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13. ABSTRACT *(Maximum 200 words)*

This report includes results from a case-control study examining the relation of serum levels of DDE, hexachlorbenzene (HCB), and polychlorinated biphenyls (PCBs) to risk of postmenopausal breast cancer. The study sample included 154 primary, incident, histologically confirmed, breast cancer cases and 192 community controls. In the entire sample there was no evidence of an adverse effect associated with serum levels of DDE, HCB, and several PCB exposure measures: total PCBs, total number of detected PCB congener peaks, and PCB congener groups. We consistently observed effect modification by history of lactation with respect to all exposures. Among women who never breastfed, higher serum levels DDE (OR=1.90; CI 0.79-6.04) and HCB (OR=2.85; CI 0.89-9.01) was associated with evidence of increased risk of breast cancer. Similar effects were observed for higher levels of total PCBs (OR=2.92; CI 0.95-11.35), moderately chlorinated PCB congeners (OR=2.86; CI 0.75-10.90), higher chlorinated PCB congeners (OR=4.15; CI 0.99-10.55), and greater number of detected PCB congeners (OR=3.57; CI 1.12-11.34). Among women who had breastfed, we found no excess risk of breast cancer related to any of these exposure variables.

In addition, this report includes a detailed evaluation of five frameworks for grouping PCB congener data into meaningful analytic units.

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FOREWORD

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During the past funding year this postdoctoral research has focussed on the association between serum organochlorine levels and postmenopausal breast cancer. Results from this research were presented at the annual meetings of the American Association for Cancer Research, Society for Epidemiologic Research, and the International Society for Environmental Epidemiology. This work was awarded with an American Association for Cancer Research Young Investigator Award, the Best Student Abstract Award of the International Society for Environmental Epidemiology, as well as the Dean's Award for Outstanding Dissertation Research of the University at Buffalo. In addition, efforts were made to evaluate the utility of frameworks for grouping polychlorinated biphenyl (PCB) congeners into meaningful analytic units. Manuscripts covering each research project have been submitted to *Cancer Epidemiology Biomarkes and Prevention* and *Environmental Health Perspectives*, respectively.

PART ONE – 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 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.

	Total			Never Lactated[1]			Ever Lactated		
Characteristic	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%	ns	61%	57%	
Rural	4%	16%	0.01	11%	17%		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] 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.

	Total		Never Lactated [1]		Ever Lactated	
Measure in ng/g of serum	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.

	Total (n=346)			Never Lactated (n=107) [1]			Ever Lactated (n=191)		
Measures in ng/g of serum	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)									
2	54	60	1.0	15	23	1.0	29	29	1.0
3 (high)	46	69	1.01 (0.56-1.86)	13	17	1.95 (0.58-6.67)	30	44	0.76 (0.35-1.63)
	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)									
2	62	61	1.0	16	27	1.0	37	26	1.0
3 (high)	40	66	0.56 (0.30-1.04)	12	14	1.26 (0.40-3.97)	23	44	0.32 (0.14-0.71)
	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.

Table 4

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

	Total (n=346)			Never Lactated (n=107)[1]			Ever Lactated (n=191)		
Measures in ng/g of serum	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						
1 (low)	56	59	2.04 (1.09-3.83)		na			na	
2 (high)	53	63	1.40 (0.76-2.59)						
			p=0.78						

Moderately chlorinated PCBs [2,10]									
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]									
									1.0
1(low)	54	63	1.0	13	20	1.0	32	34	0.96 (0.41-2.23)
2	43	66	0.79 (0.42-1.52)	11	24	0.51 (0.15-1.69)	24	34	1.00 (0.40-2.49)
3 (high)	54	62	1.19 (0.60-2.36)	21	17	1.53 (0.47-4.95)	27	38	
									p=0.94
			p=0.35			p=0.12			

[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 TWO – PCB CONGENER GROUPING

