ABSTRACT

Organochlorine pesticides persist in the environment and bioaccumulate in adipose tissues. Because of their suspected estrogenic action, organochlorines may increase breast cancer risk, specifically in postmenopausal women with low endogenous estrogen levels. We examined plasma hexachlorobenzene (HCB) and trans-nonachlor (chloradane) levels in relation to breast cancer risk in a population-based, case control study of African American women (292 cases, 270 controls) and white women (456 cases, 389 controls) in North Carolina. Mean and median levels of HCB and trans-nonachlor were similar in African American and white women. We did not observe case-control differences in plasma levels of these compounds in premenopausal women. However, among postmenopausal African American and white women, the mean level of lipid-adjusted HCB was 0.02μg/g (SD 0.05) in cases and 0.01μg/g (SD 0.01) in controls (P = 0.03). There was no difference in mean levels of trans-nonachlor in post menopausal cases (0.08μg/g, SD 0.08) versus controls (0.08μg/g, SD0.08) (P=0.32). In postmenopausal women, the adjusted odds ratio for breast cancer comparing the highest to lowest third of HCB was 1.6 (95% Confidence Interval 1.1-2.5) (Trend test P = 0.02). The corresponding odds ration for Trans-nonachlor in postmenopausal women was 1.2 (95% CI 0.8-1.9) (Trend test P=0.36) No differences in natural age at menopause was found for HCB and trans-nonachlor between cases and controls. No significant increases in cancer risk were found in relation to HCB and trans-nonachlor exposure and estrogen receptor status in cases. Our research demonstrates a relationship between hexachlorobenzene exposure and breast cancer in postmenopausal women, but does not provide evidence for this association in pre-menopausal women.

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INTRODUCTION

The purpose of this report is to investigate the relationship of environmental organochlorine exposure to the development of breast cancer. Little definitive information is available regarding the potential long term hazards of these chemicals. We investigated blood serum residues of organochlorines, specifically hexachlorobenzene and trans-nonachlor, in the Carolina Breast Cancer Study, a population based case-control study covering 24 rural counties in North Carolina.

In the year 2003, an estimated 211,300 new cases of invasive breast cancer were identified in the United States, accounting for 32% of new cancer cases in women. North Carolina alone accounted for approximately 6,000 of these new cases. According to the American Cancer Society, death rates for breast cancer in North Carolina from 1995 to 1999 were 28.7 women per 100,000, slightly below the national average of 28.8 (American Cancer Society, 2003). Because of the high incidence and mortality of this disease, it is an important public health concern and warrants research to investigate its development and potential causes.

Family history can only account for approximately 20% of breast cancer cases, and only 7% can truly be linked to mutations in major genes such as BRCA1 and BRCA2 (Miller, 1996). The causation and influences affecting the development of the remaining breast cancer cases are hotly debated topics. Environmental exposures, particularly organochlorine pesticides, have been investigated extensively as risk factors for breast cancer, with conflicting evidence linking exposure to breast cancer risk.

Organochlorines as a chemical group are relatively stable in the terrestrial environment, highly lipophilic, and are sequestered in the body's adipose tissue. Organochlorines have estrogenic properties and may affect the development of breast cancer. Although estrogen is an endogenous chemical, its influence in menopause has

been linked to an increase in risk for breast cancer. Women with natural menopause later in life have an increased risk for breast cancer while early onset of menopause is protective against breast cancer (Miller, 1996). Secondly, hormones may influence the development of cancer; specifically, estrogen is influential in tumor growth. Furthermore, estrogen also can act as a cell proliferation agent (Miller, 1996). Because of estrogen's predicted influence in the development of breast cancer, premenopausal and postmenopausal women may face different risks when considering organochlorine exposure.

Epidemiologist set out to study organochlorine's influence in the development of breast cancer with differing results. A large scale study showed an increase in breast cancer risk in relation to organochlorine exposure, specifically DDE [1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene] and PCBs[polychlorinated biphenyls] (Wolf *et al*, 1993). Another study of white, black and Asian women in the San Francisco area, detected no evidence that exposure to DDE and PCBs increased the risk of cancer although higher levels of organochlorines were found in Asian and black women (Kreiger *et al*, 1994). In response to the conflicting results of studies concerning exposure, five large epidemiological studies from the northeastern United States were combined to investigate the influence of organochlorines on breast cancer incidence. In summarizing these results, Laden *et al* found no association of breast cancer with DDE or PCBs in the combined data. (Laden *et al*, 2001).

Numerous epidemiologic studies concerning organochlorine exposure and breast cancer have provided conflicting outcomes regarding the risk of breast cancer in relation to organochlorine exposure. Therefore, we investigated the exposure levels of two organochlorines, hexachlorobenzene and trans-nonachlor, and their association with increased breast cancer risk in pre- and postmenopausal African American and white

women. We hypothesized that postmenopausal women may be at a greater risk than premenopausal women from exposure to weakly estrogenic compounds due to their relatively low levels of endogenous estrogen.

HEXACHLOROBENZENE

Hexachlorobenzene (HCB) is a synthetic substance used widely as a pesticide until 1965. Internationally, it was used as a fungicide for seed dressings until the late 1970's (IPCS, 1997). Possible large scale HCB exposure sources to third world populations resulted from HCB use as well as other chlorinated pesticides containing small amounts of HCB as an impurity (Bailey *et al*, 2001). HCB was last sold in the United States as a final product in 1984 (ATSDR, 2003). Currently, HCB is created through the production of pyrotechnics, synthetic rubber, and as byproducts of industrial manufacturing of chemicals such as carbon tetrachloride, perchloroethylene, trichloroethylene and chlorinated benzenes. Additionally, municipal incineration processes form HCB as an impurity (ATSDR, 2003).

HCB is a very simple and stable molecule, consisting of one benzene ring bonded to six chlorine molecules (Figure 1). HCB, because of its chemical stability, is one of the most persistent organochlorine environmental pollutants. Half life of the chemical varies depending on its location. Ambient air half life for a temporal region similar to North Carolina is 1.94 years. HCB's half life in surface water is estimated to be between 2.7 to 5.7 years and 5.3-11.4 years in ground water. Half life values in soil range from 3 to 6 years (ATSDR, 2003).

Human exposure levels to hexachlorobenzene vary by geographical region internationally. It is estimated that daily intake of HCB ranges between 0.0004 and 0.003 μg/kg body weight (IPCS, 1997). The ATSDR estimates an annual average intake of HCB by all routes of exposure for adults in the United States at 68 μg (ATSDR, 2003).

"Levels of HCB in human adipose tissue from around the world are generally less than 1 mg/kg" (IPCS, 1997). Bioaccumulation of HCB occurs in lipid rich tissues such as the breasts and ovaries and has been detected in blood and breast milk (ATSDR, 2003).

Biotransformation and metabolism of HCB occur slowly because of its chemical stability and ability to cross lipid membranes and be stored in fatty tissues. A majority of ingested HCB is excreted unchanged in fecal material (ATSDR, 2003). The hepatic cytochrome P450 enzymes CYP3A1, CYP3A2 and CYP3A4 are responsible for the metabolism of HCB to pentachlorophenol (PCP). HCB can be conjugated with glutathione to yield pentachlorothiophenol (PCBT). Pentachlorobenzene, a minor metabolite, is produced by reductive dechlorination. Consequently, according to the IPCS, the major metabolic products of HCB found are pentachlorophenol, pentachlorothiophenol and a secondary metabolite of pentachlorophenol, tetra-chlorohydroquinone (IPCS, 1998). In 1997, Figueras *et al*, studied a population of 100 human subjects exposed to high airborne levels of HCB in Flix, Spain. This study provided insight into the metabolism of HCB. Figure 2 shows the representative metabolic pathway of HCB deduced from this study. Although the population studied had elevated HCB levels in blood serum samples in comparison to other European populations, an increase was not shown in HCB metabolites.

Hexachlorobenzene has shown effects as a reproductive toxin in animal models. Alvarez *et al* found that "long term HCB administration induced alterations in female Wistar rat oestrus cycle characteristics, resulting in irregularity of cycles, characterized mainly as prolonged periods of oestrus" (Alvarez *et al*, 2000).

