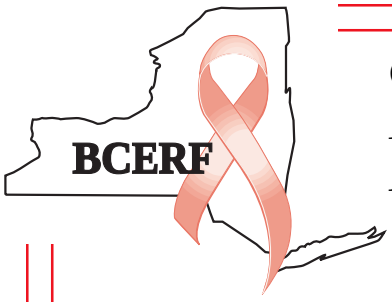

Cornell University
Program on Breast Cancer and Environmental Risk Factors in New York State (BCERF)*

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Critical Evaluation of Chlordane's Breast Cancer Risk

by

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Table of Contents-Chlordane Critical Evaluation

List of Tables

Critical Evaluation of Chlordane's Breast Cancer Risk

Authors Note: A separate Critical Evaluation has been prepared on heptachlor and heptachlor epoxide. The reader is encouraged to read the attached document, Appendix B, which includes and explanation of the BCERF Breast Cancer Risk Classification System, before reading this Critical Evaluation.

I. Chemical Information:

A. Common Name: chlordane

B. Chemical Name: 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene (IUPAC) (Meister, 1997)

C. Chemical Formula: $C_{10}H_6Cl_8$ (Worthing, 1991)

D. Trade Names: Okta-Klor ® (Chevron Chemical Co.); Corodane ® (PPG Industries); Velsicol 1068®; Octachlor® (Velsicol Chemical Co.) (Meister, 1997; Smith, 1991)

E. CAS Registry Numbers: Technical chlordane and major chlordane isomers:

1. Non-stereospecific chlordane, 57-74-9 (39400-80-1;53637-13-1)*
2. Technical-grade chlordane, 12789-036
3. *cis* -chlordane or α-chlordane, 5103-71-9 (22212-52-8; 26703-86-6; 28140-46-7)*
4. *trans* -chlordane or β-chlordane, 5103-74-2 (17436-70-3; 28181-89-7)*
5. γ-chlordane, 5566-34-7

*Replaced CAS registry numbers in parenthesis (ATSDR, 1994; IARC, 1991)

F. Chemical Structure of Chlordane :

(IARC, 1991)

II. History of Use, Usage and Nomenclature:

Chlordane is a member of the class of chlorinated cyclodiene insecticides. Structurally similar compounds include aldrin, isoaldrin, dieldrin, endrin, heptachlor, isobenzan, and alpha-endosulfan (Smith, 1991). Technical chlordane is actually a mixture of at least 26 chemicals, including chlordane isomers, other chlorinated hydrocarbons and chlordane by-products (IARC, 1991). It has been estimated that technical chlordane may have contained as many as 140 related compounds (ATSDR, 1994). The major components of technical chlordane are the *cis* and *trans* stereo isomers. Depending on the manufacturing process, these two isomers constituted up to 85% of technical chlordane (ATSDR, 1994). One available formula for technical chlordane is: *trans*-isomer (24%), *cis*-isomer (19%), chlorodene isomers (21.5%), heptachlor (10%), nonachlor (7%), and other compounds (27.5%) (IARC, 1991). The major persistent metabolite of *cis*-chlordane is oxychlordane. *Trans*-chlordane can be metabolized to heptachlor which is readily oxidized to the stable breakdown product, heptachlor epoxide (ATSDR, 1994). Nonachlor, a very persistent component of technical chlordane, has been detected in tissues, blood and milk of humans and animals. Since the components and isomers of chlordane are lipophilic, they are mainly stored in adipose tissue, and can bio-concentrate in the food chain (IARC, 1991).