INTRODUCTION

Polychlorinated biphenyls (PCBs) are persistent, lipophilic compounds that are ubiquitous in the environment. The number of chlorine atoms introduced into the biphenyl molecule determines the structure of each PCB. Isomeric PCBs are those with the same molecular weight, i.e., number of halogen substitutions. There are 209 possible PCB congeners or homologues. PCB congeners with five to seven chlorine atoms per molecule are most likely to bioaccumulate. These moderately chlorinated isomer groups (penta-, hexa-, and hepta- chlorobiphenyls) account for 112 of the 209 congeners. They were synthesized in high proportions in many commercial preparations and are likely to be prevalent in the environment. The more highly chlorinated congeners are generally less available to organisms both because they bind more tightly with soils and sediments and because they are present in lower quantities in the environment. Congeners with fewer chlorines are more readily metabolized and eliminated (1). In addition to differences in bioaccumulation, certain PCB congeners also differ with respect to endocrine (2,3), neurotoxic (4,5), cytochrome P450 enzyme induction (1) and dioxin-like (6,7) activity.

PCBs have been implicated in the etiology of a variety of health effects, including reproductive and perinatal outcomes (8,9), developmental outcomes (10), neurological (11,12), cognitive (13,14), and behavioral (15,16) development, as well as cancer (17,18).

Improvements in analytic methods have led to more detailed PCB data available for epidemiologic studies. Total PCB level, as one measurement or the sum of a few

prevalent congeners, has been replaced by toxicologic values for 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, it is generally inappropriate to analyze individual congeners in relation to an outcome due to issues of multiple comparison (19). Thus, it is important to group these congeners into meaningful analytic units.

The purpose of this paper was to explore the utility of five potential frameworks for grouping PCB congener data into meaningful analytic units. Our attempts to develop these frameworks have shown us that the utility of a framework is not purely based on biological significance, but also on data availability. In other words, a framework with high biological significance that proposes groups of PCB congeners that are rarely detected in humans is less useful than one with less biologic significance which proposes groups of individual PCBs that are commonly detected.

We explored grouping frameworks based on biochemical characteristics (degree of chlorination), toxicological characteristics (cytochrome P450 enzyme induction activity), and statistical characteristics (factor loadings). In addition, we applied to our data the frameworks proposed by McFarland & Clarke (1), based on environmental significance, and by Wolff et al. (20), designed for epidemiological studies.

MATERIALS AND METHODS

The study sample was a subset of the control group of the Western New York breast cancer study. A more detailed description of the study population was published elsewhere (21). Briefly, the sample included 192 healthy female residents of Erie and

Niagara counties in western New York, who agreed to provide a fasting blood sample and were subsequently selected for toxicological analyses. All participants were Caucasian and ranged in age between 45-81 years.

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. (22). 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.

RESULTS

PCB congeners, which were detected in this study population, identified by IUPAC number and summarized by isomer group, are shown in Table 1. No mono- or deca-biphenyls were detected in the sera of our participants, due to 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.

Using biochemical characteristics to group the congeners resulted in three groups based on the degree of chlorination of the individual PCBs. The lower chlorinated PCB congener group 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 2,

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.

The second approach involved grouping PCB congeners with respect to their cytochrome P-450 enzyme induction properties, that is by phenobarbital-type (PB-type), 3-methylchlolanthrene-type (3-MC-type), or mixed-type induction activity (Table 2). PCBs with 3-MC-type induction activity are of particular interest because they have been associated with the highest toxicological potential (1). 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 one measurement.

The third approach involved the use of factor analysis to generate uncorrelated groups of PCB congeners (Table 3). In order to avoid distortion of results, 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, regardless of direction ($\pm$). 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, and all the underlying assumptions of this statistical technique were met, these five groups of congeners do not appear to follow any

meaningful pattern, such as Arochlor exposure profile, degree of chlorination, enzyme induction, carcinogenic or estrogenic activity.

The fourth approach is based on the environmental significance of the PCBs considering potential toxicity, frequency of occurrence, and abundance in animal tissue (1). 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 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. All participants had detectable levels for congeners in groups 1b, 2, and 3, but only a small proportion for group 1a, which is identical to the 3-MC congener group, discussed above. None of the PCBs in group 4 were detected in our participants, which was not surprising, since this group was characterized by congeners with few environmental occurrences.

