (3).

Evidence from laboratory studies has demonstrated a complex diversity of biological effects associated with these compounds. DDE, HCB, mirex, and some PCB congeners have been associated with induction of cytochrome p450 enzymes (4-9), which may or may not be associated with estrogenic (10-14) and antiestrogenic effects (12) shown in some investigations. Studies have also noted changes immune responses (15-17) and tumor promoting effects (4, 7,18-20).

The body of evidence on the effect of organochlorines on breast cancer risk is limited (21-27). Several studies compared organochlorine concentrations in neoplastic tissue with tissue levels of women with benign breast disease or women who died in accidents (21-25). Results from these mammary tissue studies are inconsistent and were hampered by several methodological limitations, including small sample size and absent or inadequate control for known breast cancer risk factors.

Only recently, two epidemiological studies examined this risk relationship with more precision (26,27). Wolff et al. (26) examined DDE and total PCB concentrations in sera from 58 breast cancer cases and 171 controls. Although risk was associated with higher levels of serum DDE, there was no evidence of a linear relationship with greater body burden of PCBs. Increased risk, either statistically significant or of borderline significance, was observed for all PCB exposure levels above the lowest. A nested case-control study was also conducted by Krieger and colleagues (27) consisting of 150 breast cancer cases from three ethnic groups (Caucasian, African-American, and Asian) and 150 matched controls, all of whom had blood samples stored in the late 1960's. No excess

risks of breast cancer in association with serum levels of DDE and total PCBs were observed for the total sample, although there was some evidence for nonsignificant increases in risk for Caucasian and African-American women with higher DDE levels.

In this case-control this study, we examined the overall association between serum levels of DDE, HCB, mirex, total PCBs and PCB congener groups, and risk of postmenopausal breast cancer, and possible effect modification by history of lactation. This research adds to the existing body of evidence in several ways: first, the effect of organochlorines on breast cancer risk was examined in a group homogenous with respect to menopausal status, postmenopausal women; second, because of a larger sample size, we were able to determine the effects of these compounds in subgroups of women, defined by history of lactation; third, because of the availability of the extensive questionnaire data we were able to adjust risk estimates for all known and suspected confounders; fourth, all risk estimates were adjusted for serum lipids; and fifth, we were able to examine the effect of PCBs in much greater detail, due to the availability of congener specific data.

MATERIALS AND METHODS

The effect of environmental exposure to organochlorines on breast cancer risk was examined in a subset of participants from a case-control study of postmenopausal breast cancer. A more detailed description of this study population has been published elsewhere (28). Briefly, women in this study were enrolled from 1986 to 1991 in Erie and Niagara counties in Western New York. Included were 439 postmenopausal breast cancer cases and 494 postmenopausal community controls between the ages of 41 and 85 years. All participants were Caucasian. Cases were identified from most area hospitals and were

14

interviewed within two months of diagnosis. Of the eligible 777 women with breast cancer, 439 (56.5 percent) were interviewed. The primary reason for nonparticipation was physician refusal. Controls were 1076 female residents of the two counties who were from Health Care Finance Administration and New York State Department of Motor Vehicles records. 494 (45.9 percent) of the eligible postmenopausal women agreed to participate. Cases and controls were interviewed in-person by trained interviewers. The interview took approximately two hours to complete and included assessment of medical history, reproductive history, occupational history, exposure to exogenous hormones, family history of cancer, and a food frequency questionnaire that assessed usual intake in the year two years prior to the interview.

Of women who provided usable interviews, approximately 63% agreed to donate a blood sample. Blood was drawn for 262 postmenopausal breast cancer cases and 319 controls. Blood samples from most women with breast cancer were collected within three months of surgery. Blood was processed immediately in the laboratory staffed by a trained lab technician and placed in a freezer. Since collection, the saved samples have remained frozen at -70°C. A subset of the stored blood specimens was utilized for these toxicological analyses: 154 women with postmenopausal breast cancer and 192 controls. Cases were only included in the final study sample if their blood was drawn before chemotherapy or radiation, and within three months of surgery. Controls were frequency matched to cases by date of blood draw (±3 months) and age (±3 years).

Laboratory methods The laboratory analyses were performed by the Toxicology Research Center Analytical Laboratory at SUNYAB (TRC). The concentrations of DDE, HCB, mirex, and 56 PCB peaks, representing 73 congeners, in the serum samples were determined

by the method of Greizerstein et al. (29). The procedures include standardized extraction,

clean up and quantification by high resolution gas chromatography (GC) and comprehensive

quality assurance program to minimize systematic and erratic errors. Trained and blinded

laboratory technicians used 2 grams of serum to determine levels of DDE, HCB, mirex, and

PCB congeners measured in ng/g of serum. The sample was mixed with solutions

containing IUPAC isomers #46 and #142 (surrogate standards). Methanol was added to

precipitate the proteins and the resulting mixture was extracted with hexane. The extract was

concentrated and then cleaned by passing through a Florisil column. The eluate was

evaporated to a small volume and isomers #30 and #204 were added as internal standards.

An aliquot of the mixture was injected into the GC equipped with an electron capture

detector. Quantification was based on calibration standards and response factors calculated

using purchased reference materials. The quality control activities consisted of analyses of

samples in batches of six to ten simultaneously with quality control (QC) samples. The

quality assurance program checked that the procedures were under control by the use of

control charts and set the criteria for data acceptability. The limit of detection (LOD) for

each analyte was determined as the mean of background noise plus three standard deviations

in five reagent blank samples. The TRC participates in the Great Lakes Research Program

Quality Assurance/Quality Control Program sponsored by the Agency for Toxic Substances

and Disease Registry (ATSDR) and in the Northeast/Mid-Atlantic Breast Cancer QA/QC

Study.

Statistical analyses Risk of breast cancer was examined for serum DDE, HCB, mirex,

and PCBs. The effect of PCBs on risk was examined using several measures of PCB

exposure. First, the detected levels of the 56 PCB peaks were added to obtain a measure of

total PCBs. Second, the number of detected PCB peaks was determined to obtain an alternative measure of total PCB exposure. Third, three groups of PCB congeners were determined by adding the detected levels of lower chlorinated PCB congeners (IUPAC #s 6, 7+9, 18, 19, 22, 23, 33, 15+17, 16+32, 25+50, 31+28, 40, 45, 49, 52, 55, 60, 64, 70, 42+59, 47+48, 66+95, and 77+110), moderately chlorinated PCB congeners (IUPAC #s 87, 97, 99, 101, 118, 151+82, 129, 134, 135, 136, 138, 147, 149, 153, 141+179, 128+167, 171+156, 172, 176, 177, 180, 183, 185, 187, 188, 174+181), and higher chlorinated PCB congeners (IUPAC #s 194, 195, 200, 203, 205, 206). Grouping was based on biological activity, as well as on data availability. In our initial attempts to group these compounds, e.g. by enzyme induction or estrogenic activity, we discovered that most women had no detectable levels for these highly reactive congeners. Thus, our decision to group by degree of chlorination was in part driven by the fact that we had available data for all participants.

Descriptive analyses included Student t-tests of means for cases and controls for lifestyle, reproductive, and dietary variables, and chi square tests for categorical variables. Multivariate analysis of variance (MANOVA) was employed to obtain age and lipid adjusted means for the organochlorine variables. Odds ratios (ORs) were calculated by unconditional logistic regression with 95% confidence intervals (CIs) computed from the standard error of the regression coefficient. Most exposure categories were examined in tertiles, based on the distribution of the individual compounds in the controls. Serum mirex levels were dichotomized into detectable and non-detectable levels (referent) because of the small number of women with detectable levels (27.3%). For the lower chlorinated congeners, women with levels below the LOD (30.1%) served as the referent

group and were compared to women in the lower and upper halves of women with detectable levels. ORs were adjusted for potential confounders, including age, education, family history of breast cancer, parity, quetelet index, duration of lactation, age at first birth, years since last pregnancy, fruit and vegetable intake, and serum lipids. Covariates were only included in the final regression model if they were established risk factors in these data or changed the observed risk estimate by at least 15 percent. Lipid adjustment was modeled after the method described by Phillips et al. (30).

To determine and describe effect modification by lactation, which was expected, due to differences in elimination of organochlorines, women were also stratified by lactation history, excluding nulliparous women (n=48). Subgroup analyses were not performed for the lower chlorinated PCB congeners because lactation is not likely to be an important route of excretion of these compounds in that they are metabolized within weeks to months after exposure (31,32).

RESULTS

Descriptive characteristics for cases and controls are shown in Table 1 for the total study population and stratified by history of lactation. In the total study population in this nested study, cases and controls did not differ significantly with respect to demographic, reproductive, and dietary variables. Cases were more likely to have a positive family history of breast cancer, and less likely to reside in rural areas than controls. Among women who had never lactated, cases and controls were very similar for most of these descriptive variables, with the exception of significantly older age of menopause for cases. Similarly, among women who had lactated, we observed few differences between the study groups. Cases were more likely to have a family history of

breast cancer, consumed fewer vegetables, and were less likely to reside in rural areas than controls.

Serum concentrations, adjusted for age and serum lipids, of DDE, HCB, mirex, and PCBs in the entire study population and in the lactation groups are presented in Table 2. In the total sample, cases tended to have slightly higher mean serum organochlorine concentrations than controls, with the exception of HCB. Among women who never lactated cases had higher serum levels of all compounds under investigation. Although these differences in means were of greater magnitude than in the total study sample, none were statistically significant. In contrast, among women who had ever lactated, cases had slightly lower levels than controls of most of these compounds, with the exception of total number of PCB congener peaks detected and higher chlorinated PCBs.

Risk of postmenopausal breast cancer associated with environmental exposure to DDE, HCB, and mirex is shown in Table 3. In the total sample there was no evidence of greater risk of breast cancer for women with the highest serum levels of DDE (third tertile OR=1.34; 95% CI 0.71-2.55) and HCB (third tertile OR=0.81; 95% CI 0.43-1.53), or with detectable levels of mirex (OR=1.37; 95% CI 0.78-2.39). Among women who never lactated there was a suggestion of excess risk among women in the second and third tertile of the DDE distribution (OR=1.95; 95% CI 0.58-6.67 and OR=1.83; 95% CI 0.63-5.33, respectively). However, further adjustment for serum PCB levels reduced the magnitude of these estimates (OR=1.61; 95% CI 0.61-6.01 and OR=1.32; 95% CI 0.59-4.08, respectively [data not shown]). Similarly, in this group there was some evidence for an increase in risk as a function of increasing HCB levels (third tertile OR=1.79; 95% CI 0.59-5.40), but again, further adjustment for serum PCBs substantially reduced this

estimate (OR=1.21; 95% CI 0.50-4.11, data not shown). Among women who never

lactated, those with detectable levels of mirex had a marginally significant two-fold

increase in risk compared to women with no detectable levels. This effect persisted after

serum PCBs and DDE levels were entered into the model. Among women who ever

lactated there was no association with risk and serum DDE and mirex. As for HCB,

inverse associations were observed among women with higher HCB levels, which were

most pronounced and statistically significant for women in the second tertile of the

distribution (OR=0.32; 95% CI 0.14-0.71).

The effect of PCB body burden on breast cancer risk is presented in Table 4. In

the entire study population, women with the highest serum PCB levels or the greatest

number of detected PCB congeners were not at greater risk of breast cancer when

compared to women with the lowest levels or fewer detected peaks (OR=1.14; 95% CI

0.61-2.15 and OR=1.34; 95% CI 0.72-2.47, respectively). However, among women who

never lactated there was some indication of increasing risk with increasing serum PCBs

(third tertile OR=2.87; 95% CI 1.01-7.29) and increasing number of detected peaks (third

tertile OR=3.31; 95% CI 1.04-11.3). These effects were still observed after adjustment

for serum DDE and HCB (data not shown). For women who had ever lactated there was

no evidence for an adverse effect of these exposures on risk.

As for PCB congener groups, we observed some evidence of greater risk of breast

cancer for women with detectable levels of lower chlorinated PCBs compared to those

without detectable levels, although this effect was most pronounced for women in the

lower half of the distribution (OR=2.04; 95% CI 1.09-3.83). When all women with

detectable levels were compared to women without detectable levels the OR was 1.61

and the 95% CI was 1.07-3.51. The ORs were similar after adjustment for serum DDE and HCB levels. No such effect was observed for the moderately chlorinated PCBs (third tertile OR=1.37; 95% CI 0.73-2.59) or the higher chlorinated PCBs (third tertile OR=1.19; 95% CI 0.60-2.36). Among women who never lactated, higher levels of moderately chlorinated PCBs (third tertile OR=3.57; 95% CI 1.10-8.60), but not higher chlorinated PCBs (third tertile OR=1.53; 95% CI 0.47-4.98) were associated with increased risk of breast cancer. Among women who had ever lactated there was no evidence for an increase in breast cancer risk in relation to higher serum concentrations of moderately or higher chlorinated PCBs.

DISCUSSION

Results from this case-control study did not indicate that environmental exposure to DDE, HCB, mirex, and PCBs was related to breast cancer risk in the entire study sample, with the exception of a modest increase in risk associated with having detectable levels of lower chlorinated PCB congeners. However, among parous women who never lactated there was some evidence of greater risk for women with elevated levels of mirex, total PCBs, and moderately chlorinated PCBs, as well as for those with the highest number of detected PCB congeners. Furthermore, among women who had ever lactated, we found no association with environmental organochlorine exposure and breast cancer risk.