Technical chlordane was a versatile contact, and stomach insecticide introduced in the United States (U.S.) in 1947 (IARC, 1979). This persistent pesticide was primarily used to protect buildings, gardens and lawns from subterranean insects and termites (IARC, 1991; Tomlin, 1994; Weidhass et al., 1966). Chlordane has been used to control insects in vegetable crops, small grain, maize, potatoes, sugarcane, sugar beets, fruits, cotton and jute (Smith, 1991). It was used to pretreat seed corn to prevent damage by the corn maggot. Chlordane was also used to pretreat soil to control the cabbage maggot, cutworms and wire worms (Muka et al., 1966). Wood products were treated with chlordane to prevent infestation of insect pests (Worthing, 1991). In the 1950s, chlordane was used as a herbicide to control crabgrass in turf, marketed under the name of Velvet Green (Hanna, 1958). During the mid-1970s, 4.3 million pounds (lbs) of chlordane was used in the U.S., with 35% used by commercial pesticide applicators; 30% used for home, lawn and garden pest control; 27.5% for fruits and vegetables (mostly corn, tomatoes, and potatoes) ; and 1.2% on ornamental shrubs (IARC, 1979). After the cancellation of many agricultural and home application uses in 1978, chlordane was used primarily to control subterranean termites, applied as a liquid that was poured or injected around the

foundations of buildings (ATSDR, 1994). It has been estimated that 3.5 to 4.0 million lbs of chlordane were distributed in 1986. Although chlordane is no longer used in the U.S. (see section below on "Current Regulatory Status"), it is still manufactured in the U.S. for export. No information was available on current export volumes (ATSDR, 1994).

III. Current Regulatory Status:

A. Regulatory Status:
In March 1978, because of chlordane's oncogenicity in laboratory animals and concerns about cancer risk in humans exposed to this pesticide, all but termiticide uses of chlordane were either voluntarily cancelled by the primary manufacturer, Velsicol Chemical Co., or were suspended for failure to meet U.S. Environmental Protection Agency (EPA) requirements (USEPA, 1990). Cancellation included the use of chlordane on food crops, lawns, and gardens and as a fumigant agent. Minor uses for treating nonfood plants was cancelled by the EPA in 1983. During the next 5 years, chlordane was used primarily to control termites through its application around the foundation of buildings. Commercial use was permitted also for fire ant control in power transformers. On April 15, 1988, the EPA prohibited the sale, distribution and shipment of existing stocks of all chlordane products. EPA issued a final cancellation of all commercial and domestic uses of chlordane at this time, with the exception of existing stocks of termiticide products in possession of homeowners, and commercial use of existing stocks for fire ant control in power transformers (ATSDR, 1994; USEPA, 1990).

B. Drinking Water Standards and Health Advisories:
The EPA has set the Maximum Contaminant Level (MCL) of chlordane in drinking water at 0.002 mg/L (USEPA, 1996). The MCL is an enforceable limit on the maximum allowable concentration of a chemical in public water supplies.

Health Advisory (HA)* levels for chlordane in drinking water are as follows (USEPA, 1996):

10 kg child
- One day = 0.06 mg/L
- Ten day = 0.06 mg/L

70 kg adult
- Long term and lifetime HAs for adults are not available.

*The HAs are nonenforceable limits of the concentration of a chemical in the drinking water that is not expected to cause any adverse noncarcinogenic health effects when consumed for no more than the time period specified, with a margin of safety (USEPA, 1996).

C. Workplace Regulations:
The Occupational Safety and Health Administration (OSHA) has set the maximum allowable level of chlordane in workplace air at 0.5 mg/m^3 for 8 hours per day and 40 hours per workweek; this is also the exposure limit recommended by the National Institute of Occupational Safety and Health (NIOSH) (ATSDR, 1994).

D. Food Residue Tolerances and Action Levels:
The Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) are responsible for monitoring the levels of chlordane residues and its breakdown products in domestic and imported foods, and animal feed. The EPA sets tolerances of the maximum amount of a residue that is permitted in or on the food. Because animal cancer bioassays have shown that chlordane is a possible carcinogen, the tolerance for residues of chlordane and its breakdown products in foods has been set at zero. Since chlordane and related breakdown products persist in soil, the FDA established "action levels" for unavoidable residues of these chemicals in raw agricultural products. These action levels represent limits of the contaminant at or above which the FDA can take legal action to remove commodities containing these violative residues from the market place. The action levels for residues of chlordane isomers (*cis*- and *trans*-chlordane; alpha-, beta-, and gamma-chlordane), components of technical chlordane (*cis*- and *trans*-nonachlor and chlordene) and its metabolite (oxychlordane), include: 0.1 ppm in fruits, vegetables and nuts; 0.1 ppm in animal feed; and 0.3 ppm in rendered animal fat and the edible portion of fish (FDA, 1994).