Carcinogenicity

The EPA's Integrated Risk Information System lists HCB as a possible human carcinogen (Group 2B) based on animal data and limited human exposure information.

Sufficient data exist to classify HCB as an animal carcinogen; the main effects occur in the liver, with cancers of the kidney, thyroid, parathyroid and adrenal gland also reported (ATSDR, 2003). Results of carcinogenicity vary according to species. Clabral *et al* found increases in the incidence of hepatomas in Syrian golden hamsters fed doses of HCB over a lifetime (Clabral *et al*, 1977). In another study, female Argus rats fed HCB for 90 weeks developed 100% liver tumors compared to 0% in controls. Wistar rats in the same study developed statistically significant levels of liver tumors in comparison to controls (Smith *et al*, 1980). An additional study found significant increases in multiple forms of liver cancers in rats being fed HCB for up to two years. Female rats appeared to be more susceptible than males (Erturk *et al*, 1986).

Animal studies investigating direct effects of HCB exposure suggest a non-genotoxic carcinogenic mechanism of action, thus acting as a promoter for cancer development. Regardless of the species model, most studies investigating HCB exposure showed little DNA binding capacity or mutagenic capacity (ATSDR, 2003). Canonero *et al* provided conflicting evidence and concluded that HCB should be considered a weak genotoxic carcinogen following observation that HCB forms micronuclei and fragments DNA in human and rat hepatocytes (Canonero *et al*, 1997).

Specific short term human HCB exposure data was assessed by two follow up studies in response to ingestion of grain containing high levels of HCB in Turkey from 1955-1961. No increase in incidence of cancer was noted from the Cripps *et al* or Peters *et al* follow-up studies (Cripps *et al*, 1984) (Peters *et al*, 1982). It is noted that these studies were designed to investigate acute HCB toxicity and not increases in the incidence of cancer in these populations (ATSDR, 2003).

Although little strong evidence associating HCB exposure to genotoxic damage exists, there is ample evidence that its major primary metabolite, pentachlorophenol

(PCP), does inflict genotoxic damage through its quinoid derivatives; tetrachloro-1,4-benzoquinone(Cl_4BQ) and tetrachlorohydroquionone (Cl_4HQ). A study performed on calf thymus DNA showed that these two HCB metabolites induce direct and oxidative damage on DNA (Lin *et al*, 2001). The same research group found that HCB metabolites induced apurinic/apyrimidinic (AP) sites in human HeLa S3 tumor cells (Lin *et al*, 2001).

Epidemiologic studies investigating the association of breast cancer risk with levels of HCB have provided contradictory results. Dewailly *et al* found a statistically significant difference in HCB plasma concentrations in cases verses controls (Dewailly *et al*, 1994). More recently, researchers found a significant difference in serum HCB concentrations above a threshold value of 0.5ppb in women with breast cancer verses controls. Odds ratios for breast cancer were higher for women with HCB concentrations above 0.5ppb in comparison to controls (Charlier *et al*, 2002). Liliegren *et al* found a statistically significant case-control difference in HCB levels among pre and post menopausal women in cases versus controls. The highest odds ratios were found for postmenopausal cases with estrogen-receptor positive breast cancer (Liliegren *et al*, 1998). Contradictory to the results of these researchers, other studies have found no relation of increased levels of HCB and breast cancer (Dorgan *et al*, 1999; Myosich *et al*, 1998; Zheng *et al*, 1999).

CHLORDANE

Chlordane, also known as T-nonachlor, Octachlor and Velsicol, was first introduced as a pesticide in 1948 and discontinued for this use in 1978. By 1983 Chlordane was approved for termite extermination only, and finally discontinued in the United States in 1988 (ATSDR, 1994). Chlordane is still produced in the United States for export, but only in two facilities, one in Memphis, Tennessee and the other in Stennis, Mississippi (ATSDR, 1994). The main exposure to chlordane is through air within houses

treated with chlordane as a termicide, as it steadily vaporizes up to ten years after treatment. The EPA estimates that approximately 52 million people have been exposed to chlordane in their residences. United States populations are additionally exposed to chlordane through the soil, water and food sources contaminated when chlordane was used as an agricultural pesticide (ATSDR, 1994).

Chlordane is a viscous liquid mixture of over 140 chemicals, the major constituents being trans-chlordane, cis-chlordane, B-chlordene, heptachlor, and trans-nonachlor (Figures 3-7). Chlordane can persist in soil for over 20 years. Although average dermal exposures are difficult to establish, the 1991 FDA Total Diet Study estimates dietary chlordane intake as 0.0013 μg/kg/day for infants and 0.0005-0.0015 μg/kg/day for teenagers and adults (FDA 1991). Chlordane is very lipid soluble and can bioaccumulate throughout the food chain.

The metabolism of chlordane in humans is not well understood. Animal data provides the best understanding of the metabolic pathway (Figure 8). Metabolism of chlordane in rats results first in "dichlorochlordene via dehydrogenation followed by epoxidation to oxychlordene and subsequent hydroxylation (Route A). There was also direct hydroxylation of both the cis and trans-isomers to 1-exo-hydroxydihydrochlordene with excretion in the feces and urine as the glucuronide (Route B)" (Pesticide Residues, 1982). Trans- and cis- isomers of chlordane are metabolized at different rates with the cis- isomer being eliminated from the body faster. (INCHEM, 1982).

Research suggests that the various components of technical chlordane are metabolized at different rates, with some of these remaining in the body for extended periods of time. Hirasawa and Takizawa found that the cis and trans isomers of chlordane reached a threshold of accumulation and decreased over the course of their 29 day rat study. They noted that trans-nonachlor isomer was not readily metabolized and tended to

accumulate and persist in the body (Hirasawa *et al*, 1978). Trans-nonachlor has also been detected in mother's milk and tends to store in fat cells for extended periods of time. As with most chemicals investigated using animal models, there are species differences that affect the metabolism and action of a chemical. Tashiro *et al* found that trans-nonachlor was metabolized by rat liver microsomal preparations but not by human liver samples (Tashiro *et al*, 1978). The inability of human cells to metabolize trans-nonachlor has prompted researchers to look for the role of chronic exposure's influence in the development of cancer in humans.

Carcinogenesis

Chlordane is listed as by the EPA as a group 2B, possible human carcinogen (IRIS, 1998). This classification was reached by consideration of laboratory animal carcinogenicity data. Chlordane is suggested to act as a tumor promoting agent, due to inadequate evidence of genotoxicity. Some research provides evidence that chlordane may induce mutations while a majority of the data suggests non-genotoxic action (ATSDR, 1994). Numerous studies linked chlordane exposure to animal carcinogenesis. For example, Khasawinah and Grutsch found that five different strains of mice developed benign and malignant liver tumors after administration of technical chlordane (Khasawinah *et al*, 1998). Human exposure data are less conclusive. Cantor *et al's* case control study of non-Hodgkins lymphoma in Iowa found increased odds of disease associated with exposure to chlordane (Cantor *et al*, 1992). In contrast to theses results, one study of leukemia cases in Iowa and Minnesota found no association of cancer in relation to chlordane exposure (Brown *et al*, 1990). In another study of multiple myeloma cases found no association of cancer and chlordane exposure as well. (Brown *et al*, 1993)

Chronic exposure of chlordane and its constituents has been continually studied in relation to cancers related to hormone activity. Trans-nonachlor is considered to be very

persistent in the human body and is detected in blood serum and lipid rich tissues. As with other organochlorines, trans-nonachlor may have estrogen like activity in the human body. Klotz *et al* used a combination of assays to assess the estrogenic activity of a number of organochlorines. They concluded that trans-nonachlor has weak estrogenic activity (Klotz *et al*, 1996). Epidemiological studies have investigated the association of trans-nonachlor and various types of hormone influenced cancer. Recently, in a case-control study of 157 subjects, Ritchie *et al* did not find statistically significant differences in levels of trans-nonachlor between prostate cancer cases and controls. Odds ratios were also non-significant (Richie *et al*, 2003). Gammon *et al* used a combination of trans-nonachlor and oxychlordane residues to evaluate the association of chlordane exposure and breast cancer. No association of breast cancer and chlordane exposure was found. Furthermore, no association was found when the data was stratified by menopausal status or estrogen receptor status (Gammon *et al*, 2002). Several other recent epidemiological studies found no increase in risk of breast cancer in relation to trans-nonachlor levels (Demers *et al*, 2000; Ward *et al*, 2000; Zheng *et al*, 2000). Despite these studies, question still exists about the influence of this chemical on breast cancer.