Elevated serum levels of DDE were not associated with breast cancer risk, although there was some evidence for greater risk with higher levels among women who never lactated. Attenuation of this effect when serum PCB levels were included into the model suggests that the observed risk was largely driven by the association of DDE with

serum PCB levels. These findings are in contrast to those reported by Wolff et al. (26), who observed a nearly four-fold increase in risk for women with the highest levels of DDE, which was unaffected by inclusion of PCB levels into the regression model. Similarly, Krieger et al. (27) observed a nonsignificant two-fold increase in risk of breast cancer among white women with the highest DDE body burden.

The effect of HCB on breast cancer risk has not been previously reported in studies employing an epidemiologic study design. In three studies, mammary adipose tissue levels of breast cancer patients were compared to those of controls with benign breast disease (24,25) or accident fatalities (23). None of these studies reported a significant difference in HCB concentrations between breast cancer cases and controls. The absence of an adverse effect of HCB exposure in our data was consistent with these earlier studies.

The association between body burden of mirex and breast cancer risk has not been explored previously. In these data, no increase in risk was observed for detectable levels in the entire study population, but a borderline significant increase in risk became apparent when the sample was restricted to parous women who never lactated. The latter finding needs to be considered with caution, for the observed increase in risk was based on very small numbers of women with detectable levels of this compound (n=35). Future investigations should examine the association of mirex with breast cancer risk in population with greater exposure levels (e.g., fish consumers or women residing in the southern US).

In these data, we observed a modest increase in risk for women with detectable levels of lower chlorinated PCBs. Among the never lactating parous women with higher

levels of total PCBs, moderately chlorinated PCBs, or greater numbers of detected congener peaks there was some increase in risk. The investigation of the lower chlorinated congeners in relation to risk was problematic, since these compounds are metabolized rapidly and measured levels reflect only recent exposure (33). However, these congeners have been associated with greater toxicological activity, including estrogenic activity, than some of the higher chlorinated congeners (32). The rationale for examining these lower chlorinated PCBs was based on this biological significance, as well as on the assumption that exposure to these compounds was likely to be chronic in nature, not changing significantly over time. Therefore, serum levels at the time of the interview were assumed to be representative of lifetime exposure including the time critical for tumorigenesis. In these data, a statistically significant increase in risk (OR=1.66) was observed for women with detectable levels of these lower chlorinated compounds in comparison to women with no detectable levels. There was, however, no evidence of a dose-response relationship. These results need to be interpreted with great caution, because of the underlying assumption that current serum levels of these compounds actually reflect levels at the biologically relevant time period. Furthermore, measurement of these rapidly metabolized congeners was more likely to be subject to laboratory error than measurement of the more stable and persistent congeners. On the other hand, there is no reason to believe that measurement error was different for cases and controls, in that the laboratory technicians were blinded to disease status. Nondifferential misclassification may have attenuated the true risk estimate. Clearly, the effect of these congeners on breast cancer risk needs to be explored in a prospective study

design, where it may be possible to obtain serum levels of these compounds from a time period that precedes disease onset by an appropriate induction period.

The observed effects of total PCBs, moderately chlorinated PCBs, and number of detected peaks warrants further comment. Number of detected peaks was examined in addition to the measure of total serum PCB levels to determine whether prevalence of a greater variety of congeners affects risk differently than total amount of these compounds. In our data, there was no evidence of such a difference. Furthermore, the greatest contributor to serum PCB levels were the moderately chlorinated PCBs; thus all three PCB exposure measures (total PCBs, moderately chlorinated PCBs, and number of PCB peaks) were highly correlated, and observed risk elevations among women who never lactated were likely to reflect the same effect. However, the observation that all three PCB exposures produced similar risk associations among parous women who never lactated strengthens the notion that environmental exposure to PCBs may be related to risk in this group.

As indicated above, the two previous epidemiologic studies did not report an adverse effect of serum PCB levels (26,27). However, the designs of these studies differed from that of this research in some important aspects. First, both studies combined pre-and postmenopausal breast cancer patients in their case group. There is some evidence that risk factors differ by menopausal status (34-36). Second, risk estimates in the earlier studies were not adjusted for serum lipids; such adjustment had some impact on the magnitude of the ORs in this study. Third, statistical adjustment for potential confounders such as parity (26,27) and duration of lactation (27) was lacking in these investigations.

In general, our results suggest that an adverse effect of organochlorine body burden on breast cancer risk, if present at all, is restricted to parous women who never lactated. The observed effect modification may be explained in three ways. First, organochlorine body burden may have been measured more accurately among women who had never breastfed an infant. Serum levels in this group may represent a more valid measure of chronic exposure, uninterrupted by elimination of these compounds through lactation. Second, women who had ever breastfed an infant may not be at increased risk of breast cancer, if they eliminated a substantial amount of organochlorine body burden at a biologically relevant period of time. Third, lactation in itself may contribute to the terminal differentiation of the mammary epithelium, resulting in larger compartments of nonproliferating cells (37,38). Women who lactated may be less susceptible to the potentially adverse effect of PCBs. In this study population, history of lactation was associated with a slight reduction of breast cancer risk (39).

There are several limitations associated with this research that need to be considered in interpreting these findings. The low participation rates in the case and control group may have introduced error due to selection bias. Among the breast cancer case group, nonparticipation may have resulted in a case sample that is not representative of all women with breast cancer. However, the majority of nonparticipation of cases was due to their physicians' refusal to allow the patients to contacted by the interviewers. Thus, nonparticipation of the cases may reflect physician characteristics, rather than patient characteristics, which may minimize the potential influence of bias on these results. Nonparticipation of controls was of equal concern, which may have resulted in a more motivated and possibly more health conscious control group. Results from a brief

telephone interview comparing a sample of controls who refused to participate with a sample of those who agreed to participate, indicated that these groups did not differ with regard to dietary intake of fruits, vegetables, and meats, nor did they differ with respect to cigarette use (28). It is unknown whether nonparticipation among cases and controls was related to exposure to PCBs. Selection bias may have been additionally introduced among the participants included in the toxicological analyses. Participants who are agreed to provide a blood sample were more likely to have breastfed an infant than those who refused, for both the case and control group. Among participants who were selected for toxicological analyses, however, this difference with respect to lactation history was more pronounced in the case group than in the control group. Thus, in the sample available for this study, breast cancer cases with a history of lactation were likely to be overrepresented.

Another concern in interpreting these findings relates to the small number of women in both lactation groups. When these groups of women were divided into tertiles, the numbers in the cells became small and the corresponding risk estimates became unstable. Despite these sample size restrictions, a statistically significant increase in risk was observed for several PCB exposures. Lack of statistical power may have prevented the detection of more subtle risk elevations in association with organochlorine exposure in the entire sample.

Two further limitations of this study relate to the use of blood serum levels of these compounds as a method of exposure assessment. With regard to studying breast cancer, the obvious tissue of choice would be mammary adipose tissue. Serum organochlorine levels can only function as a surrogate measure of body burden in the

target tissue. Variations in serum organochlorines have been observed with respect to serum lipids (40). There is, however, good evidence that lipid adjusted serum measures estimate the tissue levels (40-42). A more serious concern with respect to use of blood serum levels involves the fact that the blood sample in the case group was obtained after diagnosis of breast cancer. It could therefore not be determined whether PCB levels were associated with the disease process, or were the result of metabolic changes associated with disease progression. While cancer treatment may affect organochlorine levels (43), case selection for toxicological analysis excluded those, who underwent chemotherapy or radiation therapy before the blood sample was selected. However, there may still be an effect of the disease process on measured levels of these compounds.

In conclusion, results from this study do not indicate that environmental organochlorine exposure is a risk factor for breast cancer in postmenopausal women, although there may be an effect of PCBs for parous women who had never lactated. The observed effects in the latter group should be considered with caution, since they are based on a small number of participants. Clearly, the role of organochlorine exposure in breast cancer etiology needs to be explored further in future research efforts. Ideally, a long term prospective study design should be utilized to examine serum levels of these compounds that reflect body burden of a time period preceding the onset of this disease; thus, ruling out a potential effect of the disease process itself on measured levels of these compounds. A prospective study could also examine the effect of the lower chlorinated congeners with more precision, avoiding the assumption that present levels of these PCBs reflect past exposure. Future research, case-control studies as well as cohort studies, should also aim at examining this association among premenopausal women. Finally,

future studies should employ similar epidemiologic (e.g., determination of PCB congener group; treatment of samples with measures below the limit of detection) and laboratory (e.g., proficiency testing; sample acquisition and storage) methodologies, to ensure comparability of results across studies.

REFERENCES

1. American Cancer Society. Cancer Facts and Figures, 1995. Atlanta, GA: American Cancer Society, 1995.

2. Kelsey JL & Horn-Ross PL. Breast cancer: Magnitude of the problem and descriptive epidemiology. Epidemiol Rev 1993; 15:7-16.

3. Jensen AA. Levels and trends of environmental chemicals in human milk. In: Chemical Contaminants in Human Milk. Boca Raton, Fla: CRC Press; 1991:199-207.

4. International Agency for Research on Cancer. DDT and associated compounds. IARC Monogr 1992; 53:179-249.

5. Safe S, Bandiera S, Sawyer T, Robertson L, Safe L, Parkinson A, et al. PCBs: structure-function relationships and mechanism of action. Environ Health Perspect 1985; 60:47-56.

6. Safe S. Polychlorinated biphenyls (PCBs): mutagenicity and carcinogenicity. Mutat Res 1989; 224:79-88.

7. Morris CR & Cabral JRP. Hexachlorobenzene: Proceedings of an International Symposium. IARC Scientific Publications 1986; 77:13-18.

8. Waters EM, Huff JE, Gerstner HB. Mirex. An Overview. Environ Res 1977; 14:212-222.

9. Lewandowski M, Levi P, Hodgson E. Induction of Cytochrome p-450 isozymes by Mirex and Chordecone. J Biochem Toxicol 1989; 4:195-199.

10. Bulger WH & Kupfer D. Estrogenic action of DDT analogs. Am J Ind Med 1983; 4:163-173.

11. McKinney & Waller CL. Polychlorinated biphenyls as hormonally active structural analogues. Environ Health Perspect 1994; 102:290-4.

12. Jansen HT, Cooke PS, Poecelli J, Lui TC, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: in vitro and in vivo studies. Reprod Toxicol 1993; 7:237-41.

13. Foster WG, McMahon A, Villeneuve DC, Jarrell JF. Hexachlorobenzene (HCB) suppresses circulating progesterone concentrations during the luteal phase in the cynomolgus monkey. J Appl Toxicol 1992; 12:13-9.

14. Foster WG, Pentick JA, McMahon A, Lecavalier PR. Ovarian toxicity of hexachlorobenzene (HCB) in the superovulated female rat. J of Biochem Toxicol 1992; 7:1-11.

15. Banerjee BD. Effects of sub-chronic DDT exposure on humoral and cell-mediated immune responses in albino rats. Bull Environ Contam Toxicol 1987; 39:827-834.

16. Kimbrough RD. Laboratory and human studies on polychlorinated biphenyls (PCBs) and related compounds. Environ Health Perspect 1985; 59:99-106.

17. Kimbrough RD. Human heath effects of polychlorinated biphenyls (PCBs) and polybromated biphenyls (PBBs). Annu Rev in Pharmacol and Toxicol 1987; 27:87-111.

18. Norback DH, Weltman RH. Polychlorinated biphenyl induction of hepatocellular carcinoma in the Sprague-Dawley rat. Environ Health Perspect 1985; 60:97-105.

19. Moser GJ, Meyer SA, Smart RC. The chlorinated pesticide Mirex is a novel Nonphorbol ester-type tumor promoter in mice skin. Cancer Res 1992; 52:631-636.

20. Moser GJ, Robinette CL, Smart RC. Characterization of skin tumor promotion by mirex: structure-activity relationships, sexual dimorphism and presence of Ha-*ras* mutation. Carcinogenesis 1993; 14:1155-1160.

21. Wassermann M., Nogueira DP, Tomatis L, Mirra AP, Shibata H, Arie G, et al. Organochlorine compounds in neoplastic and adjacent apparently normal breast tissue. Bull Environ Contam Toxicol 1976;15:478-484.

22. Unger M, Kiaer H, Blichert-Toft M, Olsen J, Clausen J. 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 1984; 34:24-28.

23. Mussalo-Rauhamaa H, Haesaenen E, Pyysalo H, Antervo K, Kauppila R, Pantzar P. Occurrence of β-hexachlorocyclohexane in breast cancer patients. Cancer 1990; 66:2124-2128.

24. Falck F, Ricci A, Wolff MS, Godbold J, Deckers P. Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. Arch Environ Health 1992; 47:143-146.

25. Dewailly E, Dodin S, Verreault R, Ayotte P, Sauve L, Morin J, et al. High organochlorine body burden in women with estrogen receptor-positive breast cancer. J Natl Cancer Inst 1994; 86(3):232-4.

26. Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N. Blood levels of organochlorine residues and risk of breast cancer. J Natl Cancer Inst 1993;85:648-652.

27. Krieger N, Wolff MS, Hiatt RA, Rivera M, Vogelman J, Orentreich N. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. J Natl Cancer Inst 1994; 86:589-599.