IV. Evidence of Overall Carcinogenicity (non-breast sites):

A. Human Studies:
1. Case-Reports:
Although case-reports are not sufficient to demonstrate the carcinogenicity of chemicals, they may provide useful information to justify the need for further epidemiological studies. Several studies have related case-reports of neuroblastoma and leukemia with exposure to chlordane and/or heptachlor, and are reported below.

Five cases of neuroblastoma associated with domestic exposure to chlordane during prenatal and postnatal development have been reported (Infante et al., 1978). These cases of neuroblastoma, diagnosed between 1974 and 1976, were based on self-reported exposure to chlordane. Two of the patients also had exposure to other agents, including X-rays. The time from suspected exposures to diagnosis of neuroblastoma, ranged from 2 to 4 years. This report also described three cases of leukemia, two of which were associated with chlordane or heptachlor exposure alone and one case where there were exposures to other chemicals including the pesticides 2,4-D and diazinon.

In another study, Epstein and Ozonoff (1987) presented four other cases of leukemia and exposure to chlordane and heptachlor. The age of the subjects was not provided, nor was information on the duration of exposure and the time elapsed from exposure to diagnosis. In three of these cases, the subjects had been exposed to other chemicals besides chlordane or heptachlor.

Both of these studies are limited by the lack of medical history of the individuals, incomplete exposure histories, and the Epstein and Ozonoff study (1987) lacked information on the period of time between exposure to suspect agents and diagnosis of the disease state. Moreover, most of the individuals had been exposed to other chemicals that may have influenced the etiology of these cancers. Therefore, it can not be concluded that there was a causal association between exposure to chlordane or heptachlor and neuroblastomas or leukemia in these reported cases.

2. Occupational Cohort Mortality Studies:

a. Chlordane Manufacturing Plants:

Four retrospective studies have examined the mortality of workers employed at the only chlordane manufacturing plant in the U.S., located in Marshall, Illinois (Velsicol Chemical Corporation). Chlordane was the only pesticide ever manufactured at the Velsicol plant in Marshall since 1946. The findings of these studies are summarized below.

Wang and MacMahon (1979a) followed a cohort of 570 white males employed for at least 3 months between 1946 and 1976 at the Velsicol Chemical Corporation (Corp.) chlordane manufacturing plant in Marshall, Illinois. This study was funded in part by the Velsicol Chemical Corp. The authors reported a deficit of deaths from all cancers (SMR=0.65; Standard Mortality Ratio {SMR}= the ratio of "observed" to "expected" deaths). A small but nonsignificant excess mortality from lung cancer (SMR=1.15) was also reported, but the increased risk was related to the duration of employment. Smoking history of participants was not obtained. A small excess in deaths from cerebrovascular disease (SMR=1.58) was also reported in this cohort (Wang and MacMahon, 1979a).

An update of this study was published in 1986 by Shindell and Ulrich (1986). This added nine years of observations to Wang and MacMahon's original study (1979a). The updated study included 706 white males workers who were employed for at least three months between 1946 and 1985 at the Velsicol chlordane plant. There was no excess of cancer deaths in those employed in production jobs (SMR=0.79) or nonproduction jobs (SMR=1.05) at the plant. In contrast to the Wang and MacMahon study (1979a), this study reported a nonsignificant deficit of deaths from lung cancer (SMR=0.86). There was no trend between the duration of employment and the incidence of respiratory cancer. Concentrations of oxychlordane, a chlordane metabolite, in the blood of chlordane production workers were higher than those of nonproduction workers, indicating that exposure to chlordane had occurred in the those involved in the manufacturing of chlordane.