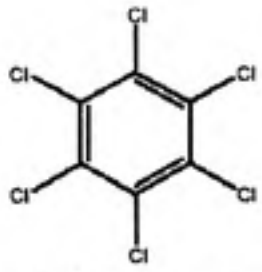

Figure 1. Hexachlorobenzene Structure

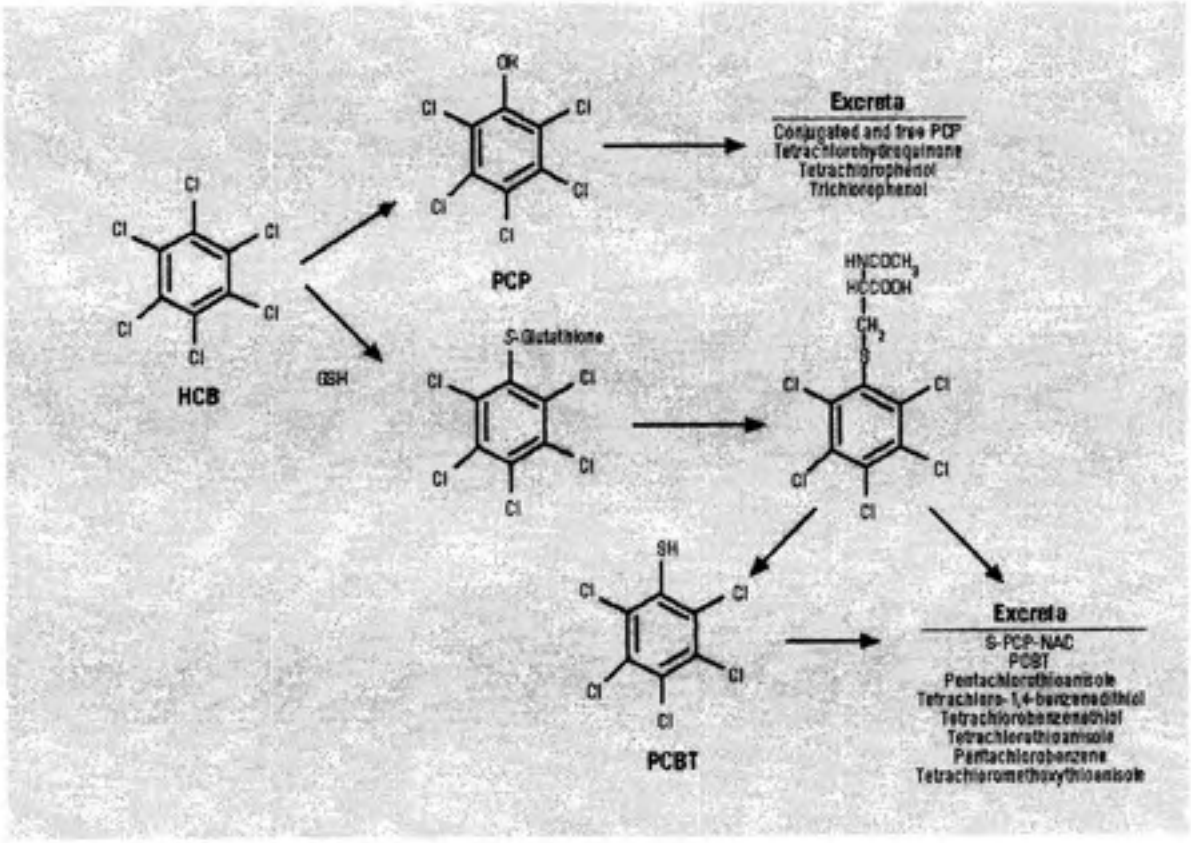

Figure 2. Metabolic Pathway of HCB (To-Figueras *et al,* 1997)

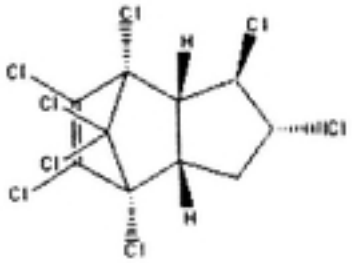

Figure 3. Trans-Chlordane

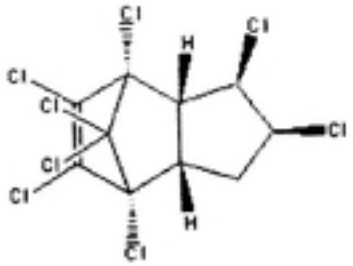

Figure 4. Cis-Chlordane

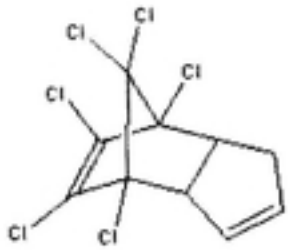

Figure 5. B-Chlordene

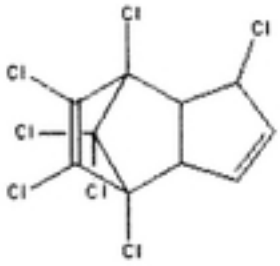

Figure 6. Heptachlor

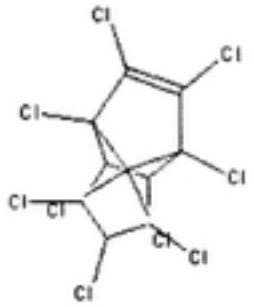

Figure 7. Trans-nonachlor Chemical Structure

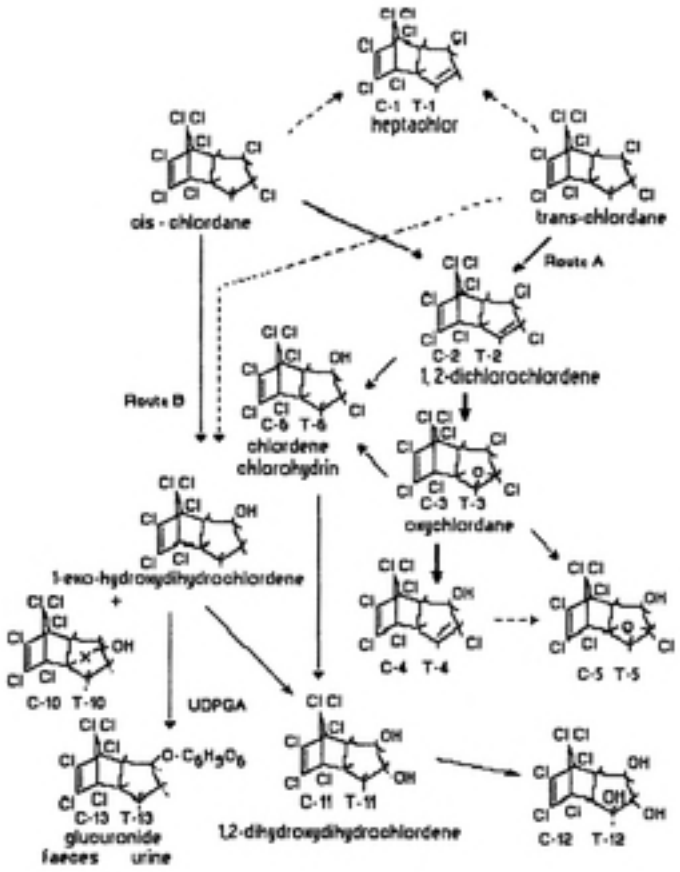

Figure 8. Proposed metabolic pathway of Chlordane (Inchem, 1982)

MATERIALS AND METHODS

Study Population

The study methods of the Carolina Breast Cancer study have been previously described (Newman, 1995). In brief, the Carolina Breast Cancer Study (CBCS) was designed to investigate the causes and risk factors associated with breast cancer in white and African American women in the eastern region of North Carolina. The CBCS is a population-based case-control study representing 24 rural North Carolina counties (Figure 9).