28. Graham S, Hellmann R, Marshall JR, Freudenheim J, Vena J, Swanson M et al. Nutritional epidemiology of postmenopausal breast cancer in western New York. Am J Epidemiol 1991; 134:552-66.

29. Greizerstein H, Gigliotti P, Vena JE, Freudenheim J, Kostyniak PJ. Standardization of a method for routine analysis of polychlorinated biphenyl congeners and selected pesticides in human serum and milk. J Anal Toxicol (in press).

30. Phillips DL, Pirkle JL, Burse VW, Bernert JT, Henderson LO, Needham LL. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ Contam Toxicol 989; 18:495-500.

31. McFarland VA and Clarke JU. Environmental occurrence, abundance, and potential Toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. Environ Health Perspect 1989; 81:225-240.

32. Wolff MS & Toniolo PG. Environmental organochlorine exposure as a potential etiologic factor in breast cancer. Environ Health Perspect 1995; 103 (Suppl): 141-145.

33. Woodruff T, Wolff MS, Davis DL, Hayward D. Organochlorine estimation in the study of cancer etiology. Environ Res 1994; 65:132-144.

34. de Waard F. Premenopausal and postmenopausal breast cancer: one disease or two? J Natl Cancer Inst 1979; 63:549-52.

35. Janerich DT & Hoff MB. Evidence for a crossover in breast cancer risk factors. Am J Epidemiol 1982; 116:737-42.

36. Velentgas P & Daling JR. Risk factors for breast cancer in younger women. Monogr – Natl Cancer Inst 1994; 16:15-24.

37. Jahnke GD & Barrett JC. Environmental causes and molecular biology of breast cancer: Future research directions. In: Huff S, ed. Cellular and molecular mechanisms of hormonal carcinogenesis: Environmental influences. New York:Wiley-Liss, 1996:455-469.

38. Russo J, Russo IH. Biological and molecular bases of mammary carcinogenesis. Lab Invest 1987; 57:112-137.

39. Freudenheim JL, Marshall JR, Graham S, Vena JE, Moysich KB, Muti P, et al. Lactation history and breast cancer risk. Am J Epidemiol (in press).

40. Needham L, Burse V, Head S, Liddle JA, Bayse D, Boozer EL, et al. Adipose tissue/serum partitioning of chlorinated hydrocarbon pesticides in humans. Chemosphere 1990; 20:975-980.

41. Brown JF & Lawton RW. Polychlorinated biphenyl (PCB) partitioning between adipose tissue and serum. Bull Environ Contam Toxicol 1984; 33:277-280.

42. Patterson DG, Needham LL, Pirkle JL, Roberts DW, Bagby J, Garret WA, et al. Correlation between serum and adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in 50 persons from Missouri. Arch Environ Contam Toxicol 1988; 17:139-143.

43. Gammon MD, Wolff MS, Neugut AL, Terry MB, Britton JA, Greenbaum E, et al. Treatment for breast cancer and blood levels of chlorinated hydrocarbons. Cancer Epidemiol Biomarkers Prev 1996; 5:467-471.

Table 1.

Characteristics of postmenopausal breast cancer cases and community controls - Western New York 1986-1991.

Characteristic	Total			Never Lactated[1]			Ever Lactated		
	Cases (n=154)	Controls (n=192)	p value	Cases (n=46)	Controls (n=61)	p value	Cases (n=85)	Controls (n=106)	p value
Age[2]	64.07 (7.69)	63.16 (7.61)	ns	63.93 (5.85)	60.75 (7.61)	ns	64.47 (8.06)	64.79 (7.18)	ns
Education (years)[2]	12.47 (2.84)	12.18 (2.58)	ns	12.52 (1.82)	12.41 (2.58)	ns	12.48 (3.01)	11.95 (2.54)	ns
Quetelet Index[2,4]	25.68 (4.94)	25.90 (5.25)	ns	26.07 (6.31)	26.13 (6.00)	ns	25.56 (4.21)	26.08 (4.74)	ns
Age at Menarche[2]	12.85 (1.56)	12.90 (1.60)	ns	12.83 (1.54)	12.89 (1.72)	ns	12.98 (1.63)	12.84 (1.51)	ns
Age at Menopause[2]	47.52 (5.66)	46.95 (5.85)	ns	47.76 (5.91)	45.46 (5.38)	0.04	47.93 (5.61)	47.48 (5.66)	ns
Age at First Pregnancy[2,5]	24.06 (4.77)	23.36 (4.34)	ns	24.41 (4.55)	24.25 (4.50)	ns	23.71 (4.72)	22.76 (4.15)	ns
Number of Livebirths[1,2]	3.23 (1.90)	3.39 (1.93)	ns	3.41 (1.65)	3.00 (1.47)	ns	3.25 (1.96)	3.72 (2.01)	ns
Years since Last Lactation[2,6]	33.75 (9.85)	33.93 (8.63)	ns	na	na	na	33.75 (9.85)	33.93 (8.63)	ns
Months of Lactation[2,6]	8.60 (13.10)	10.19 (12.4)	ns	na	na	na	8.60 (13.10)	10.19 (12.4)	ns
Benign Breast Disease[3]	23%	20%	ns	26%	15%	ns	21%	20%	ns
Family History of Breast Cancer[3]	18%	9%	0.02	13%	10%	ns	20%	9%	0.04
Fruit (g/months)[2,7]	8423 (5249)	8715 (5000)	ns	7013 (4460)	7293 (4131)	ns	8834 (5214)	9626 (5395)	ns
Vegetables (g/months)[2,7]	12680 (5226)	13983 (7341)	ns	12160 (5302)	12201 (4958)	ns	12789 (5231)	14894 (8403)	0.05
Dairy (g/months)[2,7]	8484 (6319)	7840 (6184)	ns	6967 (5138)	7716 (7437)	ns	9362 (6584)	7885 (5747)	ns

Fish (g/months)[2,7]	787 (586)	833 (621)	ns	766 (597)	877 (529)	ns	773 (582)	791 (666)	ns
Meats (g/months)[2,7]	1642 (940)	1724 (1002)	ns	1543 (843)	1800 (932)	ns	1708 (1035)	1676 (1042)	ns
Residence[3,8]									
Urban	36%	31%		33%	39%		38%	26%	
Suburban	60%	53%		56%	44%		61%	57%	
Rural	4%	16%	0.01	11%	17%	ns	1%	17%	0.001
Occupation[3,9]									
Professional	18%	17%		11%	20%		18%	11%	
Sales/Administration	44%	43%		41%	44%		44%	43%	
Service	17%	15%		22%	7%		19%	21%	
Labor	21%	25%	ns	26%	29%	ns	20%	25%	ns

[1] women with at least one livebirth, excluding 48 nulliparous women; [2] mean (SD), differences in means examined with student t-tests; [3] differences between groups examined with chi-square test; [4] weight/height^{2},[5]; [5] among women with at least one pregnancy; [6] among women who had ever breastfed an infant; [7] reflects intake two years before the interview; [8] place of residence at time of interview; [9] occupation two years before the interview; na=not applicable, ns=p>.05

Table 2

Serum concentrations of organochlorines in postmenopausal breast cancer cases and community controls - Western New York 1986-1991.

Measure in ng/g of serum	Total		Never Lactated[1]		Ever Lactated	
	Cases (n=154)	Controls (n=192)	Cases (n=46)	Controls (n=61)	Cases (n=85)	Controls (n=106)
DDE[2] Mean (SD)	11.47 (10.49)	10.77 (10.64)	13.16 (11.65)	10.82 (10.91)	10.36 (8.97)	10.44 (10.43)
HCB[2] Mean (SD)	0.41 (0.19)	0.42 (0.19)	0.45 (0.24)	0.39 (0.18)	0.39 (0.16)	0.44 (0.19)
Mirex[2] Mean (SD)	0.043 (0.09)	0.037 (0.09)	0.083 (0.12)	0.046 (0.14)	0.029 (0.06)	0.036 (0.08)
Total PCBs[2] Mean (SD)	4.29 (2.40)	4.12 (2.24)	4.63 (2.88)	4.00 (1.96)	4.27 (2.50)	4.30 (2.42)
Number of peaks detected[2] Mean (SD)	18.38 (5.40)	17.93 (4.99)	18.68 (4.63)	17.93 (5.34)	18.49 (5.98)	18.35 (4.66)
Lower chlorinated PCBs[2] Mean (SD)	0.31 (0.34)	0.29 (0.35)	na		na	
Moderately chlorinated PCBs[2] Mean (SD)	3.11 (1.71)	3.06 (1.73)	3.43 (1.77)	2.90 (1.48)	3.10 (1.67)	3.20 (1.91)
Higher chlorinated PCBs[2] Mean (SD)	0.43 (0.29)	0.40 (0.25)	0.50 (0.31)	0.40 (0.24)	0.41 (0.27)	0.40 (0.26)

[1] women with at least one livebirth, excluding 48 nulliparous women; [2] adjusted for age and serum lipids; na=not applicable

Table 3

Risk of postmenopausal breast cancer associated with environmental exposure to DDE, HCB, and mirex - Western New York 1986-1991.

Measures in ng/g of serum	Total (n=346)			Never Lactated (n=107)[1]			Ever Lactated (n=191)		
	Cases	Controls	OR[3] (95% CI)[5]	Cases	Controls	OR[4] (95% CI)[5]	Cases	Controls	OR[3] (95% CI)[5]
DDE[2,6]									
1 (low)	54	60	1.0	15	23	1.0	29	29	1.0
2	46	69	1.01 (0.56-1.86)	13	17	1.95 (0.58-6.67)	30	44	0.76 (0.35-1.63)
3 (high)	54	63	1.34 (0.71-2.55)	18	21	1.83 (0.63-5.33)	26	33	1.28 (0.54-3.05)
			p=0.25			p=0.24			p=0.44
HCB[2,7]									
1 (low)	62	61	1.0	16	27	1.0	37	26	1.0
2	40	66	0.56 (0.30-1.04)	12	14	1.26 (0.40-3.97)	23	44	0.32 (0.14-0.71)
3 (high)	52	65	0.81 (0.43-1.53)	18	20	1.79 (0.59-5.40)	25	36	0.46 (0.20-1.08)
			p=0.80			p=0.22			p=0.11
Mirex[8]									
<LOD	112	148	1.0	28	44	1.0	65	83	1.0
>LOD	42	44	1.37 (0.78-2.39)	18	17	2.42 (0.98-4.32)	20	23	1.08 (0.52-2.25)

[1] women with at least one livebirth, excluding 48 nulliparous women; [2] tertiles based on the distribution in all controls; [3] odds ratios adjusted for age, education, family history of breast cancer, parity, quetelet index, duration of lactation, age at first birth, years since last pregnancy, fruit and vegetable intake, and serum lipids; [4] odds ratios adjusted for age, education, family history of breast cancer, parity, quetelet index, age at first birth, years since last pregnancy, fruit and vegetable intake, and serum lipids; [5] 95% confidence interval; [6] DDE tertile cutpoints (ng/g of serum):first tertile=0.02-5.07, second tertile=5.10-10.98, third tertile=11.0-76.2; [7] HCB tertile cutpoints (ng/g of serum):first tertile=0-0.34, second tertile=0.35-0.44, third tertile=0.45-1.35; [8] mirex levels > LOD ranged from 0.06 to 0.99 ng/g of serum; LOD=limit of detection.

36

Table 4

Risk of postmenopausal breast cancer associated with environmental exposure to polychlorinated biphenyls (PCBs) - Western New York 1986-1991.