An occupational cohort mortality study conducted at the same chlordane plant included 327 white male workers employed for at least 6 months between 1946 and 1976 (Ditraglia et al., 1981). It is probable that men included in this cohort were also included in the Wang and MacMahon study (1979a). There was a nonsignificant decreased risk of deaths from any cancer (SMR=0.69; 95% CI 0.35-1.24), and a slight, but nonsignificant excess of deaths from lung cancer (SMR=1.1; 95% CI 0.4-2.4) in this study. There was also a statistically insignificant excess mortality from stomach cancer (SMR=3.03; 95% CI 0.61-8.85), based on a small number of deaths (3 observed).

The Ditraglia study was updated by Brown (1992) who added 11 more years of observations, providing a total follow-up of 40 years for the cohort of 405 white males. There was a slight deficit of overall cancer mortality (SMR=0.87; 95% CI=0.61-1.22), and a small but nonsignificant excess of deaths from lung (SMR=1.33; 95% CI 0.80-2.08) and stomach cancers (SMR=2.10; 95% CI 0.57-5.37). The study, however, did not analyze the death rates from these specific cancers as a function of latency and failed to provide exposure data. The authors also recognized the potential of the workers being exposed to additional chemicals used in the manufacturing process.

All four studies of these occupational cohort mortality studies used the same population-base and therefore, do not provide independent estimates of cancer risk. The validity of some of these studies is also limited by the lack of exposure data on chlordane, and lack of inclusion of biomarkers (i.e., serum oxychlordane levels) to assess chlordane exposure. There also was little information on the exposure to other chemicals in the workplace, and no information provided on history of tobacco use. Though these cohort studies did not find a significant increased risk of cancer mortality with occupational exposure to chlordane, there is an inherent difficulty in interpreting this type of study. Because cancers have a long latency period, tumors may not develop in those occupationally exposed to suspect chemicals until later in life after termination of, or change in employment.

b. Pesticide Applicators:

Risk of cancer mortality has also been studied among pesticide applicators licensed for termite control. Although these pesticide applicators had been exposed to many other chemicals, chlordane had been the chemical most widely used for termite control during their employment.

In a nationwide prospective study, Wang and MacMahon (1979b) examined the causes of death among a cohort of 16,126 urban

pesticide applicators that were employed for at least three months between 1967 and 1976 by any of three nationwide pest control companies (company names not provided). Death records were obtained through the Social Security Administration, and 269 death certificates were obtained. The SMR for all causes of death was 0.84 (95% CI 0.75-0.94), which would indicate a lower death rate than the general population. This may be indicative of a "healthy worker effect." Risk of cancer death from any type of neoplasm was also less than expected (SMR=0.83; 95% CI 0.64-1.09). However, the risk of death from bladder cancer was significantly elevated (SMR=2.77; 95% CI 1.01-7.61), but this was based on only three cases. Lung cancer deaths were slightly, but not significantly elevated (SMR=1.15; 95% CI 0.77-1.70). No information was obtained on use of tobacco products among these applicators, and without this information, risk of lung cancer deaths could not be fully interpreted.

In addition, this study also examined causes of mortality in a subset of pesticide applicators, termite control operators, who would be more likely to be exposed to chlordane and/or heptachlor. While risk of death from all cancers was not elevated among the termiticide applicators (SMR=0.83; no CI provided) risk of death was elevated, but not significantly, for skin cancer (SMR=1.48) and bladder cancer (SMR=2.45). The risk of lung cancer was not elevated in the termiticide operators (SMR=0.87), but was slightly elevated in non-termiticide pesticide applicators (SMR=1.31). It should be noted that this cohort may not have been followed for a long enough period of time to allow for a long enough latency period for cancer to develop.