Cases were identified through a rapid case ascertainment system implemented through the North Carolina Central Cancer registry. Women between the ages of 24 and 74, living in the 24 rural counties and diagnosed with invasive breast cancer for the first time between May 1, 1993 and September 30, 1995 were eligible as cases for the study (Newman, 1995). Subgroups of African American women were over sampled in comparison to white women by design to ensure equal study participation.

Controls were selected using two sources, a list from the North Carolina Division of Motor Vehicles for women under the age of 65 and a list from the Health Care Financing Administration for women between the ages of 65-74. Potential comparison subjects were randomly selected from these lists. Controls were matched to cases by race and five-year age-group.

Overall response rate for the study was 74.4% for cases and 52.8% for controls (Moorman, 1999). Trained nurses took body measurements and conducted an interview of each participant. Body measurements obtained were height, weight, and waist and hip girths. Interview questions consisted of smoking status and other known risk factors for

breast cancer, including family history of breast cancer, hormone use, alcohol consumption, reproductive and menstrual history, occupational exposures, and socio-demographic characteristics (Newman, 1995). Race was classified by self-report. For this analysis, women were classified as either African American or white. The subgroup of white women included three Asian women, seven American Indian women, and three women who described themselves as "multiracial" (Millikan, 2000). Study participants reported age at last menstrual cycle, determining menopausal status and age at menopause. Thirty six participants were labeled pre-menopausal due to not meeting the postmenopausal criteria of twelve months from last menstrual cycle. Twenty eight women were excluded from menopausal classification due to hormone use or incomplete data (Cooper, 2002). Medical records were used to obtain information for cases on stage at cancer diagnosis, estrogen receptor status, and treatment procedures for cases.

Blood samples of 10mL were also taken from each participant. Of the women interviewed, 98% provided a blood sample for analysis. Each blood sample was collected in acid citrate dextrose-anticoagulated tubes, centrifuged at 700-900 X g (2600 rpm) at –70°C. Each sample was processed within 48 hours of collection (Millikan, 2000).

Laboratory Methods

Blood plasma levels were used to estimate total body burden of HCB and trans-nonachlor. Analysis of plasma levels of organochlorine compounds was performed at the Research Triangle Institute (Research Triangle Park, NC). Laboratory methods have been previously published for PCB congeners and DDE (Millikan, 2000). Plasma samples of 2mL were treated with 1.0mL of methanol and spiked with standards of PCB 198 and

o,p'-DDT. The samples were then extracted sequentially with three 2.5 mL portions of 1:1 hexane-diethyl ether. Rotary mixing for 15 minutes at 60 rpm followed by centrifugation for 15 minutes at 1800 rpm was used to extract the plasma samples. Combined extracts were fractionated using Florisil (R) open-column chromatography. The initial fraction was eluted with 35mL of hexane and contained HCB, trans-nonachlor and the standard organochlorines. This fraction was concentrated and spiked with an external quantitation standard of octachloronaphthalene. All extracts were analyzed by gas chromatography/electron capture detection with a DB-5 column and Hewlett-Packard Model 6890 instrument. Chromatographic retention times relative to internal standards and pattern recognition were used to identify individual compounds in the sample extract.

Quantitation limits were set at 0.125 ng/g for both HCB and trans-nonachlor. Detection limits for both compounds were 0.0625 ng/g. The quantification limit was based upon the lowest point of the calibration curve, defined as the level of the lowest calibration standard that had a calculated value within 20% of its known value and a signal to noise ratio of 10:1. The lowest standard had to be within 30% of its known value.

Plasma lipid levels were determined at the Core Laboratory for Clinical Studies at Washington University School of Medicine (St. Louis, MO). Automated enzymatic assays using cholesterol esterase and lipoprotein lipase were performed on a Technicon RA-1000 analyzer. Replicate samples were included with each shipment to determine coefficients of variation (3.4% for net triglycerides and 2.1% for total cholesterol) (Millikan, 2000).

Organochlorine and lipid values were available for 748 (84%) cases and 659 (78%) controls. Insufficient plasma levels as well as interference in the sample were reasons for failure in obtaining results.

Statistical Methods

Variables for HCB and trans-nonachlor were computed using the same methods. Raw values for both pesticides were analyzed. Values less than the detection limit of 0.0625 g/mL were imputed by dividing the detection limit by the square root of 2. Log imputed HCB and trans-nonachlor values were obtained by taking the natural log of the imputed value. Imputed pesticide levels divided by total lipids provided the lipid adjusted values. Log lipid adjusted values were calculated by taking the natural log of the lipid adjusted value of each pesticide. Distributions of HCB and trans-nonachlor were skewed, thus median levels of each pesticide were compared using a Wilcoxon rank sum test for unpaired data. The association between plasma levels of HCB and trans-nonachlor and age at natural menopause in postmenopausal women was determined using ANOVA and Pearson Correlation Coefficients.

Odds ratios for breast cancer were calculated after categorizing participants into tertiles based on the distribution of controls. ORs for breast cancer and 95% confidence intervals were calculated using unconditional logistic regression. Confounders were adjusted for using PROC GENMOD of the SAS software (version 8.2; SAS Institute Cary, NC). The entire study population was adjusted for confounders for the following characteristics: age, age squared, race (white and African American), menopausal status (pre- and postmenopausal), body mass index (≤ 24.9 kg/m^2, 25-29.9, and ≥ 30.0),

parity/lactation (nulliparous, parous never breastfed, and parous ever breastfed), hormone replacement therapy (ever and never), and income (<$15,000, $15,000 to <$30,000, $ 30,000 to <$50,000, and ≥ $50,000 per year).

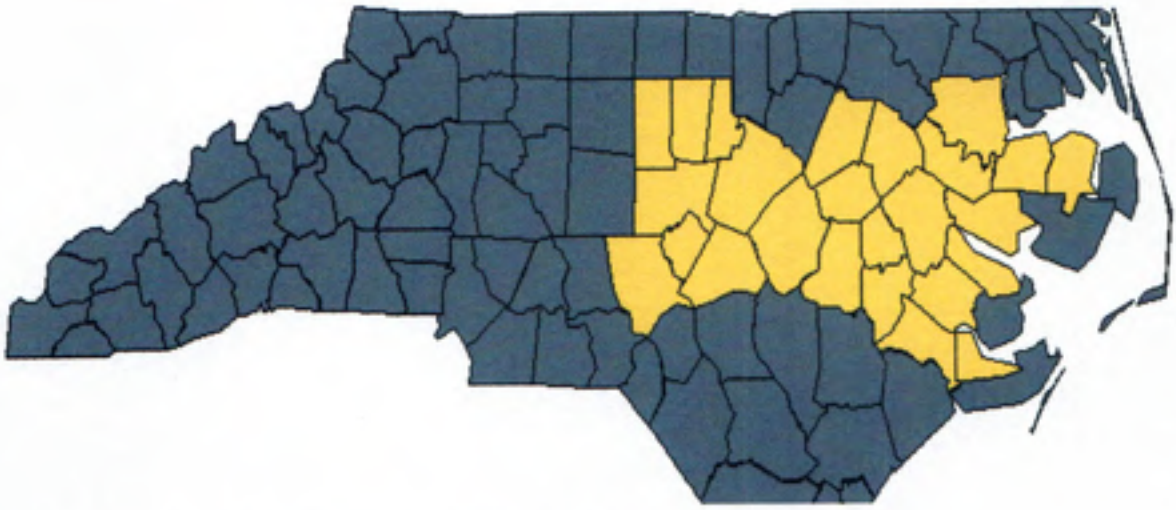

Figure 9. Location of study participants (Yellow Counties)

RESULTS

Characteristics of Carolina Breast Cancer Study participants have been presented previously (Millikan, 1998). Organochlorine data was collected for all of Phase I of the Carolina Breast Cancer study (1993-1996). The analyzed population consisted of 292 African American cases, 270 African American controls, 456 white cases, and 389 white controls. The mean age of study cases and controls respectively was 50.2 years with a range of 23-74 and 51.5 years with a range of 21-74. Premenopausal women consisted of 51.1% (n=382) of the cases and postmenopausal were 48.9 % (n=366). Controls were 46.4 % (n=306) premenopausal and 53.6 % (n=353) postmenopausal.