Measures in ng/g of serum	Total (n=346)			Never Lactated (n=107)[1]			Ever Lactated (n=191)		
	Cases	Controls	OR[4] (95% CI)[6]	Cases	Controls	OR[5] (95% CI)[6]	Cases	Controls	OR[4] (95% CI)[6]
Total PCBs [2,7]									
1(low)	53	2	1.0	11	22	1.0	32	29	1.0
2	45	68	0.70 (0.37-1.29)	15	21	1.71 (0.55-5.35)	22	41	0.38 (0.17-1.03)
3 (high)	56	61	1.14 (0.61-2.15)	20	18	2.87 (1.01-7.29)	31	36	0.71 (0.31-1.61)
			p=0.51			p=0.07			p=0.72
Number of peaks detected [2,8]									
1 (low)	48	64	1.0	10	21	1.0	30	21	1.0
2	43	64	0.88 (0.45-1.59)	14	20	1.61 (0.41-3.56)	23	37	0.63 (0.29-1.40)
3 (high)	63	64	1.34 (0.72-2.47)	22	20	3.31 (1.04-11.3)	32	38	0.82 (0.37-1.83)
			p=0.52			p=0.10			p=0.85
Lower chlorinated PCBs [3,9]									
<LOD	45	69	1.0			na			na
1 (low)	56	59	2.04 (1.09-3.83)						
2 (high)	53	63	1.40 (0.76-2.59)						
			p=0.78						

37

Moderately
chlorinated PCBs [2,10]

	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
1(low)	53	61	1.0	11	21	1.0	32	31	1.0
2	41	73	0.57 (0.03-1.07)	12	23	0.73 (0.22-2.63)	22	41	0.48 (0.23-1.07)
3 (high)	60	57	1.37 (0.73-2.59)	23	17	3.57 (1.10-8.60)	31	34	0.85 (0.37-1.95)
			p=0.69			p=0.08			p=0.44

Higher chlorinated
PCBs [2,11]

	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
1(low)	54	63	1.0	13	20	1.0	32	34	1.0
2	43	66	0.79 (0.42-1.52)	11	24	0.51 (0.15-1.69)	24	34	0.96 (0.41-2.23)
3 (high)	54	62	1.19 (0.60-2.36)	21	17	1.53 (0.47-4.95)	27	38	1.00 (0.40-2.49)
			p=0.35			p=0.12			p=0.94

[1] women with at least one livebirth, excluding 48 nulliparous women; [2] tertiles based on the distribution in all controls; [3] women with levels below the LOD were compared to those in the lower and upper half of the distribution in the controls; [4] odds ratios adjusted for age, education, family history of breast cancer, parity, quetelet index, duration of lactation, age at first birth, years since last pregnancy, fruit and vegetable intake, and serum lipids; [5] odds ratios adjusted for age, education, family history of breast cancer, parity, quetelet index, age at first birth, years since last pregnancy, fruit and vegetable intake, and serum lipids; [6] 95% confidence interval; [7] total PCB tertile cutpoints (ng/g of serum):first tertile=0.94-2.92, second tertile=2.93-4.43, third tertile=4.44-19.04; [8] number of peaks tertile cutpoints:first tertile=9-14, second tertile=15-20, third tertile=21-35; [9] lower chlorinated PCBs cutpoints (ng/g of serum):< median of detectable levels=0.01-0.31, > median=0.32-1.65; [10] moderately chlorinated PCBs tertile cutpoints (ng/g of serum):first tertile=0.47-2.19, second tertile=2.20-3.12, third tertile=3.13-15.07; [11] higher chlorinated PCBs tertile cutpoints (ng/g of serum):first tertile=0.01-0.25, second tertile=0.26-0.44; third tertile=0.45-1.30; LOD = limit of detection; na=not applicable

PART THREE – PCB CONGENER GROUPING

Introduction

Polychlorinated biphenyls (PCBs) are persistent, lipophilic compounds that are

ubiquitous in the environment. The number of chlorine atoms attached to the biphenyl molecule

determines the structure of each PCB, with 209 possible PCB congeners or homologues. PCBs

with five to seven chlorine substitutions were synthesized in high proportions in many

commercial preparations and are most prevalent in the environment. More highly chlorinated

congeners bind more tightly with soils and sediments, thus are less available to organisms.

Congeners with fewer chlorines are more readily metabolized and eliminated (McFarland &

Clarke, 1989). In addition to differences in bioaccumulation, certain PCB congeners also differ

with respect to effects on the endocrine system (Korach et al., 1988; McKinney & Waller, 1994),

neurotoxicity (Safe, 1990; Seegel & Schantz, 1994), cytochrome P450 enzyme induction

(McFarland & Clarke, 1989) and dioxin-like activity (Safe, 1993; Kutz et al., 1990).

PCBs have been associated with a variety of health effects, including reproductive,

(Battershill, 1994; Kimbrough 1995), developmental (Sauer et al, 1994), and neurological

outcomes (Huisman et al., 1995; Steenland et al., 1994), as well as effects on cognition (Chen et

al., 1992; Jocobson & Jacobson, 1996) and behavior (Chen et al., 1994; Fein et al., 1983). PCB

exposure has also been linked to some forms of human cancer (Silberhorn et al. 1990; Safe,

1994, Laden & Hunter, 1998).

Improvements in analytic methods have led to quantification of a large number of

specific PCB congeners for epidemiologic studies. Total PCB level, as one measurement or the

sum of a few prevalent congeners, has been complimented by measurements of a variety of

specific PCB congeners. The availability of congener-specific data may provide greater insight in the role of PCBs in the etiology of various health outcomes, in that congeners with specific biological activities can be examined in relation to health effects. However, examination of individual congeners may be less meaningful than groups with functional significance, leading to strengthening of toxicological associations and decreased potential for exposure misclassification (Wolff et al., 1997).

In this exploratory research we evaluated the utility of five potential frameworks for grouping PCB congener data into meaningful analytic units. These frameworks differ with respect to complexity and biological significance. Based on a sample of healthy postmenopausal women, we were interested in determining whether participants had detectable levels for the PCBs in the proposed congener groups. We explored grouping frameworks based on 1) degree of chlorination, 2) factor analysis, 3) cytochrome P450 enzyme induction activity, 4) enzyme induction and occurrence (McFarland & Clarke,1989), and 5) enzyme induction, occurrence, and other toxicological aspects (Wolff et al., 1997).

Methods

The study sample was a subset of the control group for the Western New York breast cancer study. A more detailed description of the study population has been published elsewhere (Moysich et al. 1998; Graham et al. 1991). Briefly, the sample included 192 healthy postmenopausal female residents of Erie and Niagara counties in western New York, who agreed to provide a fasting blood sample and were subsequently selected for chemical analyses. All participants were Caucasian and ranged in age between 45 and 81 years. Descriptive characteristics of the study population are shown in Table I.

Determination of serum PCB levels (56 PCB peaks, representing 71 congeners) was performed by the Toxicology Research Center using the method of Greizerstein et al. (1997). The procedures included standardized extraction, clean up and quantification by high resolution gas chromatography (GC) and a comprehensive quality assurance program to minimize systematic and erratic errors. Quantification was based on calibration standards and response factors calculated using purchased reference materials. The limit of detection (LOD) for each analyte was determined as the mean of background noise plus three standard deviations in five reagent blank samples. Partial correlation coefficients, adjusted for age, serum lipids, and duration of lactation, were computed to determine the correlations among the evaluated congener groups.

Our evaluation of the utility of these grouping frameworks was based on three factors: high proportion of participants with detectable levels for the proposed groups (data availability), low correlations among the congener groups within a framework, and the biological significance of the framework. For the latter, we considered grouping approaches based on degree of chlorination and factor analysis to be less biologically significant than the remaining three grouping approaches.

Results

Mean values, standard deviations, and limits of detection for all PCB congeners detected in this study population are shown in Table II. No mono- or deca-biphenyls were detected in the sera of our participants, likely because of their limited bioaccumulation. The majority of the detected PCBs were in the tetra-, penta, hexa, and hepta biphenyl isomer groups, as expected, consistent with their higher likelihood of bioaccumulation (Table III).

Using degree of chlorination of the individual PCBs to group the congeners, resulted in three groupings. The congener group of lower chlorinated PCBs contained all detected di-, tri, and tetra-biphenyls; the moderately chlorinated PCB group included all penta-, hexa, and hepta-biphenyls; and the higher chlorinated PCB group contained all octa- and nona-biphenyls. As is shown in Table IV, all participants had detectable levels for the moderately, and higher chlorinated PCBs, and a high proportion (68 percent) had detectable levels for the lower chlorinated PCBs. Lower chlorinated PCBs were weakly correlated with moderately (r=0.20) and higher chlorinated PCBs (r=0.18), but somewhat stronger correlations were observed between moderately and higher clorinated PCBs (r=0.51).

The second approach involved the use of factor analysis to generate uncorrelated groups of PCB congeners (Table V.). We eliminated those congeners for which less than 20 percent of the participants had detectable levels (IUPAC numbers 185,151+82, 99,135, 60, 18, 25+50, 149, 55, 45, 44, 15+17, 97, 70, 40, 59+42, 16+32, 136, 49, 52, 33, 19). Principal component analysis, followed by a varimax rotation, was employed to account for the maximum amount of the variance. Eigenvalues, scree plots, the variance explained, and interpretability of the factors were examined to determine the number of factors. Factor structures were determined by selecting items with loadings ≥0.35 (absolute value). Items loading ≥0.35 on two or more factors were

considered only in the factor with their highest score. Although these factors are relatively uncorrelated (r=-0.03 (factor 4 and factor 5) to r=0.39 (factor 1 and factor 2)) and all the underlying assumptions of this statistical technique were met, these five groups of congeners do not appear to follow any pattern with obvious biologic meaning, such as Arochlor exposure profile, degree of chlorination, enzyme induction, carcinogenic or estrogenic activity.

The third approach involved grouping PCB congeners with respect to their cytochrome P-450 enzyme induction properties, as defined by phenobarbital-type (PB-type), 3-methylchlolanthrene-type (3-MC-type), mixed-type, or unknown induction activity (Table VI.). PCBs with 3-MC-type induction activity were of particular interest because they have been associated with the highest toxicological potential (McFarland & Clarke, 1989). All women had detectable levels for the PB-type, mixed-type, and unknown induction activity groups, but only 12 percent of the sample had detectable levels for the 3-MC-type group. Furthermore, only one congener peak in the 3-MC-type group was detected, making the value dependent on a single measurement. These groups were generally weakly correlated (r<0.20), with the exception of PB-type PCBs, which were moderately correlated with PCBs of unknown activity (r=0.48) and highly correlated with mixed-type PCBs (r=0.84).

The fourth approach was based on the environmental significance of the PCBs considering potential toxicity, frequency of occurrence, and abundance in animal tissue (McFarland & Clarke, 1989). Group 1a contains the coplanar PCBs with pure 3-MC-type induction activity and group 1b contains the mixed type inducers that are abundant in environment and animal tissues. Group 2 is made up of known and predicted phenobarbital - type inducers, which are abundant in environment and animal tissues. Group 3 includes weak or non-inducing PCBs that are common in the environment, and group 4 consists of mixed-type

inducers with few reported environmental occurrences. The congeners in groups 1a and b are of the highest priority because of their greatest potential to produce biological effects. As presented in Table VII, all participants had detectable levels for congeners in groups 1b, 2, and 3, but only a small proportion had detectable levels for group 1a, which is identical to the 3-MC congener group, discussed above. None of the PCBs in group 4 was detected in our participants, which was not surprising, since this group was characterized by congeners with few environmental occurrences. These groups were generally weakly correlated (r<0.30), although group 2 was strongly correlated with group 1b (r=0.84) and moderately correlated with group 3 (r=0.50).

The final framework in this evaluation was recently proposed by Wolff and her colleagues (1997) and is based on major PCB peaks occurring in house dust or human samples. In this framework, group 1 consists of potentially estrogenic PCBs, with group 1a comprised of non-persistent, estrogenic PCBs with weak PB-type induction activity, and with group 1b comprised of persistent PCBs with weak PB-type induction activity. Group 2 includes potentially antiestrogenic, immunotoxic and dioxin-like PCBs. Specifically, group 2a consists of moderately persistent PCBs with dioxin activity and group 2b includes persistent PCBs with limited dioxin-like activity. Group 3 includes biologically persistent PB-type inducers. When this framework was applied to our data, we observed that nearly all participants had detectable levels for groups 1b, 2a+b, and group 3 (Table VII.). Even though only 40 percent of the participants had detectable levels for group 1a, the effect of these congeners could still be crudely assessed in epidemiologic studies. For the final framework, we observed moderate correlations for group 1b and group 2a (r=0.38), group 2b (r=0.52), and group 3 (r=0.56), but found that group 3 and group 2b were highly correlated (r=0.90).

Discussion

In summary, grouping a large number of PCB congeners by degree of chlorination may be the most practical approach with respect to data availability (i.e., a large proportion of participants had detectable levels for all proposed groups), but this practical characteristic is counteracted by the crudeness of this approach, in that some of the groups contain congeners with counteracting biological effects.

Grouping with respect to P450 enzyme induction activity was of less utility, due to the few individuals with measurable levels in the group with the greatest toxicological potential (3-MC-type). In addition, the two remaining biologically relevant groups (PCBs with PB-type and mixed-type induction activity) were highly correlated; thus the independent effect of these compounds could not be evaluated. Factor analysis was difficult to interpret in these data, resulting in relatively uncorrelated factors that did not seem to follow any meaningful pattern. However, the observed factors could provide insight about the patterns of exposure and retention of PCBs in the study population, due to the fact that the groups of PCBs within the observed factors were weakly correlated and may be associated with distinct routes of exposures. Environmental significance groupings were of limited utility, again due to the few individuals with measurable levels of 3-MC-type PCBs. Utility may be improved by combining groups 1a and b, although this would be accompanied by some loss of precision in measurement. In addition, the biological properties of group 1 could not be eveluated independently, due to the strong correlation between group 1b and group 2.

The grouping approach formulated by Wolff et al. (1997) was a refined and applicable alternative to the crude approach of grouping by degree of chlorination. This framework takes into account the biological properties of PCBs as well as their prevalence in the environment because it is based on major PCB peaks detected in human samples and in house dust. This last

property may suggest that PCB measures from other studies could result in reasonably high proportions of participants with detectable levels for the PCBs in the proposed groups. Again, group 2b and group 3 were highly correlated and the independent effect of these groupings cannot be established.

In our exploratory evaluation of the utility of frameworks for grouping PCB congeners, we saw that that the utility of a framework is not purely based on biological relevance, but also on data availability (e.g., sufficiently high proportions of participants with detectable levels). In other words, a framework with high biological relevance that proposes groups of PCB congeners that are rarely detected in humans may not be as robust as than one with less biologic justification which proposes groupings of individual PCBs that are commonly detected. Furthermore, collinearity among congener groups needs to be considered, in that a proposed framework with both, high biological significance and data availability is of limited value if all congener groups are highly correlated.