In a follow-up study, MacMahon et al. (1988) examined the causes of death in the same cohort of 16,124 urban pesticide applicators. This study included pesticide applicators employed between 1967 and 1984. Death records were obtained through the Social Security Administration, and 540 death certificates were obtained from the states and other registration sites (this includes the death certificates obtained in the original 1979 study). An overall excess of deaths from lung cancer was observed among the whole sample of pesticide applicators (SMR=1.35; 90% CI 1.14-1.58). However, as had been observed in the original study by Wang and MacMahon (1979b), excess of lung cancer mortality was observed among non-termite control applicators (SMR=1.58; 90% CI 1.29-1.90), but not among termite control applicators who had a higher probability of exposure to chlordane and heptachlor (SMR=0.97). Moreover, the risk of lung cancer did not increase with the length of employment. Since smoking was not controlled for as a confounding variable, it is not known if smoking patterns may have contributed to the incidence of lung cancer mortality in this cohort.

Blair et al. (1983) determined the causes of death among a cohort of 3,827 white, male pesticide applicators from Florida. Vital status of subjects was obtained through information from the Social Security Administration, Motor Vehicle Departments of Florida, and other states, telephone and street directories, post offices, and personal contacts. A small, nonsignificant increased risk of lung cancer mortality (SMR=1.35) was observed. However, unlike the study of MacMahon et al. (1988), the mortality from lung cancer among pesticide applicators increased with the number of years licensed; the SMR was 1.01 for <10 years, but increased to 1.55 for 10 to 19 years and to 2.89 for ≥ 20 years. An excess of lung cancer mortality was also seen among workers licensed for termite application (SMR=1.42). An excess mortality was also observed for brain cancer (SMR=2.0). However, cancer mortality in this study may not be specifically attributable to chlordane exposure because termiticide applicators were also licensed for the use of other organochlorine pesticides such as aldrin, DDT, heptachlor, propoxur and chlorpyrifos.

During the time chlordane was in active use, pesticide applicators were predominantly male. However, it is possible that female spouses of pesticide applicators who may have handled the contaminated clothing of applicator spouses during laundering practices may have had significant exposure to chlordane. Unfortunately, no studies are available that have followed a cohort of female spouses of termite control pesticide applicators that used predominately chlordane.

3. Case-Control Studies of Agricultural Workers:

A case-control study of men in western Washington occupationally exposed to pesticides failed to find a significant association between exposure to chlordane and the risk of developing soft tissue sarcoma, (Odds Ratio, OR=0.96; 95% CI 0.2-4.8), or non-Hodgkin's lymphoma (NHL) (OR=1.61; 95% CI 0.7-3.8). However, no information was available on the duration of exposure (Woods et al., 1987). A similar lack of a significant association between NHL and "regular" use of chlordane (OR=1.56; 95% CI 0.5-5.1) was reported in another case control study of male farmers in western Washington state (Woods and Polissar, 1989). In contrast, a study conducted in Iowa and Minnesota (Cantor et al., 1992) did find a relationship between chlordane and the risk of NHL among 622 white male farmers and 1,245 population-based controls. There was an elevated risk of NHL among farmers who had ever handled, mixed or applied chlordane as animal insecticide (OR=1.7; 95% CI 1.0-2.9) and the risk of NHL was even higher for first use prior to 1965 (OR=2.2; 95% CI 1.2-4.2). Elevated risk of NHL was also found among farmers who did not wear protective clothing or equipment when applying chlordane as animal insecticide (OR=2.2; 95% CI 1.2-4.2).

One case-control study has measured the levels of components of technical chlordane, chlordane isomers and chlordane metabolites in the adipose tissue of men and women with NHL, and in non-cancer surgical controls (Hardell et al., 1996). Mean adipose tissue

levels of *trans*-nonachlor, oxychlordane, and nonachlor III, but not *cis*-chlordane were significantly higher ($p<0.028$) in 27 cases with NHL as compared to 17 surgical controls without cancer. The authors noted that both genetic and acquired immunosuppression are established risk factors for the development of NHL in humans, suggesting that agents such as chlordane that are known to impair the immune system may be of importance in the etiology of NHL (Hardell et al., 1996).