Data on mean, median and range of plasma HCB and trans-nonachlor levels in African Americans and whites are presented in Table 1. There were no statistically significant differences in lipid adjusted plasma levels of Hexachlorobenzene and trans-nonachlor when stratified by race. Among African American subjects, cases had a mean lipid adjusted HCB blood plasma levels of 0.02 μg/g and controls had 0.01μg/g. Both African American cases and controls had a mean lipid adjusted trans-nonachlor blood plasma level of 0.07 μg/g. Whites had similar lipid adjusted HCB levels of .02μg/g and .01μg/g for cases and controls respectively. White controls had lipid adjusted trans-nonachlor levels (mean=0.06 μg/g) that were slightly lower than African American levels (mean=0.07 μg/g).

Results of plasma concentrations of HCB and trans-nonachlor stratified by menopausal status are presented in Table 2. We found no differences in mean lipid adjusted plasma levels of both pesticide residues in premenopausal women. Mean lipid adjusted HCB levels were 0.01 μg/g for both cases and controls although there was a greater range of values for cases. Lipid adjusted trans-nonachlor plasma levels were 0.04

μg/g for both cases and controls. There was a statistically significant difference in median plasma levels of lipid adjusted Hexachlorobenzene in postmenopausal women. Cases had a mean level of 0.02 μg/g with a range of 0.0 to 0.83 μg/g. while controls had a mean level of 0.01 μg/g with a range of 0.01 to 0.07 μg/g.

Lipid adjusted pesticide residue levels were divided into tertiles from lowest level of exposure to greatest based upon distributions in controls. Odds ratios for breast cancer according to tertiles of HCB and trans-nonachlor are presented in Table 3. For the study population as a whole, there was no difference in risk of breast cancer for either of the two upper tertiles versus the lowest exposure for either HCB or trans-nonachlor. When the data was stratified by race, African American women had a slight increase in the adjusted odds ratio (ORs) for the middle third for HCB [1.6 (95% CI=1.0-2.5)]. No dose response gradient was seen in African American women as the highest third of exposure of HCB had an OR of 1.3 (95% CI=0.8-2.0). There was no difference in risk in African American women for trans-nonachlor exposure. Additionally, no increase in risk was seen for white women exposed to both HCB and trans-nonachlor.

ORs for tertiles of hexachlorobenzene and trans-nonachlor, stratified by menopausal status, are presented in Table 4. There were no increases in ORs for premenopausal women for HCB. A slight, statistically nonsignificant increase in risk was seen in trans-nonachlor for the second third of premenopausal women. The adjusted OR for the second tertile was 1.4 (95% CI=1.0-2.1) but the highest tertile had a lower odds ratio of 1.1 (95% CI=0.7-1.6). Thus, no dose response relationship was found. For postmenopausal women, a significant increase of risk was found for both the second and third tertiles of HCB exposure. Adjusted odds ratio were 1.7 (95% CI=1.1-2.5) and 1.6 (95% CI= 1.1-2.5) for the middle and highest exposure levels respectively. No increases

in risk were found in postmenopausal women for any of the three levels of trans-nonachlor exposure.

We looked at age a natural menopause in relation to organochlorine levels. There was no association between age at menopause and HCB in cases ($p=0.38$) or controls ($p=0.64$). There was also no association between age at menopause and trans-nonachlor levels in cases ($p=0.58$) or controls ($p=0.48$).

Plasma organochlorine levels were also stratified by estrogen receptor (ER+ or ER-) status in breast cancer cases and presented in Table 5. Plasma levels of HCB were slightly higher in ER+ cases than ER- cases but medians were not significantly different ($p=0.20$). Trans-nonachlor levels were significantly higher in ER+ verse ER- cases ($P=0.04$). Odds ratios did not differ for ER+ and ER- breast cancer cases vs. controls. (data not shown).

Table 1.
Plasma levels of hexachlorobenzene and t-nonachlor among African American and white cases and controls in the CBCS (1993-1996)

	Cases Mean (SD)	Median	Range	**Controls** Mean (SD)	Median	Range	P-value[a]
African Americans	N = 292			N = 270			
HCB[b]	0.05 (0.08)	0	0 – 0.96	0.04 (0.06)	0	0 – 0.29	0.98
Lipid-adjusted HCB[c]	0.02 (0.01)	0.01	0 – 0.19	0.01 (0.01)	0.01	0.01-0.07	0.59
T-nonachlor[d]	0.36 (0.30)	0.26	0 – 1.73	0.35 (0.44)	0.26	0 – 5.39	0.57
Lipid-adjusted T-nonachlor[e]	0.07 (0.06)	0.05	0 – 0.37	0.07 (0.08)	0.05	0.01-1.1	0.36
Whites	N = 456			N = 389			
HCB[b]	0.05 (0.15)	0	0 – 2.79	0.04 (0.06)	0	0 – 0.30	0.65
Lipid-adjusted HCB[c]	0.02 (0.04)	0.01	0-0.83	0.01 (0.01)	0.01	0 – 0.05	0.60
T-nonachlor[d]	0.30 (0.34)	0.21	0 – 3.72	0.31 (0.33)	0.23	0 – 4.5	0.29
Lipid-adjusted T-nonachlor[e]	0.06 (0.05)	0.04	0.01 – 0.50	0.06 (0.05)	0.04	0.01-0.57	0.48

[a]Wilcoxon rank sum test comparing medians in cases and controls.
[b]Hexachlorobenzene in parts per billion (nanograms/ml), without imputation or lipid adjustment.
[c]Hexachlorobenzene adjusted for lipids (micrograms/g), with imputation.
[d]T-nonachlor (chlordane) in parts per billion (nanograms/ml), without imputation or lipid adjustment.
[e]T-nonachlor (chlordane) adjusted for lipids (micrograms/g), with imputation.

Table 2.
Plasma levels of hexachlorobenzene and t-nonachlor among cases and controls, stratified by menopausal status.

	Cases Mean (SD)	Median	Range	**Controls** Mean (SD)	Median	Range	**P-value**[a]
Premenopausal	N = 382			N = 306			
HCB[b]	0.03 (0.07)	0	0 – 0.91	0.03 (0.05)	0	0 – 0.20	0.20
Lipid-adjusted HCB[c]	0.01 (0.01)	0.01	0 – 0.20	0.01 (0.01)	0.01	0.01 – 0.04	0.24
T-nonachlor[d]	0.21 (0.22)	0.16	0 – 1.71	0.21 (0.20)	0.16	0 – 1.29	0.92
Lipid-adjusted T-nonachlor[e]	0.04 (0.04)	0.03	0 – 0.50	0.04 (0.03)	0.04	0.01 – 0.28	0.97
Postmenopausal	N = 366			N = 353			
HCB[b]	0.07 (0.17)	0.07	0 – 2.79	0.05 (0.07)	0	0 – 0.30	0.23
Lipid-adjusted HCB[c]	0.02 (0.05)	0.01	0 - 0.83	0.01 (0.01)	0.01	0.01-0.07	0.03
T-nonachlor[d]	0.44 (0.38)	0.33	0 – 3.72	0.42 (0.46)	0.34	0 – 5.39	0.70
Lipid-adjusted T-nonachlor[e]	0.08 (0.06)	0.06	0 – 0.35	0.08 (0.08)	0.06	0 – 1.1	0.32

[a]Wilcoxon rank sum test comparing medians in cases and controls.
[b]Hexachlorobenzene in parts per billion (nanograms/ml), without imputation or lipid adjustment.
[c]Hexachlorobenzene adjusted for lipids (micrograms/g), with imputation.
[d]T-nonachlor (chlordane) in parts per billion (nanograms/ml), without imputation or lipid adjustment.
[e]T-nonachlor (chlordane) adjusted for lipids (micrograms/g), with imputation.