In addition to data availability and collinearity, there are several factors that affect the utility of grouping frameworks for PCB congeners (Figure I.), including factors related to analytical chemistry, epidemiologic factors, biological significance, and disease outcome. First, we need to point out that our evaluation was based on measured PCB levels obtained from a particular study population, specifically Caucasian, postmenopausal females, with no known special exposure (e.g., contaminated fish consumption, occupational exposure). Advanced age (the mean age in this study population was 61 years) has been associated with greater PCB body burden and it is likely that more PCB peaks were detected in our sample than in a younger study population with comparable characteristics. On the other hand, it is possible that fewer peaks were detected in this population than among a group of younger individuals with high

environmental or occupational exposures. Furthermore, the accumulation and kinetics of PCBs in adult women is likely to be very different from men. There is good evidence that lactation affects PCB body burden (Jensen, 1991), as well as some consideration that pregnancy itself may affect accumulation of these compounds (Buck et al., 1997). All of these characteristics of this study population were likely to have had an impact on the observed utility of the grouping frameworks. In other words, the frameworks that were found to be useful in our population may differ in terms of utility when applied to study populations with different characteristics.

A second major factor that may have affected our evaluation of the utility of grouping frameworks involves the fact that we used serum PCB measures from a single laboratory employing particular chemical techniques. Analytical chemistry laboratories that provide PCB congener data for epidemiologic studies differ with respect to technology and funds that are available for PCB analyses. In general, laboratories with updated technology and generous funding are able to provide a greater number of PCB congeners, than those with less sophisticated technology and limited funding. Independent from technology and funding, the type of specimen (e.g., fat, milk, serum) and the quantity or size of the specimen affects the number of PCB congeners available for epidemiologic analyses, in that more congeners are likely to be detected in fat than in milk, and more in milk than in serum. Similarly, the greater the amount of the specimen available for chemical analyses, the lower the limit of detection for specific congeners and therefore the greater the likelihood of detecting a large number of congeners. The effect of these differences in laboratory techniques is clearly illustrated by the vast differences in the number of PCB congeners available for analysis among the studies currently underway to examine the association between organochlorine exposure and breast

cancer risk. As the chemical techniques differed across studies, the number of PCB peaks available for analysis ranged from 9 to 56 (unpublished data).

Another issue involves the notion that grouping of PCB congeners should be based on biologic significance. However, as shown above, theoretical approaches, based purely on biologic significance, may lose utility when applied to actual data, due to the fact that some of the most biologically active PCBs may rarely be detected in humans. Thus, epidemiologic studies that are intended to investigate the health effects of these rarely detected compounds will be hampered by limited statistical power.

Lack of generalizibility of a framework to a variety of health outcomes also affects the utility of grouping frameworks. Endocrine activity as a determinant for grouping may be useful only for health outcomes that are hormone dependent. It is therefore unlikely that one uniform framework will be applicable for all epidemiologic studies, suggesting that several frameworks may be needed, defined by the biologic mechanism of various diseases or disorders under investigation.

Finally, it is important to point out that this research was intended to explore various approaches in grouping PCB congeners, and continue the discussion on this essential issue. One important goal of epidemiologic studies with exposure biomarkers should be the comparability of chemical determinants across studies. There is some effort among toxicologists who provide PCB measurements for epidemiologic studies, to engage in proficiency programs, to ensure comparability of the biological measurements across studies. These efforts should be extended to the epidemiological side of these research projects, by determining comparable approaches in data analysis. However, as outlined above, there are many problems associated with the

development of meaningful frameworks for PCB congener data, underlining the importance of

continuing the discussion among researchers from all disciplines.

References

Battershill JM. 1994. Review of the safety assessment of polychlorinated biphenyls (PCBs) with particular reference to reproductive toxicity. Hum Exp Toxicol 13:581-597.

Buck GM, Mendola P, Vena JE, Sever LE, Sweeney AM, Kostyniak P, Greizerstein H, Olson J. Prospective pregnancy study, New York State Angler Cohort. Paper presented at the International Scientific Conference on the Effects of the Environment on Human Health in the Great Lakes and St. Lawrence River Basins. Montreal, Canada; May 1997.

Chen YCJ, Guo YL, Hsu CC, Rogan WJ. 1992. Cognitive development of Yu-Cheng ('oil disease') children prenatally exposed to heat-degraded PCBs. JAMA 268:3213-3218).

Chen YCJ, Yu MLM, Rogan WJ, Gladen BC, Hsu CC. 1994.: A 6-year follow-up of behavior and activity disorders in the Taiwan Yu-Cheng children. Am J Public Health 84:415-421.

Fein GG, Schwartz PM, Jacobson SW, Jacobson JL. 1983. Environmental toxins and behavioral development. Am Psychol 1188-1197.

Graham S, Hellmann R, Marshall JR, Freudenheim JL, Vena J, Swanson M, Zielezny M, Nemoto T, Stubbe N, Raimondo T. 1991. Nutritional epidemiology of postmenopausal breast cancer in western New York. Am J Epidemiol 134:552-66.

Greizerstein H, Gigliotti P, Vena JE, Freudenheim J, Kostyniak PJ. 1997. Standardization of a method for routine analysis of polychlorinated biphenyl congeners and selected pesticides in human serum and milk. J Anal Toxicol 21:558-566.

Huisman M, Koopman-Esseboom C, Flieder V, Hadder-Algra M, van der Paauw CG, Tuinstra LGMTh, Weisglas-Kuperus N, Sauer PJJ, Touwen BCL, Boersma ER. 1995. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neurological development. Early Hum Dev 41:111-127.

Jacobson JL & Jacobson SW. 1996. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. New Engl J Med 335:783-789.

Jensen AA. 1991.Levels and trends of environmental chemicals in human milk. In: Chemical Contaminants in Human Milk. (Jensen AA, ed) Boca Raton, Fla:CRC Press.p 199-207.

Kimbrough RD. 1995. Polychlorinated biphenyls (PCBs) and human health: an update. Crit Rev Toxicol 25:133-163.

Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD. 1988. Estrogen receptor-binding activity of polychlorinated biphenyls:conformationally restricted probes. Mol Pharmacol 33:120-126.

Kutz FW, Barnes DG, Bottimore DP, Greim H, Bretthauer EW. 1990. The international toxicity equivalent factor (I-TEF) methods of risk assessment for complex mixtures of dioxins and related compounds. Chemosphere 20:751-757.

Laden F & Hunter DJ. 1998. Environmental risk factors and female breast cancer. Annu Rev Public Health 19:101-23.

McFarland VA & Clarke JU. 1989. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. Environ Health Perspect 81:225-239.

McKinney JD & Waller CL. 1994. Polychlorinated biphenyls as hormonally active structural analogues. Environ Health Perspect 102:290-297.

Moysich KB, Ambrosone CB, Vena JE, Shields PG, Mendola P, Kostyniak P, Greizerstein H, Graham S, Marshall JR, Schisterman EF, Freudenheim JL. 1998. Environmental organochlorine exposure and postmenopausal breast cancer risk. Cancer Epidemiology Biomarkers and Prevention 7:181-188.

Safe SH. 1990. Polychlorinated biphenyls, dibenzo-p-dioxins, dibenzufurans, and related compounds: environmental mechanistic considerations which support the development of toxicology equivalent factors. Crit Rev Toxicol 21:51-88.

Safe SH. 1993. Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. Environ Health Perspect100:259-268.

Safe SH. 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24:87-149.

Sauer PJJ, Huisman M, Koopman-Esseboom C, Morse DC, Smits-van Prooije AE, van de Berg KJ, Tuinstra LGMTh, van der Paauw GG, Boersma ER, Weisglas-Kuperus N, Lammers JHCM, Kulig BM, & Brouwer A. 1994. Effects of polychlorinated biphenyls (PCBs) and dioxins on growth and development. Hum Exp Toxicol 13:900-6.

Seegel RF, Schantz SL. 1994. Neurochemical and behavioral sequelae of exposure to dioxins and PCBs. In:Dioxins and health (Schecter A, ed). New York:Plenum Press. P 409-434.

Silberhorn EM, Glauert HP, Robertson LW. 1990. Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. Crit Rev Toxicol 20:440-496.

Steenland K, Jenkins B, Ames GR, O'Malley M, Chrislip D, Russo J. 1994. Chronic Neurological Sequelae to organophosphate pesticide poisoning. Am J Public Health 84:731-736.

Wolff MS, Camann D, Gammon M, Stellman SD. 1997. Proposed PCB congener groupings for epidemiological studies. Environ Health Perspect 105:13-14.

Table I.

Characteristics of 192 postmenopausal residents - Western New York 1986-1991.

Characteristic	n=192
Age (years)[1]	63.16 (7.61)
Education (years) [1]	12.18 (2.58)
Quetelet Index [1,2]	25.90 (5.25)
Number of Livebirths[1,3]	3.39 (1.93)
Years since Last Lactation[2,4]	33.93 (8.63)
Months of Lactation[1,4]	10.19 (12.4)

Residence [5]
   Urban
   Suburban
   Rural
	
	 31%
	 53%
	 16%

Occupation [6]
   Professional
   Sales/Administration
   Service
   Labor
	
	 17%
	 43%
	 15%
	 25%

[1] mean (SD); [2] weight/height^2; [3] women with at least one livebirth; [4] among women who had ever breastfed an infant; [5] place of residence at time of interview; [6] occupation two years before the interview

Table II.

Polychlorinated biphenyl (PCB) congeners detected in a sample of 192 postmenopausal women – Western New York 1986-1991.

PCB congener IUPAC No. [1]	LOD[2]	%>LOD[3] (n)	mean[4] (sd)	minimum	maximum
#6	0.11	11% (21)	0.18 (0.04)	0.12	0.26
#7+9	0.02	38% (74)	0.14 (0.10)	0.02	0.66
#15+17	0.22	5% (10)	0.35 (0.26)	0.23	1.06
#16+32	0.28	4% (8)	0.31 (0.02)	0.28	0.35
#18	0.48	8% (15)	0.52 (0.03)	0.48	0.57
#19	0.23	1% (2)	0.38 (0.05)	0.34	0.41
#22	0.11	16% (32)	0.16 (0.05)	0.11	0.29
#25+50	0.32	7% (13)	0.45 (0.26)	0.11	1.14
#31+28	0.21	38% (73)	0.34 (0.13)	0.21	0.80
#33	0.45	1% (2)	0.36 (0.23)	0.20	0.52
#40	0.09	4% (7)	0.21 (0.14)	0.09	0.45
#44	0.16	5% (10)	0.21 (0.05)	0.17	0.30
#45	0.08	7% (13)	0.10 (0.02)	0.08	0.14
#47+48	0.03	37% (72)	0.65 (0.30)	0.03	0.14
#49	0.70	2% (4)	0.89 (0.14)	0.73	1.03
#52	0.24	2% (3)	0.29 (0.04)	0.25	0.32
#55	0.03	7% (14)	0.04 (0.01)	0.03	0.06
#59+42	0.14	4% (7)	0.17 (0.02)	0.14	0.21
#60	0.07	8% (15)	0.11 (0.05)	0.07	0.21
#66+95	0.19	20% (38)	0.25 (0.06)	0.19	0.49
#70	0.11	4% (7)	0.14 (0.05)	0.10	0.23
#77+110	0.01	12% (23)	0.02 (0.01)	0.01	0.04
#87	0.03	15% (28)	0.06 (0.02)	0.04	0.12

#97	0.05	4% (7)	0.07 (0.01)	0.06	0.09
#99	0.63	9% (17)	0.90 (0.14)	0.64	1.10
#101	0.15	16% (30)	0.20 (0.06)	0.15	0.39
#105+132	0.04	77% (148)	0.11 (0.07)	0.05	0.50
#118	0.09	97% (187)	0.44 (0.31)	0.09	2.48
#128	0.01	31% (60)	0.04 (0.03)	0.01	0.18
#129	0.09	14% (27)	0.16 (0.09)	0.09	0.47
#134	0.02	15% (29)	0.04 (0.03)	0.02	0.15
#135	0.03	8% (16)	0.05 (0.02)	0.03	0.09
#136	0.08	2% (4)	0.11 (0.01)	0.09	0.12
#138	0.05	98% (189)	0.67 (0.44)	0.11	4.00
#141+179	0.03	38% (74)	0.05 (0.03)	0.03	0.17
#147	0.01	22% (42)	0.03 (0.02)	0.01	0.16
#149	0.08	7% (13)	0.12 (0.05)	0.08	0.25
#151+82	0.05	9% (18)	0.08 (0.05)	0.05	0.24
#153	0.09	100% (193)	0.83 (0.48)	0.10	4.77
#171+156	0.05	69% (133)	0.14 (0.10)	0.05	0.88
#172	0.06	11% (21)	0.14 (0.10)	0.06	0.42
#174+181	0.02	12% (23)	0.06 (0.03)	0.03	0.13
#176	0.01	63% (122)	0.11 (0.17)	0.01	1.12
#177	0.02	89% (172)	0.06 (0.08)	0.04	0.99
#180	0.02	100% (193)	0.49 (0.25)	0.07	2.22
#183	0.03	62% (120)	0.09 (0.15)	0.03	0.99
#185	0.02	9% (18)	0.07 (0.06)	0.02	0.30
#187	0.03	81% (156)	0.18 (0.17)	0.02	1.65
#188	0.03	81% (156)	0.08 (0.04)	0.30	0.30
#194	0.03	96% (186)	0.14 (0.12)	0.03	0.99
#195	0.02	57% (110)	0.07 (0.04)	0.02	0.99
#200	0.01	88% (169)	0.31 (0.08)	0.01	0.99
#203+196	0.01	96% (186)	0.11 (0.06)	0.02	0.40

| #205 | 0.01 | 13% (25) | 0.07 (0.12) | 0.01 | 0.41 |
| #206 | 0.08 | 63% (121) | 0.16 (0.08) | 0.08 | 0.51 |

[1] ng/g of serum

[2] limit of detection

[3] percent with levels above the limit of detection

[4] mean values based on participants with levels above the limit of detection

Table III.