The final framework in this evaluation was recently proposed by Wolff and her colleagues (20) and is based on major PCB peaks occurring in house dust or human samples. Briefly, in this framework, group 1 consists of potentially estrogenic PCBs, with non-persistent, estrogenic PCBs with weak PB-type induction activity in group 1a and persistent PCBs with weak PB-type induction activity in group 1b. 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 4). 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, by dividing study samples into groups of participants with and without detectable levels.

DISCUSSION

In summary, the most practical approach in these data for reducing a large number of PCB congeners into meaningful analytic units appeared to be grouping by degree of chlorination. A large proportion of participants had detectable levels for all proposed groups. However, 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). Factor analysis was difficult to interpret in these data, resulting in uncorrelated factors that could provide insight about the patterns of exposure and retention of PCBs in the study population. 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.

The grouping approach formulated by Wolff et al. (20) was a refined and applicable alternative to 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.

There are several issues that need to be addressed in the context of evaluating grouping frameworks for PCB congeners. First, we need to point out that measured PCB levels were 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 (23), as well as some consideration that pregnancy itself may affect accumulation of these compounds (24). All of these characteristics of this study population were likely to have had an impact on the observed utility of the grouping frameworks. I other words, the framework that were found to be useful in our population may be of little utility when applied to study populations with different characteristics.

Another issue involves the notion that grouping of PCB congeners requires consideration of biologic significance as well as data availability. 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.

Lack of generalizibility of a framework to a variety of health outcomes is also a problem in the development of grouping frameworks. Grouping PCBs with respect to endocrine activity may be useful for health outcomes that are hormone dependent but not for those that are not. 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.

Furthermore, the uniform utilization of PCB congener frameworks is complicated by differences in laboratory methods, resulting in differences in availability of the number of PCB congeners. For instance, among the seven studies currently underway to examine the association between organochlorine exposure and breast cancer risk, the number of PCB peaks available for analysis ranges from 9 to 56 (25). Thus, even if frameworks exist that are applicable over a range of health outcomes, they may not be useful for studies with a limited number of PCB congeners available for analysis.

Finally, it is important to point out that this research was intended to explore various approaches in grouping PCB congeners, and subsequently continue the discussion on this essential issue. The ultimate goal of this discussion should be the comparability of epidemiological findings across a number of studies. There is some effort among toxicologists who provide PCB measures for epidemiologic studies, to engage in proficiency programs, to ensure comparability of the biological measurements across studies (25). 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

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

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

3. McKinney JD & Waller CL. Polychlorinated biphenyls as hormonally active structural analogues. Environmental Health Perspectives 1994; 102:290-297.

4. Safe SH. Polychlorinated biphenyls, dibenzo-p-dioxins, dibenzufurans, and related compounds: environmental mechanistic considerations which support the development of toxicology equivalent factors. Critical Reviews in Toxicology 1990;21:51-88.

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

6. Safe SH. Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. Environmental Health Perspectives 1993;100:259-268.

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

8. Battershill JM. Review of the safety assessment of polychlorinated biphenyls (PCBs) with particular reference to reproductive toxicity. Human and Experimental Toxicology 1994;13:581-597.

9. Kimbrough RD. Polychlorinated biphenyls (PCBs) and human health: an update. Critical Reviews in Toxicology 1995;25:133-163.

10. Sauer PJJ, Huisman M, Koopman-Esseboom C, Morse DC, Smits-van Prooije AE, van de Berg KJ, et al. Human and Experimental Toxicology 1994; 13:900-6.

11. Huisman M, Koopman-Esseboom C, Flieder V, Hadder-Algra M, van der Paauw CG, Tuinstra LGM, et al. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neurological development. Early Human Development 1995;41:111-127.

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

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

14. Jacobson JL & Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. New England Journal of Medicine 1996;335:783-789.

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

16. Fein GG, Schwartz PM, Jacobson SW, Jacobson JL. Environmental toxins and behavioral development. American Psychologist 1983;1188-1197.

17. Silberhorn EM, Glauert HP, Robertson LW. Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. Critical Reviews in Toxicology 1990;20:440-496.

18. Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Critical Reviews in Toxicology 1994;24:87-149.

19. Rothman KJ. Fundamentals of epidemiologic data analysis. In:Modern Epidemiology. Boston:Little, Brown and Company, 1986;131-151.