Table 3.
Odds ratios for lipid-adjusted hexachlorobenzene and t-nonachlor and breast cancer, overall and stratified by race.

	Cases	Controls	OR[a] (95% CI)	OR[b] (95% CI)
All participants				
HCB[c]				
<0.009	227	219	Referent	Referent
0.009 to < 0.013	263	220	1.1 (0.9-1.5)	1.1 (0.8-1.5)
>= 0.013	258	220	1.1 (0.9-1.5)	1.1 (0.9-1.5)
Trend test				P = 0.39
T-nonachlor[d]				
<0.035	268	219	Referent	Referent
0.035 to < 0.063	223	220	0.9 (0.7-1.2)	0.9 (0.7-1.2)
>= 0.063	257	220	1.1 (0.8-1.4)	1.1 (0.8-1.5)
Trend test				P = 0.71
African Americans				
HCB[c]				
<0.009	80	90	Referent	Referent
0.009 to < 0.014	115	90	1.4 (1.0-2.2)	1.6 (1.0-2.5)
>= 0.014	97	90	1.2 (0.8-1.8)	1.3 (0.8-2.0)
Trend test				P = 0.32
T-nonachlor[d]				
<0.038	96	90	Referent	Referent
0.038 to < 0.069	85	90	1.0 (0.6-1.5)	1.0 (0.6-1.5)
>= 0.069	111	90	1.2 (0.7-2.0)	1.2 (0.7-2.0)
Trend test				P = 0.55
Whites				
HCB[c]				
<0.009	146	129	Referent	Referent
0.009 to < 0.013	147	130	1.0 (0.7-1.4)	0.9 (0.6-1.3)
>= 0.013	163	130	1.2 (0.8-1.6)	1.1 (0.7-1.5)
Trend test				P = 0.72
T-nonachlor[d]				
<0.033	161	129	Referent	Referent
0.033 to < 0.059	154	130	1.0 (0.7-1.5)	1.0 (0.7-1.5)
>= 0.059	141	130	1.0 (0.7-1.5)	1.1 (0.7-1.6)
Trend test				P = 0.70

[a]Adjusted for offsets, age, age-squared, and race (all participants).
[b]Adjusted for offsets, age, age-squared, race (all participants), menopausal status, BMI, parity/lactation, HRT use, and income.
[c]Hexachlorobenzene adjusted for lipids in micrograms/g lipid, with imputation.
[d]T-nonachlor adjusted for lipids in micrograms/g lipid, with imputation.

Table 4.
Odds ratios for lipid-adjusted hexachlorobenzene and t-nonachlor and breast cancer, stratified by menopausal status.

	Cases	Controls	OR[a] (95% CI)	OR[b] (95% CI)
Premenopausal				
HCB[c]				
<0.009	143	102	Referent	Referent
0.009 to < 0.012	121	101	0.8 (0.6-1.2)	0.8 (0.5-1.2)
>= 0.012	118	103	0.8 (0.6-1.2)	0.7 (0.5-1.1)
Trend test				P = 0.10
T-nonachlor[d]				
<0.025	115	102	Referent	Referent
0.025 to < 0.046	153	102	1.4 (1.0-2.0)	1.4 (1.0-2.1)
>= 0.046	114	102	1.1 (0.7-1.6)	1.1 (0.7-1.6)
Trend test				P = 0.73
Postmenopausal				
HCB[c]				
<0.009	88	118	Referent	Referent
0.009 to < 0.015	144	117	1.6 (1.1-2.4)	1.7 (1.1-2.5)
>= 0.015	134	118	1.5 (1.1-2.3)	1.6 (1.1-2.5)
Trend test				P = 0.02
T-nonachlor[d]				
<0.046	116	118	Referent	Referent
0.046 to < 0.079	117	117	1.0 (0.7-1.5)	1.1 (0.7-1.6)
>= 0.079	133	118	1.2 (0.8-1.7)	1.2 (0.8-1.9)
Trend test				P = 0.36

[a]Adjusted for offsets, age, age-squared, and race (all participants).
[b]Adjusted for offsets, age, age-squared, race (all participants), menopausal status, BMI, parity/lactation, HRT use, and income.
[c]Hexachlorobenzene adjusted for lipids in micrograms/g lipid, with imputation.
[d]T-nonachlor adjusted for lipids in micrograms/g lipid, with imputation.

Table 5.
Plasma levels of HCB and trans-nonachlor in estrogen receptor positive and estrogen receptor negative cases

	ER+ Cases n=404	ER- Cases n=282	P value[a]
Lipid Adjusted HCB[c]			
Mean (SD)	0.02 (0.04)	0.01 (0.01)	
Median	0.01	0.01	P=0.20
Range	0.0-0.83	0.0-0.20	
Lipid-Adjusted trans-nonachlor[e]			
Mean (SD)	0.7 (0.06)	0.06 (0.05)	
Median	0.05	0.04	P=0.04
Range	0.01-0.5	0.01-0.37	

[a]Wilcoxin rank sum test comparing medians in ER+ and ER- cases
[c]Hexachlorobenzene adjusted for lipids (micrograms/g), with imputation
[e]Trans-nonachlor adjusted for lipids (micrograms/g), with imputation

DISCUSSION

The influence of weak estrogenic compounds on breast cancer risk is a hotly debated topic. The Carolina Breast Cancer Study has been used to investigate the influence of organochlorine compounds, specifically DDE and PCB congeners, showing no significant increases in risk in association with these compounds (Millikan, 2000). To explore the effect of other organochlorine compounds in this population, we examined the influence of hexachlorobenzene and trans-nonachlor on the development of breast cancer, particularly in pre- and postmenopausal women. There were no differences in median plasma levels of trans-nonachlor when stratified by race or menopausal status. Additionally, no increased odds of breast cancer was found when trans-nonachlor levels were stratified into tertiles. There was a significant difference in median plasma concentrations of HCB in postmenopausal cases versus controls. Furthermore, when data was stratified by HCB level exposure, postmenopausal women in the highest two exposure levels experienced increased odds of breast cancer of 1.6 and 1.5, respectively.

Mixed results have been published concerning these compounds in relation to breast cancer. Three small case control studies found increased HCB levels among cases (Dewailly, 1994, Liljegren, 1998, Charlier, 2003). A majority of studies have found no statistically significant differences in HCB or trans-nonachlor levels in relation to the development of breast cancer (Moysich, 1998, Aronson, 2000, Zheng, 1999, Dorgan, 1999). No significant association of trans-nonachlor and breast cancer has been found to date (Zheng, 2000, Gammon, 2002, Demers, 2000).

Prior studies were limited in their population size. In the largest study to date for either of these compounds, Gammon *et al,* investigated trans-nonachlor (597 cases and

397 controls). The current study had an advantage with the larger sample size (748 cases and 659 controls), racial diversity and equal numbers of pre- and postmenopausal women.

Plasma levels of HCB and trans-nonachlor in our study were similar to other studies. Lipid adjusted mean serum values of trans-nonachlor were 0.07 μg/g lipids in African American cases and controls and 0.06 μg/g lipids in white cases and controls. Gammon *et al* measured chlordane serum levels using a combination of trans-nonachlor and oxychlordane, another constituent in chlordane. Lipid adjusted levels were slightly higher than ours at 0.095 μg/g for cases and 0.096 μg/g for controls (Gammon, 2002). Ward *et al* found substantially lower plasma concentrations of 0.012 μg/g and 0.010 μg/g in Norwegian white cases and controls, respectively (Ward, 2000).