Isomer Groups of polychlorinated biphenyl (PCB) congeners detected in a sample of 192

postmenopausal women – Western New York 1986-1991.

Isomer Group	PCB Congeners (IUPAC #s)	Percent with Detectable Levels
Mono-		0
Di-	#6, #7+9	41
Tri-	#18, #19, #22, #15+17, #16+32, #25+50, #31+28, #33	46
Tetra-	#40, #42+59, #45, #44, #47+48, #49, #52, #55, #60, , #66+95, #70, #77+110	61
Penta-	#82+151,#87, #97, #99, #101, #105+132, #118	97
Hexa-	#128, #129, #134, #135, #136, #138, #141+179, #147, #149, #153, , #156+171	100
Hepta-	#172, #176, #177, #180, #183, #185, #187, #188, #174+181	100
Octa-	#194, #195, #200, #203+196, #205	100
Nona-	#206	63
Deca-		0

Table IV.

Polychlorinated biphenyl (PCB) congeners detected in a sample of 192 postmenopausal women grouped by degree of chlorination– Western New York 1986-1991.

Congener Group	PCB Congeners (IUPAC #s)	Percent with Detectable Levels	Mean[1] (SD) [Range]
Lower Chlorinated Congeners	#18, #19, #22, #15+17, #16+32, #25+50, #31+28, #33, #40, #45, #44, #49, #52, #55, #60, , #70, #42+59, #47+48, #66+95, #77+110	68	0.28 (0.35) [0.00-1.65]
Moderately Chlorinated Congeners	#82+151,#87, #97, #99, #101, #105+132, #118, #128, #129, #134, #135, #136, #138, #141+179, #147, #149, #153, #156+171, #172, #176, #177, #180, #183, #185, #187, #188, #174+181	100	3.04 (1.73) [0.47-15.07]
Higher Chlorinated Congeners	#194, #195, #200, #203+196, #205, #206	100	0.40 (0.25) [0.01-1.30]

[1] ng/g of serum

Table V.

Factor structures resulting from exploratory factor analysis of polychlorinated biphenyl (PCB)

measures of 192 postmenopausal women - Western New York 1986-1991.

	PCB Congeners (IUPAC #s)	Item Loading
Factor 1 % Variance=23.7	# 203+196 # 180 # 187 # 194 # 206 # 156+171 # 172 # 195	.83 .77 .70 .69 .62 .48 .45 .44
Factor 2 % Variance=7.8	# 118 # 105+132 # 138 # 147 # 153 # 188	.84 .83 .74 .72 .66 .58
Factor 3 % Variance=7.6	# 7+9 # 87 # 134 # 6 # 47+48 # 177	.74 .69 .62 .48 .46 .45
Factor 4 % Variance=6.6	# 22 # 31+28 # 101 # 66+95 # 141+179 # 128 #174+181 # 129	.75 .57 .54 .52 .51 .46 .42 .39

Factor 5		
% Variance=5.5	# 176	.86
	# 200	.74
	# 77+110	.49
	# 183	.37
	# 205	.35

Table VI.

Polychlorinated biphenyl (PCB) congeners detected in a sample of 192 postmenopausal women grouped by cytochrome P450 enzyme induction activity– Western New York 1986-1991.

Congener Group	PCB Congeners (IUPAC #s)	Percent with Detectable Levels	Mean[1] (SD) [Range]
PB-type	#15+17, #52, #66+95, #87, #99, #101, #136, #82+151, #153, #180, #183, #194, #195, #203+196, #205, #206	100	2.07 (1.18) [0.41-10.07]
3-MC-type	#77+110	12	0.002 (0.007) [0.00-0.04]
Mixed-type	#138, #118, #128, #156+171	100	1.19 (0.75) [0.12-4.70)
No Known Activity	#6, #7+9, #16+32, #18, #19, #22, #25+50, #31+28, #33, #40, #45, #47+48, #49, #55, #42+59, #60, #70, #97, #129, #134, #135, #141+179, #147, #149, #176, #177, #185, #187, #188, #200	100	0.83 (0.66) [0.05-4.31]

[1] ng/g of serum

Table VII.

Polychlorinated biphenyl (PCB) congeners detected in a sample of 192 postmenopausal women grouped by frameworks proposed by

McFarland and Clarke (1989) and Wolff et al. (1997) – Western New York 1986-1991.

Congener Group	PCB Congeners (IUPAC #s)		Percent with Detectable Levels	Mean[1] (SD) [Range]
	Detected in Sample	Not Detected in Sample		
	McFarland and Clarke (1989)			
Group 1 A **Pure 3-MC inducers**	#77+110	#126, #169	12	0.002 (0.007) [0.00-0.04]
Group 1 B *Mixed-type inducers, abundant in environment*	#118, #128, #138, #156+171	#105, #170	100	1.19 (0.75) [0.12-4.70]
Group 2 *Known and predicted PB-type inducers, abundant in environment*	#87, #99, #101, #153+132, #180, #183, #194		100	1.64 (0.92) [0.21-8.4]
Group 3 *Weak or non-inducing, abundant in environment*	#18, #49, #52, #70, #151+82, #177, #187	#44, #74, #201	100	0.26 (0.20) [0.01-2.63]
Group 4 *Mixed-type inducers,*		#37, #81, #114, #119, #123, #157, #158, #168, #189	0	

Rare in environment				
	Wolff et al. (1997)			
Group 1 – *Potentially estrogenic*				
Group 1 A *Weak PB-type inducers, estrogenic, not persistent*	#44, #52, #31+28, #70		40	0.17 (0.26) [0.00-1.57]
Group 1 B *Weak PB-type inducers, persistent*	#101, #187, #174, #177	#201	98	0.23 (0.22) [0.00-2.35]
Group 2 - *Potentially antiestrogenic and immunotoxic, dioxin-like*				
Group 2 A *Non-ortho and mono-ortho substituted, moderately persistent*	#66+95, #77+110, #105+132, #118, #156+171	#74, #126, #169, #167	98	0.56 (0.41) 0.00-2.65
Group 2 B *Di-ortho substituted, limited dioxin activity, persistent*	#128, #138	#170	100	0.67 (0.46) [0.04-4.05]
Group 3 *PB-, CYP1A1-, and CYP2B- inducers, persistent*	#99, #153, #180, #203+196, #183		100	1.57 (0.88) [0.23-7.83]

PART FOUR - CYTOCHROME P4501A1 POLYMORPHISM, POLYCHLORINATED BIPHENYL EXPOSURE AND POSTMENOPAUSAL BREAST CANCER RISK

Introduction

It has been proposed that higher body burden of polychlorinated biphenyls (PCBs) may increase the risk of breast cancer because of their hypothesized estrogenic (1), tumor promoting (2), immune modulating (3) and enzyme inducing properties (4). To date, however, findings from epidemiologic investigations of this relation have been inconclusive, with several studies showing no association with risk, and others only among specific subgroups of individuals (5-13).

In laboratory studies, PCBs are potent inducers of cytochrome P450 1A1 (*CYP1A1*), a drug metabolizing gene, involved in the activation of potentially genotoxic endogenous and exogenous substances (14,15). There is wide inter-individual variation in *CYP1A1* activity and several genetic polymorphisms are present. Approximately 10-15 % of Caucasians carry a *CYP1A1* valine for isoleucine substitution allele (16). Although no difference in enzymatic activity of the variant type compared to the wild type has been demonstrated (17), there is evidence that the *CYP1A1* variant genotype results in *CYP1A1* activity that is more inducible in lymphocytes (18). Greater activity may lead to enhanced carcinogen activation and steroid hormone metabolism, and may therefore be potentially related to risk for breast cancer. The *CYP1A1* valine for isoleucine substitution has been associated with greater risk of lung cancer in Japanese (19). For breast cancer, there is no evidence for an overall association between this genetic polymorphism and breast cancer risk (20-24), although two studies reported subgroup effects among light smokers (22) or among women who had commenced smoking before age 18 (24).

In this case-control study, we sought to examine whether body burden of PCBs modified the association between *CYP1A1* genotype and breast cancer risk.

Materials and Methods
Study Population This research utilized a subset of data collected as part of a case-control study (1986-1991) of 933 postmenopausal Caucasian women in Western New York; detailed methods have been previously reported (25). The protocol for the study was reviewed by the Institutional Review Board of the State University of New York at Buffalo, and of participating hospitals. Informed consent was received from all participants. Women diagnosed with incident, primary, histologically confirmed breast cancer (n=439) were frequency-matched by age and county of residence with controls (n=494), randomly selected from the New York State Motor Vehicle lists (<65 years) and the Health Care Finance Administration rolls (≥65 years). Interview data included medical, reproductive and life style histories. Of women who provided usable interviews, approximately 63% agreed to donate a blood sample. Blood was drawn for 262 postmenopausal breast cancer cases and 319 controls. Controls providing a blood sample did not differ from those who did not with respect to age, years of education, age at menarche, prevalence of benign breast disease or family history of breast cancer, and fruit, vegetable, or meat consumption, although they tended to have fewer years of smoking, consume fewer alcoholic beverages and undergo menopause at an older age.

A subset of stored blood specimens was utilized for toxicological and genetic analyses: 154 women with postmenopausal breast cancer and 191 controls. Cases were only included in the final study sample if their blood was drawn before chemotherapy or radiation and within three months of surgery. Controls were frequency matched to cases by date of blood draw (± 3 months) and age (± 3 years). Controls who provided a blood sample but were not selected for these analyses were more likely to consume red meat and alcoholic beverages than those who

were selected, but the two groups differed little with regard to demographic, reproductive or lifestyle charateristics.

Polychlorinated Biphenyl Analysis PCB concentrations were determined by the method of Greizerstein et al. (26). Total PCBs were calculated from summing the detected levels of 56 congener peaks, measured in the serum (based on 73 individual congeners). Participants with non-detectable levels for individual PCBs were assigned zero for these compounds. The procedures include standardized extraction, clean up and quantification by high resolution gas chromatography and comprehensive quality assurance program to minimize systematic and random errors. The sample was mixed with solutions containing IUPAC isomers #46 and #142 (surrogate standards). Methanol was added to precipitate the proteins and the resulting mixture was extracted with hexane. The extract was concentrated and then cleaned by passing through a Florisil column. The eluate was then evaporated to a small volume and isomers #30 and #204 were added as internal standards. An aliquot of the mixture was injected into the gas chromatographic system equipped with an electron capture detector. Quantification was based on calibration standards and response factors calculated using purchased reference materials. Quality control activities consisted of analyses of samples in batches of six to ten simultaneously with quality control samples. The coefficients of variation of the quality control samples for individual PCB congeners ranged from 2.5% (IUPAC #180) to 6.2% (IUPAC #52). The coefficient of variation for total PCBs was 1.8%. The quality assurance program checked that the procedures were under control by the use of control charts and set criteria for data acceptability. The limit of detection for each analyte was determined as the mean of background noise plus three standard deviations in five reagent blank samples.

Genetic Analysis As previously described (22), DNA was extracted from blood clots obtained following centrifugation and removal of serum, which had been stored at $-70°$ C. The CYP1A1 isoleucine to valine substitution in exon 7 was determined simultaneously with that of glutathione S-transferase (GSTM1), as previously detailed (22). Briefly, using primers specific for GSTM1 and for CYP1A1, where a base was substituted to introduce an NcoI restriction site, genes were amplified using PCR. The amplified PCR product was subjected to restriction enzyme analysis with NcoI (New England Biolabs, Beverly, MA), according to the manufacture's instructions. A simultaneous restriction enzyme digestion was also conducted with HinfI (New England Biolabs, Beverly, MA), which cleaved the GSTM1 fragment so that this band did not overlap the cleaved CYP1A1 fragment. Analysis by gel electrophoresis (4.0% agarose; 3:1 NuSieve; FMC Bioproducts, Rockland ME: Agarose, Gibco BRL, Gaithersburg, MD) revealed 232- and 31-bp fragments for wild-type alleles (isoleucine) or a single 263-bp fragment when the mutation (valine) was present. The assay was validated by confirming polymorphic Mendelian inheritance patterns in seven human family cell lines (n=134 family members), encompassing three generations (data not shown; samples were obtained from NIGMS Human Genetic Mutant Cell Repository, Coriell Institute, Camden, NJ). Genotyping results were read by two independent investigators and genotyping for 20% of the subjects were repeated for quality control. The investigators were blinded to each other's interpretations and to case-control status.