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

21. Graham S, Hellmann R, Marshall JR et al. Nutritional epidemiology of postmenopausal breast cancer in western New York. American Journal of Epidemiology 1991; 134:552-66.

22. 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).

23. 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.

24. 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.

25. Timing of environmental exposures in breast cancer and northeast/mid-Atlantic breast cancer programs, NIESH, 25-26 September 1996.

Table 1

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, #64, #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 2.

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

Congener Group	PCB Congeners (IUPAC #s)	Percent with Detectable Levels
Degree of Chlorination		
Lower Chlorinated Congeners	#18, #19, #22, #15+17, #16+32, #25+50, #31+28, #33, #40, #45, #44, #49, #52, #55, #60, #64, #70, #42+59, #47+48, #66+95, #77+110	68
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
Higher Chlorinated Congeners	#194, #195, #200, #203+196, #205, #206	100
Cytochrome P450 Induction Activity		
PB-type	#15+17, #52, #66+95, #87, #99, #101, #136, #82+151, #153, #180, #183, #194, #195, #203+196, #205, #206	100
3-MC-type	#77+110	12
Mixed-type	#138, #118, #128, #156+171	100
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

Table 3

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	.82981
	# 180	.77283
	# 187	.70407
	# 194	.69883
	# 206	.62273
	# 156+171	.48505
	# 172	.45829
	# 195	.44947
Factor 2		
% Variance=7.8	# 118	.84253
	# 105+132	.83215
	# 138	.74527
	# 147	.72743
	# 153	.66349
	# 188	.58972
Factor 3		
% Variance=7.6	# 7+9	.74536
	# 87	.69466
	# 134	.62540
	# 6	.48076
	# 47+48	.46713
	# 177	.45644
Factor 4		
% Variance=6.6	# 22	.75545
	# 31+28	.57676
	# 101	.54841
	# 66+95	.52922
	# 141+179	.51153
	# 128	.46491
	#174+181	.42415
	# 129	.39163
Factor 5		
% Variance=5.5	# 176	.86534
	# 200	.74887
	# 77+110	.49235
	# 183	.37884
	# 205	.35862

Table 4

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*
	Available for Analysis	Not Available for Analysis	
McFarland and Clarke (1989)			
Group 1 A	#77+110	#126, #169	12
Group 1 B	#118, #128, #138, #156+171	#105, #170	100
Group 2	#87, #99, #101, #153+132, #180, #183, #194		100
Group 3	#18, #49, #52, #70, #151+82, #177, #187	#44, #74, #201	100
Group 4		#37, #81, #114, #119, #123, #157, #158, #168, #189	0
Wolff et al. (1997)			
Group 1 A	#44, #52, #31+28, #70		40
Group 1 B	#101, #187, #174, #177	#201	98
Group 2 A	#66+95, #77+110, #105+132, #118, #156+171	#74, #126, #169, #167	98
Group 2 B	#128, #138	#170	100
Group 3	#99, #153, #180, #203+196, #183		100

OUTLINE FOR FUTURE ANALYSES

The final part of this postdoctoral research will involve an examination of the association between organochlorine exposure on breast cancer risk among subgroups of women with different capacities to metabolize and detoxify environmental carcinogens. This effort will be conducted in collaboration with Dr. Christine Ambrosone from the National Center for Toxicological research and Dr. Peter Shields from the National Cancer Institute. In addition, we will closely work with Dr. James Olson from the Department of Toxicology at the State University of New York at Buffalo. Genetic information in this study population is available for several polymorphic genes: *N-acetyltransferase 1 (NAT1), N-acetyltransferase 2 (NAT2) Glutathione S-Transferase* μ *(GSTμ), Cytochrome P450 1A1 (CYP1A1), Cytochrome P450 1A2 (CYP1A2),* and *Cytochrome P450 2D6 (CYP2D6).*

In addition, we will attempt to examine whether the effect of organochlorine exposure on breast cancer risk is different for estrogen receptor positive and estrogen receptor negative breast cancer. Unfortunately, at this point we were only able to ascertain the estrogen receptor status of about 50 percent of our case group, which may not allow for a detailed examination of this risk association.