Mean plasma serum hexachlorobenzene levels were similar in both African American and white cases (0.02 μg/g) and controls (0.01 μg/g). A majority of studies that investigated the levels of HCB utilized breast adipose tissue levels. Zheng *et al* found largely higher values, with cases and controls having 21.0 ng/g and 19.0 ng/g, respectively (Zheng, 1994). Aronson *et al's* mean levels of HCB were similar, 32.0 μg/kg for cases and 30.1 μg/kg for controls (Aronson, 2000). Litergen *et al* displayed substantially higher mean concentrations of lipid adjusted HCB residues in cases (0.073 μg/g) verses controls (0.048 μg/g) (Litergen, 1998).

When trans-nonachlor exposure data was stratified into tertiles, African American women in the second level of exposure had 40% greater odds of having breast cancer, although the results were non-significant and showed no dose response relationship. White women had a slight increase in risk for the highest tertile, but this difference was

non-significant as well. No other studies compared levels of HCB or trans-nonachlor between African American and white subjects, mainly due to the low representation of minorities in the studies. Kreiger *et al* investigated differences between ethnic groups in regards to the organochlorines DDE, DDT and PCBs, finding higher levels among Asian and African American women. (Kreiger, 1994). It is interesting that 78% of the African American women in their study were born in the American South, while the white subjects were predominantly from California. One study states that people residing in the South are more likely to have higher serum organochlorine levels in comparison to women from other regions of the United States (Stehr-Green, 1989). This may possibly account for the increased organochlorine levels found among this ethnic group in the Kreiger *et al* study. Our study addressed this issue and showed a slight difference in trans-nonachlor levels across racial groups with African American women having higher serum concentration of trans-nonachlor.

We stratified our data by pre- and postmenopausal status. No differences were found for trans-nonachlor between cases and controls in both pre- and postmenopausal women. Median trans-nonachlor levels of postmenopausal cases and controls (0.06 μg/g) were higher than the levels of the premenopausal women (cases=0.04 μg/g, controls=0.04 μg/g). When divided into tertiles, premenopausal women in the middle exposure level showed a non significant increase in risk [OR= 1.4 (95%CI=1.0-2.0] but no dose response trend was present. Postmenopausal women showed no significant increase in risk for trans-nonachlor. The higher levels of HCB in postmenopausal women could be a result of greater life-long exposure to HCB due to its use as a fungicide during their lifetime.

Estrogen receptor positive cases had a statistically significant difference in trans-nonachlor levels in comparison to estrogen negative cases (P=0.04) but had no difference in risk of breast cancer. Gammon *et al* stratified trans-nonachlor (Chlordane) data by menopausal status and estrogen receptor status, resulting in no significant increase in odds of cancer for either pre or postmenopausal women and ER+ or ER- cases (Gammon, 2002). Despite the weak estrogenic properties of trans-nonachlor, our study agrees with previous evidence that this organochlorine does not contribute to the development of breast cancer.

Postmenopausal cases had an increased lipid adjusted HCB level in comparison to controls. When exposure levels were separated into tertiles, statistically significant increased ORs were found in the highest two levels of exposure. Postmenopausal women with adjusted HCB exposure in the middle level had 70% greater odds of breast cancer than women in the lowest tertile. The highest level of HCB exposure had a 60% increase in odds of breast cancer compared to the lowest level. Liljegren *et al* also found case-control differences in lipid adjusted plasma HCB levels in postmenopausal women (Liljegren, 1998). Cases had a mean concentration of 87.3 ng/g and controls were 55.9 ug/g. Their study found a non-significant increase in odds ratios for breast cancer in postmenopausal women. Their results were further stratified by estrogen receptor status of cases, finding a significant increased OR in estrogen positive cases versus controls. We did not find a difference in risk of breast cancer in ER+ and ER- cases. All three studies that found increased odds of breast cancer in relation to HCB used controls that had been investigated for benign breast lesions. In contrast, Zheng *et al* also used benign breast disease controls and found no association of HCB levels and breast cancer (Zheng,

1994). Our study used population controls, allowing extrapolation of the results to the general population. Furthermore, the studies that found increases in risk also had very small sample sizes. This study utilized the largest sample size to date investigating HCB exposure and breast cancer risk.

An increase in age at natural menopause is related to increased odds of developing breast cancer (Miller, 1996). It may be possible that exposure to organochlorine chemicals elevate the age at natural menopause, thus increasing risk of breast cancer. We evaluated the age at natural menopause for cases and controls in relation to HCB and trans-nonachlor levels, finding no differences in natural age of menopause. Exposure to higher levels of organochlorines did not increase age at menopause.

One limitation of our study was that women were enrolled after diagnosis and treatment of breast cancer. Research has suggested that that treatment of breast cancer may change the serum levels of organochlorines (Gammon, 1996). This possibility was previously investigated for total PCB's and DDE in relation to weight loss or gain in the Carolina Breast Cancer study, finding no differences in ORs for these chemicals.

Although hexachlorobenzene and trans-nonachlor are no longer used as pesticides in the United States, they are still introduced into the environment as byproducts of industrial processes and impurities of other pesticides. Furthermore, they are still detected in the food chain as well as human adipose tissue, breast milk, and blood serum (ATSDR, 2003). The national status and trends program (NS&T) for marine environmental quality published a report in 2002 providing information on concentrations of chemicals in North and South Carolina coastal sediments and shellfish. The report found mean

concentrations of HCB at 0.23 ng/g and Chlordane (sum of heptachlor, hepatachlorepoxide, trans-nonachlor, cis-chlordane) of 10 ng/g. Interestingly, the highest sediment levels in this study for both of these chemicals are found in the Morehead City/ New Bern area of North Carolina. Although mean levels have decreased since the cessation of these chemicals as pesticides, they remain as a contaminant in our environment and may pose a potential health concern for the people of North Carolina (Cantillo, 2002).

Investigation into the influence of hexachlorobenzene on breast cancer will provide a greater understanding of the mechanism of the development of breast cancer. Our study showed a weak association between HCB and breast cancer in postmenopausal women. This evidence must be further investigated by epidemiologic studies to better understand the effects of these two chemicals.

FUTURE DIRECTIONS

This report investigates the influence of two organochlorines, which may mimic the effects of estrogen and contribute to the development of breast cancer. Our study shows a weak association between HCB exposure and risk of breast cancer in postmenopausal women. Gene-environment interactions may provide additional information on the mechanism of carcinogenicity. Research suggests that HCB may be weakly genotoxic and could be metabolized through an epoxide intermediate (Canonero, 1997; Rietjens, 1997). Additionally, Lin *et al* has provided evidence that PCP, a primary HCB metabolite, is further biodegraded and can induce direct DNA damage as well as damage through reactive oxygen species (Lin, 2001). Hexachlorobenzene is predominantly metabolized by three cytochrome p450 enzymes, CYP3A1, CYP3A2, and

CYP3A4. CYP3A4 accounts for 30-60% of liver cytochrome P450 enzymes and has been reported to have increased expression in breast tumors (Amirimani, 2003) (Kapucuoglu, 2003). There is a single nucleotide polymorphism of an A to G substitution (CYP3A4*1B) located in the promoter region of the CYP3A4 gene. The function of this SNP is not completely understood, but the variant was associated with an increased risk of prostate cancer in one study (Rebbeck, 1998). Conversely, a later case-control study showed no significant increase in risk for breast cancer in relation to the CYP3A4*1B variant allele. (Spurdle, 2002). Both of the aforementioned studies only associated the genotype with cancer, and did not evaluate interactions with environmental factors such as pesticide exposure. Our laboratory is currently completing genotyping on the CYP3A4*1B polymorphism and will investigate the gene-environment interactions of this SNP and hexachlorobenzene exposure.

Risk of breast cancer stratified by HCB exposure and menopausal status for specific DNA repair genetic polymorphisms was presented with HCB exposure data at the American Association of Cancer Researchers special conference in Kona, Hawaii (Appendix I). Genotype data for three DNA repair pathways, direct repair, nucleotide excision repair, and double strand break repair, were evaluated and stratified by HCB exposure tertiles. Statistically significant odds ratios for breast cancer versus controls were observed for postmenopausal women in the two highest HCB exposure levels for variant genotypes of the DNA repair genes examined (Appendix II). Future research will consist of stratifying hexachlorobenzene exposure with polymorphisms in related metabolic enzymes as well as DNA repair genes to examine the mechanism of breast cancer carcinogenesis.