Statistical Analyses Descriptive analyses included Student t-tests of means for cases and controls for lifestyle, reproductive, and dietary variables, and chi square tests for categorical variables. We carefully examined cases and controls with respect to age and date of blood draw and attempted to individually match study participants on these variables. However, the width of

the frequency matching strata did not allow for individual matching. Therefore, we used unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs). ORs were adjusted for potential confounders, including age, education, family history of breast cancer, parity, quetelet index, duration of lactation, age at first birth, serum lipids and smoking status (nonsmokers; light smokers, less than 29 pack-years; and heavy smokers, 29 or more pack-years). Covariates were only included in the final regression model if they were established risk factors in these data or changed the observed risk estimate by at least 15 percent. Lipid adjustment was modeled after the method described by Phillips et al. (27), where serum triglycerides and total cholesterol levels were used to estimate total serum lipid content. *CYP1A1* was investigated by collapsing the categories for women who were homozygous (Val:Val) with those who were heterozygous (Ile:Val) for the valine allele because of the small number of women in the first group (n=6). Participants were designated as having either high or low PCB body burden based on a level above or below the median for the controls. Levels ranged from 0.75-3.72 ng/g of serum in the low PCB group, and from 3.73-19.04 ng/g of serum in the high PCB group.

Results

The effect of PCBs on risk (13) and of *CYP1A1* (22) have been examined in detailed elsewhere. Shown here are those associations in this sub-sample with both *CYP1A1* and PCB determinations. Women with higher body burden of PCBs were not at greater risk of breast cancer than women with lower levels, and women with at least one valine *CYP1A1* allele (Ile; Val or Val; Val), were at slightly elevated risk compared to women who were homozygous for the isoleucine allele (Ile;Ile) (Table 1). The test for statistical interaction between PCB body burden and *CYP1A1* genotype approached statistical significance (p=0.13). In Table 2, the combined effect of of PCB body burden and *CYP1A1* genotype is shown. Women with lower

serum PCB levels and *CYP1A1* Ile:Ile served as the reference category. Compared to this group, women with lower PCB levels and *CYP1A1* valine genotype were not at greater risk for breast cancer (adjusted OR=0.88; 95% CI 0.29-2.70), nor were women with elevated PCB levels and *CYP1A1* Ile:Ile (OR=1.08; 95% CI 0.62-1.89). In contrast, there was evidence of increased risk for women with high PCB body burden and at least one valine allele when compared to women with lower serum PCB levels and who were homozygous for the isoleucine allele (OR=2.96; 95% CI 1.18-7.45). We repeated these analyses in two PCB congener groups, defined by *CYP1A1*-induction activity (IUPAC #'s 60, 105, 118, 128, 138, 153, 180 and 203) and by potential estrogenic activity (IUPAC #'s 18, 47, 52, 77, 136 and 153). The OR's for women with elevated PCB body burden and at least one valine allele were 2.87 (95% CI 1.19-7.30) and 2.75 (95% CI 1.09-7.21), respectively (data not shown). This correspondence in the observed risk estimates is likely to be an effect of the high correlations between total PCB levels and measured levels of these specific congener (Pearson r > 0.90).

Discussion

In this study of associations between serum levels of PCBs, *CYP1A1* genotype and breast cancer risk, we found that among women with elevated PCB body burden, the *CYP1A1* polymorphism statistically significantly increased risk. No effect of *CYP1A1* was noted among women with lower serum levels of PCBs or women with the Ile:Ile genotype. To our knowledge, this is the first study to examine the effect of *CYP1A1* in relation to PCB exposure on breast cancer risk. A number of previous investigations found no main effect between *CYP1A1* and breast cancer among Caucasian women (20-24), despite laboratory evidence for a role of polycyclic aromatic hydrocarbons, which are activated by *CYP1A1*, in mammary carcinogenesis. *CYP1A1* is also known to be involved in the metabolism of estradiol to the possibly mutagenic catechol estrogens (28), and increased metabolism related to the polymorphism could increase

breast cancer risk through that mechanism. It is possible that such an association was masked by variability in *CYP1A1* induction, due to heterogeneity in exposures to PCBs and other *CYP1A1* inducers.

As indicated above, results of investigations of PCBs and breast cancer have been mixed; a number of studies did not observe an increase in breast cancer risk associated with elevated PCB levels (6,7,11,12). Interestingly, in one of those studies (12), there was suggestion of an association between exposure and risk among African-American women. Investigators have identified novel *CYP1A1* alleles in African-Americans, and the MspI allele, which is thought to be in linkage disequilibrium with the exon 7 substitution, is three times as common among African-Americans than among Caucasians (16), and has been found to be associated with increased breast cancer risk (21). It is possible, that risk associated with PCB exposure among African-American women may be related to the increased prevalence of mutant alleles within this population.

The role of PCBs as P450 inducers in experimental animal has been so widely acknowledged that the commercial form is often used in animal studies to ensure and hasten neoplasms initiated by known carcinogens. Repeated studies, using a number of different bioassays and target organs have all confirmed the role of PCBs as promoters of carcinogenesis (4). Caucasian postmenopausal women with elevated PCB body burden and the *CYP1A1* isoleucine to valine substitution are possibly at greater risk of breast cancer due to the PCB mediated enhanced induction of polymorphic *CYP1A1*, leading to increased activation of environmental carcinogens and subsequently resulting in the production of reactive intermediates and DNA damage. Thus, by inducing *CYP1A1*, PCBs and other inducers can trigger the activation of xenobiotics, such as those found in tobacco, into mutagenic compounds. This

notion is supported by our observation that risk was significantly increased among women with elevated PCB body burden and the *CYP1A1* polymorphism who had ever smoked cigarettes (OR=7.74, 95% CI, 1.12-53.90), but not among women who had never smoked (OR=1.43, 95% CI, 0.53-3.87) (data not shown). It should be pointed out, however, that these risk estimates are based on very small numbers, as indicated by the width of the corresponding confidence intervals, and should be interpreted with caution.

Alternatively, there is some evidence that P450-mediated metabolism of lower chlorinated PCBs, which have been associated with greater toxicological activity, may lead to further metabolism by peroxidases to DNA-reactive metabolites (29). It is possible that women with both elevated PCB body burden and the potentially more inducible *CYP1A1* variant genotype are at greater risk for DNA damage, and subsequently for breast cancer. In fact, in our previous analyses, we observed a modest increase in risk (OR=1.66) for women with detectable levels of lower chlorinated PCBs (13). We attempted to examine the effect of lower chlorinated PCBs in this research, but only a small proportion of participants had detectable levels for these congeners, resulting in very small numbers, which did not permit further stratification by *CYP1A1* genotype.

Sample size was a major limitation in this study, as in many molecular epidemiologic studies. Our findings were based on small numbers, resulting in possibly unstable risk estimates. It is possible that the modifying effect of PCB body burden may be a result of sampling variation. Further, it was not possible to examine the effect of PCB body burden on the association between *CYP1A1* and risk in more detail, restricting the analysis to crude stratification into low and high PCB body groups. A larger sample size would allow for an examination of a potential threshold effect at which PCB body burden affects the association

between *CYP1A1* and risk. Finally, due to sample size restrictions, it was not feasible to explore the previously identified interactions of PCBs with lactation status (13) and *CYP1A1* genotype and smoking status (22) in this study of both PCBs and *CYP1A1* genotype.

Overall, our results indicate that the *CYP1A1* isoleucine to valine substitution may be a risk factor for breast cancer among women with elevated PCB body burden. If these findings are confirmed, the lack of an association between PCBs and breast cancer in some studies may, in part, be explained by the fact that only a small proportion of the study population was susceptible to the adverse effects of PCB exposure, that is, those with the *CYP1A1* polymorphism.

References

1. McKinney & Waller CL. Polychlorinated biphenyls as hormonally active structural analogues. Environ Health Perspect 1994; 102:290-4.

2. Norback DH, Weltman RH. Polychlorinated biphenyl induction of hepatocellular carcinoma in the Sprague-Dawley rat. Environ Health Perspect 1985; 60:97-105.

3. Kimbrough RD. Human heath effects of polychlorinated biphenyls (PCBs) and polybromated biphenyls (PBBs). Annu Rev in Pharmacol and Toxicol 1987; 27:87-111.

4. Safe S. Polychlorinated biphenyls (PCBs): mutagenicity and carcinogenicity. Mutat Res 1989; 224:79-88.

5. Wassermann M., Nogueira DP, Tomatis L, Mirra AP, Shibata H, Arie G, Cucso S, Wassermann D. Organochlorine compounds in neoplastic and adjacent apparently normal breast tissue. Bull Environ Contam Toxicol 1976;15:478-484.

6. Unger M, Kiaer H, Blichert-Toft M, Olsen J, Clausen J. 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 1984; 34:24-28.

7. Mussalo-Rauhamaa H, Haesaenen E, Pyysalo H, Antervo K, Kauppila R, Pantzar P. Occurrence of β-hexachlorocyclohexane in breast cancer patients. Cancer 1990; 66:2124-2128.

8. Falck F, Ricci A, Wolff MS, Godbold J, Deckers P. Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. Arch Environ Health 1992; 47:143-146.

9. Dewailly E, Dodin S, Verreault R, Ayotte P, Sauve L, Morin J, Brisson J. High organochlorine body burden in women with estrogen receptor-positive breast cancer. J Natl Cancer Inst 1994; 86(3):232-4.

10. Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N. Blood levels of organochlorine residues and risk of breast cancer. J Natl Cancer Inst 1993;85:648-652.

11. Krieger N, Wolff MS, Hiatt RA, Rivera M, Vogelman J, Orentreich N. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. J Natl Cancer Inst 1994; 86:589-599.

12. Hunter DJ, Hankinson SE, Laden F, Colditz GA, Manson JE, Willett WC, Speizer FE, and Wolff MS. Plasma organochlorine levels and the risk of breast cancer. N Engl J med 1997;337:1253-8.

13. Moysich KB, Ambrosone CB, Vena JE, Shields PG, Mendola P, Kostyniak P, Greizerstein H, Graham S, Marshall JR, Schisterman EF, Freudenheim JL. Environmental organochlorine exposure and postmenopausal breast cancer risk. Cancer Epidemiology Biomarkers and Prevention 1998; 7:181-188.

14. Drahushuk AT, Choy CO, Kumar S, McReynolds JH, Olson JR. Modulation of cytochrome P450 by 5,5'-bis-triflouromethyl-2,2'-dicholrobiphenyl, a unique environmental contaminant. Toxicology 1997; 120:197-205.

15. Bandiera SM, Torok SM, Letcher RJ, Norstrom RJ. Immunoquantification of cytochromes P450 1A and P450 2B and comparison with chorinated hydrcarbon levels in archived polar bear liver samples. Chemosphere 1997; 34: 1469-79.

16. Shields PG, Caporaso NE, Falk RT, Sugimura H, Trivers GE, Trump BF, Hoover RN, Weston A, Harris CC. Lung cancer, race and a *CYP1A1* polymorphism. Cancer Epidemiology Biomarker Prevention 1993;2: 481-485.

17. Zhang ZY, Fasco MJ, Huang I, Guegenrich FP, and Kaminski LS. Characterization of purified human recombinant cytochrome P450-Ile^{462} and Val^{462} : assessment of a role of the rare allele in carcinogenesis. Cancer Res 1996; 56:3926-3933.

18. Cosma G, Crofts F, Taioli E, Toniolo P, Garte S. Relationship between genotype and function of the human *CYP1A1* gene. J Toxicol Environ Health 1993; 40:309-16.

19. Kawajiri K, Nakachi K, Imai K, Yoshii A, Shinoda N, and Watanabe J. Identification of genetically high risk individuals to lung cancer by polymorphisms of the cytochrome P450IA1 gene. FEBS Lett. 1990;263:131-136.

20. Rebbeck TR, Rosvold EA, Duggan DJ, Zhang J, Buetow KH. Genetics of *CYP1A1*: Coamplification of specific alleles by polymerase chain reaction and association with breast cancer. Cancer Epidemiology Biomarker Prevention 1994;3:511-514.

21. Taioli E, Trachman J, Chen X, Toniolo P, and Garte SJ. A *CYP1A1* restriction fragment length polymorphism is associated with breast cancer in African-American women. Cancer Research 1995;55:3757-3758.

22. Ambrosone CB, Freudenheim JL, Graham S, Marshall JR, Vena JE, Brasure JR, Laughlin R, Nemoto T, Michalek AM, Harrington A, Ford TD, Shields PG. Cytochrome P4501A1 and glutathione S-transferase (MI) genetic polymorphism and postmenopausal breast cancer risk. Cancer Res 1995; 55:3483-3485.

23. Bailey LR, Roodi N, Verrier CS, Yee CJ, Dupont WD, and Pari FF. Breast Cancer and *CYP1A1*, GSTM1, and GSTT1 polymorphisms: Evidence for a lack of association in Caucasians and African-Americans. Cancer Res 1998; 58:65-70.

24. Ishibe N, Hankinson SE, Colditz GA, Spiegelman D, Willett WC, Speizer FE, Kelsey KT, Hunter DJ. Cigarette smoking, cytochrome P450 1A1 polymorphisms, and breast cancer risk in the Nurses' Health Study. Cancer Res 1998; 58:667-71.