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Hexachlorobenzene (HCB), T-nonachlor (chlordane) and breast cancer risk among African-American and white women in North Carolina

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ABSTRACT

We examined plasma hexachlorobenzene (HCB) and T-nonachlor (chlordane) levels in relation to breast cancer risk in a population-based, case-control study of African American women (292 cases, 270 controls) and white women (456 cases, 389 controls) in North Carolina. Mean and median levels of HCB and T-nonachlor were similar in African American and white women. We did not observe case-control differences in plasma levels of these compounds in premenopausal women. However among postmenopausal African American and white women, the mean level of lipid-adjusted HCB was 0.02 (SD: 0.03) in cases and 0.01 (SD: 0.01) in controls (P = 0.03). There was no difference in mean levels of T-nonachlor in post menopausal cases (0.08, SD: 0.06) versus controls (0.08, SD0.06) (Pval:12). In postmenopausal women, the adjusted odds ratio for breast cancer comparing the highest to lowest third of HCB was 1.6 (95% Confidence Interval 1.1-2.5) (Trend test P = 0.02). The corresponding odds ratios for T-nonachlor in postmenopausal women was 1.2 (95% CI 0.8-1.9) (Trend test Pval:30). Joint effects of plasma levels of each compound and polymorphisms in CYP1A1, Glutathione S-transferases M1, T1 and M1, as well as a panel of DNA repair genes were examined. Results suggest that HCB exposure may increase risk of breast cancer among subgroups of women, but the effects of such exposure are quite weak.

BACKGROUND

Study Population

Carolina Breast Cancer Study

1993 -1996 (Phase I) 1996 - 2001 (Phase II)

- Case Control Study of women in 24 eastern and central counties of North Carolina
- Cases: Central Cancer Registry. Phase I invasive breast cancer;
- Controls: DMV and HCFA
- Population consists of half African American, half non- African American
- Half below 50 years of age, half above 50 years of age

Pesticides

- Pesticide data collected for Phase I of CBCS study (989 cases, 841 controls).
- Hexachlorobenzene (HCB)- Widely used as a seed fungicide and pesticide until 1965. Currently present as industrial process byproduct. Bioaccumulates in adipose tissue.
- T-nonachlor- Major constituent of chlordane, a compound used as a pesticide from 1948 to 1988. Bioaccumulates in adipose tissue.

BACKGROUND

Metabolism

- **CYP1A1** gene encodes aryl hydrocarbon hydroxlase (AHH), an enzyme involved in activation of aromatic hydrocarbons
- The M1 allele, a T to C substitution at nucleotide 3801 is associated with higher inducibility of the CYP1A1 gene and higher levels of AHH activity
- M1 allele is associated with increased risk for many cancer types.

DNA Repair

- **MGMT**- encodes O6-methylguanine DNA methyltransferase, an enzyme involved in direct repair of DNA adducts. The Phe amino acid at codon 84 is associated with stronger effects for smoking in the CBCS study
- **XPD** encodes a helicase involved in the Nucleotide excision pathway. The Gln allele at codon 751 is associated with decreased DNA repair function.
- **XPC** encodes for an enzyme involved in the nucleotide excision repair pathway. And repair of bulky adducts. Higher risk for smoking was associated with the Gln amino acid at codon 939 was found in the CBCS study.
- **NBS1** encodes for an enzyme involved in the double strand DNA repair. The Glu/Gln genotype at codon 185 was associated with increased risk with smoking.
- **BRCA2** is involved in a number of functions in the body, notably the double strand DNA repair pathway. The His allele has been associated with increased risk of breast and ovarian cancer. It was also associated with increased risk of breast cancer due to ionizing radiation in the CBCS study.

METHODS

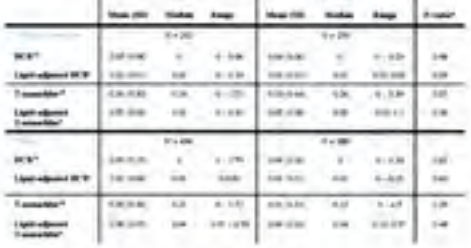
- Pesticide levels were measured by Research Triangle Institute, RTP, NC
- Organochlorine dose was analyzed by gas chromatography/electron capture using a DB-5 column and Hewlett-Packard Model 6890 model.

- Genotyping performed in the High Throughput Genotyping Core UNC-CH
- All genotypes were identified with Applied Biosystems 7700 Taqman®
- All results were verified with Hardy Weinberg Equilibrium tests as well as a random repeat of 10 percent of all samples.

- Odds ratios for breast cancer were calculated using logistic regression in SAS version 6.11, incorporating offset terms to account for sampling probabilities.

RESULTS

Plasma levels of HCB and t-nonachlor among African American and whites in cases and controls



Plasma levels of HCB and t-nonachlor stratified by postmenopausal status

Odds ratios for HCB and t-nonachlor and breast cancer stratified by race and menopausal status

Odds ratios for lipid adjusted HCB and breast cancer in postmenopausal women, stratified by genotype

CONCLUSIONS

- Plasma levels of HCB were elevated in postmenopausal breast cancer cases compared to controls. No difference was found for t-nonachlor.
- Odds ratios for breast cancer were elevated for the highest tertiles of HCB among postmenopausal women.
- Odds ratios for HCB were elevated for postmenopausal women with CYP1A1 M2 genotype as well as a panel of DNA repair gene variants. No strong differences were observed according to GST M1, P1, T1 genotypes.
- These results suggest a possible mechanism of action for HCB involving reactive intermediates and DNA damage.

Appendix II.

Odds ratios for lipid adjusted HCB and breast cancer in postmenopausal women, stratified by genotype

Genotype	T1 <0.009	T2 0.009 to <0.015	T3 ≥ 0.015	Trend test
CYP1A1 Wild Type OR[a] (95% CI)	Referent	1.8 (1.1-3.0)	1.5 (0.9-2.5)	P = 0.18
CYP1A1 Any M1 OR[a] (95% CI)	Referent	**2.3 (1.0-5.5)**	**2.3 (1.0-5.7)**	**P = 0.07**
MGMT 84 Leu/Leu OR[a] (95% CI)	Referent	1.5 (0.9-2.4)	1.3 (0.8-2.2)	P = 0.25
MGMT 84 Any Phe OR[a] (95% CI)	Referent	**2.8 (1.0-7.5)**	**4.6 (1.6-13.4)**	**P = 0.006**
XPD751 Lys/Lys OR[a] (95% CI)	Referent	1.0 (0.5-2.0)	1.3 (0.7-2.5)	P = 0.44
XPD751 Any Gln OR[a] (95% CI)	Referent	**2.4 (1.4-4.3)**	**2.3 (1.3-4.1)**	**P = 0.006**
XPC939 Lys/Lys OR[a] (95% CI)	Referent	1.3 (0.7-2.4)	1.4 (0.7-2.6)	P = 0.33
XPC939 Any Gln OR[a] (95% CI)	Referent	**2.3 (1.3-4.1)**	**2.2 (1.2-4.0)**	**P = 0.02**
NBS1 185 Any Gln OR[a] (95% CI)	Referent	1.2 (0.6-2.1)	1.3 (0.7-2.5)	P = 0.43
NBS1 185 Glu/Glu OR[a] (95% CI)	Referent	**2.3 (1.3-4.2)**	**2.2 (1.2-3.9)**	**P = 0.01**
BRCA2 372 Asn/Asn OR[a] (95% CI)	Referent	1.8 (1.1-3.1)	1.3 (0.8-2.3)	P = 0.30
BRCA2 372 Any His OR[a] (95% CI)	Referent	**1.9 (0.9-3.7)**	**2.4 (1.25-5.0)**	**P = 0.02**

a Adjusted for offset, age, age –squared, menopausal status, BMI, parity/lactation, HRT use, and income
b Hexachlorobenzene adjusted for lipids in micrograms/g lipid, with imputation