25. Graham, S., Hellman, R., Marshall, J., Freudenheim, J., Vena, J., Swanson, M., Zielezny, M., Nemoto, T., Stubbe, N., and Raimondo, T. Nutritional epidemiology of postmenopausal breast cancer in Western New York. Am. J. Epidemiology, 134: 552-566, 1991.

26. Greizerstein H, Gigliotti P, Vena JE, Freudenheim J, Kostyniak PJ. Standardization of a method for routine analysis of polychlorinated biphenyl congeners and selected pesticides in human serum and milk. J Anal Toxicol 1997;21:558-566.

27. Phillips DL, Pirkle JL, Burse VW, Bernert JT, Henderson LO, Needham LL. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ Contam Toxicol 989; 18:495-500.

28. Suchar LA, Chang RL, Thomas PE, Rosen RT, Lech J, Conney AH. Effects of phenobarbital, dexamethasone, and 3-methylcholanthrene administration on the metabolism of 17 beta-estradiol by liver microsomes from female rats. Endocrinology 1996;137:663-676.

29. Oakley GG, Devanaboyina US, Robertson LW, Gupta RC. Oxidative DNA damage induced by activation of polychlorinated biphenyls (PCBs) – implications for PCB-induced oxidative stress in breast cancer. Chemical Research in Toxicology 1996; 9:1285-1292.

Table 1.

Risk of postmenopausal breast cancer associated with PCB body burden - Western New York 1986-1991.

	Case	Control	Crude OR[1]	95% CI[2]	Adj. OR[3]	95% CI
Polychlorinated Biphenyls						
Low[4]	70 (46)	96 (50)	1.0		1.0	
High[5]	84 (54)	95 (50)	1.12	0.79-1.86	1.27	0.76-2.14
Total	154 (45)	191 (55)				
Cytochrome P450 1A1 Genotype						
Ile:Ile	127 (83)	168 (88)	1.0		1.0	
Ile:Val, or Val:Val	27 (17)	23 (12)	1.56	0.86-2.85	1.79	0.91-3.55
Total	154 (45)	191 (55)				

[1] odds ratio
[2] confidence interval
[3] odds ratios adjusted for age, education, serum lipids, age at menopause, family history of breast cancer, age at first birth, parity, duration of lactation, body mass index, and smoking status
[4] 0.75-3.72 ng/g of serum
[5] 3.73-19.04 ng/g of serum

Table 2.

Risk of postmenopausal breast cancer by *CYP1A1* polymorphism and PCB body burden -
Western New York 1986-1991.

PCB/*CYP1A1*	Case	Control	Crude OR[1]	95% CI[2]	Adj. OR[3]	95% CI
PCB low[4]-Ile:Ile	62 (41)	85 (45)	1.0		1.0	
PCB low[4]-Ile:Val/Val:Val	8 (5)	11 (6)	1.0	0.37-2.62	0.88	0.29-2.70
PCB high[5]-Ile:Ile	65 (42)	83 (43)	1.07	0.68-1.70	1.08	0.62-1.89
PCB high[5]-Ile:Val/Val:Val	19 (12)	12 (6)	2.17	0.98-4.78	2.9	1.18-7.45
Total	154 (45)	191 (55)				

[1] odds ratio
[2] confidence interval
[3] odds ratios adjusted for age, education, serum lipids, age at menopause, family history of breast cancer, age at first birth, parity, duration of lactation, body mass index, and smoking status[4] homozygous (Ile;Ile) for the *CYP1A1* wild-type alleles
[4] 0.75-3.72 ng/g of serum
[5] 3.73-19.04 ng/g of serum

BIBLIOGRAPHY

Meeting Abstracts

Ambrosone CB, Freudenheim JL, Marshall JR, Graham S, Vena JE, Breasure JR, Michalek AM, Bowman ED, Harrington AM, Shields PG. N-acetyltransferase (NAT), cigarette smoking, and breast cancer risk. Proc Am Assoc Ca Res 1995; 36:283.

Shields PG, Blomeke B, Kato S, Bowman ED, Harrington AM, Freudenheim JL, Marshall JR, Vena JE, Ambrosone CB. Genetic polymorphisms and increased cancer risk. (Abstract) Genetics and Susceptibility: Impact on Risk Assessment Conference, 1994.

Ambrosone CB, Freudenheim JL, Marshall JR, Graham S, Vena JE, Breasure JR, Michalek AM, Bowman ED, Harrington AM, Shields PG. N-acetyltransferase (NAT), cigarette smoking, and breast cancer risk. Proc Am Assoc Ca Res 1995; 36:283.

Ambrosone CB, Freudenheim JL, Graham S, Vena JE, Brasure JR, Harrington AM, Ford T, Shields PG. Polymorphisms in carcinogen-metabolizing genes and breast cancer susceptibility. International Genetic Epidemiology Society Meeting, 1995.

Ambrosone C, Freudenheim JL, Graham S, Vena JE, Brasure JR, Harrington AM, Ford T, Shields PG. Cigarette smoking, N-acetyltransferase genetic polymorphisms and breast cancer risk. American College of Epidemiology, 1995.

Freudenheim JL, Graham S, Marshall JR, Vena J, Moysich KB, Muti P, Laughlin R, Nemoto T. Lactation and risk of breast cancer: A case-control study. Am J Epidemiol 1996;143:S32.

Moysich KB, Ambrosone CB, Vena J, Marshall JR, Graham S, Laughlin R, Kostyniak P, Greizerstein H, Mendola P, Freudenheim JL. Dietary correlates of serum DDE, HCB and total PCB levels in postmenopausal women. Meeting of the International Society for Environmental Epidemiology, Edmonton, 1996.

Ambrosone CB, Kato S, bowman ED, Harrington AM, Blomeke B, Freudenheim JL, Graham S, Marshall JR, Vena JE, Brasure JR, Shields PG. Chemicals and Cancer. European J Cancer Prev 5:25-27, 1996.

Freudenheim JL, Ambrosone CB, Moysich KB, Vena JE, Marshall JR, Graham S, Laughlin R, Nemoto T, Shields PG. Alcohol intake and breast cancer risk: Effect of alcohol metabolism by alcohol dehydrogenase 3. Proc Am Assoc Ca Res 1997; 38:619.

Moysich KB, Ambrosone CB, Vena JE, Kostyniak P, Shields PG, Marshall JR, Graham S, Laughlin R, Greizerstein H, Freudenheim JL. Serum polychlorinated biphenyls (PCBs) and postmenopausal breast cancer risk. Proc Am Assoc Ca Res 1997; 38:627.

Freudenheim JL, Ambrosone CB, Moysich KB, Vena JE, Marshall JR, Graham S, Nemoto T, Shields, PG. Breast cancer, alcohol and the alcohol dehydrogenase 3 polymorphism. Society for Epidemiologic Research Meeting, 1997.

Moysich KB, Ambrosone CB, Vena JE, Shields PG, Marshall JR, Graham S, Kostyniak P, Greizerstein H, Freudenheim JL. Serum organochlorine levels and breast cancer risk. Society for Epidemiologic Research Meeting, 1997.

Moysich KB, Ambrosone CB, Vena JE, Shields PG, Marshall JR, Graham S, Kostyniak P, Greizerstein H, Freudenheim JL. Serum organochlorine levels and risk of postmenopausal breast cancer. International Society for Environmental Epidemiology, 1997.

Moysich KB, Ambrosone CB, Vena JE, Mendola P, Schisterman EF, Marshall JR, Graham S, Kostyniak P, Greizerstein H, Freudenheim JL. Evaluating Three Frameworks for Group Polychlorinated Biphenyls (PCBs) into Meaningful Analytic Units. International Society for Environmental Epidemiology, 1997.

Moysich KB, Ambrosone CB, Vena JE, Shields PG, Marshall JR, Graham S, Kostyniak P, Greizerstein H, Schisterman EF, and Freudenheim JL. Environmental exposure to organochlorines and postmenopausal breast cancer risk. Poster presented at the Era of Hope Department of Defense Breast Cancer Meeting, Washington, DC, October 1997.

Moysich KB, Shields PG, Freudenheim JL, Vena JE, Kostyniak P, Greizerstein H, Schisterman EF, Marshall JR, Graham S and Ambrosone CB. Polychlorinated biphenyls, cytochrome P4501A1 polymorphism, and postmenopausal breast cancer risk. Paper presented at the Annual Meeting of the American Association for Cancer Research, New Orleans, Louisiana, April 1998.

Moysich KB, Shields PG, Freudenheim JL, Vena JE, Kostyniak P, Greizerstein H, Schisterman EF, Marshall JR, Graham S and Ambrosone CB. Polychlorinated biphenyls, cytochrome P4501A1 polymorphism, and postmenopausal breast cancer risk. Paper presented at the Annual Meeting of the Society for Epidemiologic Research, Chicago, IL, June 1998

Moysich KB, Shields PG, Freudenheim JL, Vena JE, Kostyniak P, Greizerstein H, Schisterman EF, Marshall JR, Graham S and Ambrosone CB. Cytochrome P4501A1 and postmenopausal breast cancer: Effect of Environmental PCB exposure. Paper presented at the Annual Conference of the International Society of Environmental Epidemiology, Boston, MA, August 1998.

Papers

Ambrosone CB, Freudenheim JL, Graham S, Marshall JR, Vena JE, Brasure JR, Michalek AM, Laughlin R, Nemoto T, Gillenwater KA, Harrington AM, Sheilds PG. Cigarette smoking, N-acetyltransferase genetic polymorphisms and breast cancer risk. JAMA 1996; 276:494-501.

Shields PG, Ambrosone CB, Graham S, Bowman ED, Harrington AM, Gillenwater KA, Marshall JR, Vena JE, Laughlin R, Nemoto T, Freudenheim JL. A cytochrome P450II E1 genetic

polymorphism (CYP2E1) and tobacco smoking in breast cancer. Molecular Carcinogenesis 1996; 17:144-50.

Ambrosone CB, Freudenheim JL, Graham S, Marshall JR, Vena JE, Brasure JR, Laughlin R, Nemoto T, Michalek AM, Harrington A, Ford TD, Shields PG. Cytochrome P4501A1 and glutathione S-transferase (MI) genetic polymorphism and postmenopausal breast cancer risk. Cancer Res 1995; 55:3483-3485.

Ambrosone C, Marshall JR, Vena JE, Laughlin R, Graham S, Nemoto T, Freudenheim JL. Interaction of family history of breast cancer and dietary antioxidants with breast cancer risk (New York, United States). Cancer Causes Control 1995; 6:407-415.

Freudenheim JL, Graham S, Moysich KB, Marshall JR, Vena JE, Muti P, Laughlin R, Nemoto T. Lactation and Risk of Breast Cancer: A Case-Control Study. American Journal of Epidemiology 1997;146:932-8.

Moysich KB, Ambrosone CB, Vena J, Mendola P, Marshall JR, Graham S, Laughlin R, Shields PG, Kostyniak P, Greizerstein H, Schisterman EF, and Freudenheim JL. Environmental organochlorine exposure and postmenopausal breast cancer risk. Cancer Epidemiology Biomarkers and Prevention 1998;7:181-188.

Moysich KB, Mendola P, Schisterman EF, Ambrosone CB, Freudenheim JL, Vena J, Kostyniak P, Greizerstein, H Graham S, Marshall JR. Evaluating five frameworks for grouping polychlorinated biphenyl congener data into meaningful analytic units. American Journal of Industrial Medicine (in press).

Moysich KB, Shields PG, Freudenheim JL, Vena JE, Kostyniak P, Greizerstein H, Schisterman EF, Marshall JR, Graham S and Ambrosone CB. Polychlorinated biphenyls, cytochrome P4501A1 polymorphism, and postmenopausal breast cancer risk Cancer Epidemiology Biomarkers and Prevention (in press).

Mendola P, Moysich KB, Freudenheim JL, Shields PG, Schisterman EF, Vena JE, Marshall JR, Graham S, Nemoto T, and Ambrosone CB. Risk of recurrent spontaneous abortion, cigarette smoking and genetic polymorphisms in NAT2 and GSTM1. Epidemiology 1998; 9(6):666-668.

Schisterman EF, Buck GM, Mendola P, Vena JE, Trevisan M, Moysich KB, Perez K Utility of the Bayesian Approach for Reproductive Epidemiology: Estimating the Probability of Low Birth Weight Based on Prior Knowledge. Epidemiology (submitted).

Freudenheim JL, Ambrosone CB, Moysich KB, Vena JE, Graham S, Marshall JR, Muti P, Laughlin R, Nemoto T, Harty L, Crits A, and Shields PG. Polymorphic alcohol Dehydrogenase 3 and risk of breast cancer associated with alcohol consumption. Journal of the National Cancer Institute (submitted).

Schisterman EF, Buck GM, Moysich KB. Estimation of the correlation coefficient using the Bayesian approach and its applications for epidemiologic research. (submitted).

Awards and Honors

Christine Ambrosone, American College of Epidemiology Student Prize Paper 1995, "Cigarette smoking, N-acetyltransferase genetic polymorphisms and breast cancer risk."

Christine Ambrosone, Women in Cancer Research Brigid G. Leventhal Training Award, 1995.

Kirsten Moysich, Young Investigators Award, American Association for Cancer Research, 1997.

Kirsten Moysich, Best Student Abstract Award, International Society for Environmental Epidemiology, 1997.

Kirsten Moysich, School of Medicine and Biomedical Sciences Dean's Award for best dissertation, 1997.