A case-control study of white, male agricultural workers residing in Iowa and Minnesota examined the risk of leukemia in relation to using specific animal insecticides. The risk of developing leukemia was slightly, but not significantly elevated (OR=1.3; 95% CI 0.7-2.3) in men that had ever mixed, handled, or applied chlordane (19 cases and 38 controls). A further analysis of the number of days per year chlordane was used on animals and risk of leukemia found a OR of 1.1 (95% CI 0.4-2.8) in men that had used chlordane for 1 to 4 days a year. Risk of leukemia was elevated but was not statistically significant (OR=3.2; 95% CI 0.9-11.0) when chlordane was handled more than 10 days per year (Brown et al., 1990).

A case-control study of 173 white men with multiple myeloma (MM) and 650 controls was conducted in Iowa to evaluate the association of MM, agricultural risk factors, and the exposure to individual pesticides, including chlordane (Brown et al., 1993). Cases were identified from the Iowa Health Registry, and included all white men 30 years and older diagnosed during 1981-84 with MM. Controls were selected from a population without lymphatic or hematopoetic cancer by random digit dialing, Medicare records, and state death certificate files. A standardized questionnaire was used to obtain information on farm activities, pesticide use, use of pesticide protective equipment, and first and last year the pesticide was used. However, information was not collected on the frequency of pesticide use. Vital status and age were included in logistic models to adjust for potential confounding. Other factors, including smoking and education were evaluated, but were not found to be confounders. A nonsignificant OR of 1.6 (95% CI 0.7-3.6) for the development of MM was observed for the nine cases and 29 controls that had used chlordane as an animal insecticide in this study. Over all, ORs were not significantly elevated among farmers for the use of any pesticide, herbicide, insecticide or fungicide in this study.

4. Summary, Human Studies (non-breast sites):

There is insufficient evidence to show a causal relationship between chlordane exposure and the risk of cancer at various sites in humans. Although there have been several case reports of neuroblastomas in children pre- and postnatally exposed to chlordane, there are no case-control studies that have substantiated these findings (Infante et al., 1978). There have been several case reports of leukemia in individuals exposed to chlordane, but most of these individuals were exposed to other chemicals as well (Epstein and Ozonoff, 1987; Infante et al., 1978). Case-control studies of male pesticide applicators or farmers exposed to chlordane found a slightly elevated, though not statistically significantly increased risk of leukemia (Blair et al., 1983; Brown et al., 1990).

The majority of studies evaluating workplace exposures to chlordane and the risk of lung cancer have not been able to demonstrate statistically significantly elevated lung cancer mortality in male chlordane manufacturing plant workers (Brown, 1992; Ditraglia et al., 1981; Shindell and Ulrich, 1987; Wang and MacMahon, 1979a) or termiticide pest control applicators (MacMahon et al., 1988; Wang and MacMahon, 1979b). However, one study did find a significant increased risk of lung cancer mortality in pesticide applicators who had used chlordane over 20 years, suggesting that prolonged exposures to chlordane may increase risk of lung cancer mortality (Blair et al., 1983).

There is concern that chlordane exposure may affect the risk of NHL, though there is only limited evidence of such a relationship in agricultural workers (Hardell et al., 1996; Cantor et al., 1992). Others have not reported an increased risk of NHL (Woods and Polissar, 1989; Woods et al., 1987) or soft-tissue sarcoma (Woods et al., 1987) in male agricultural workers exposed to chlordane.

B. Experimental Animal Studies:

1. Mice:

Several studies have demonstrated the heptocarcinogenicity of chlordane in multiple strains of male and female mice. These studies are presented below.

Epstein (1976) re-evaluated the unpublished lifetime mouse oncogenic studies conducted by the International Research and Development Corporation (IRDC). Charles River CD-1 mice (100 of each sex) were maintained starting at 6 weeks of age on diets containing either 0, 5, 25, or 50 ppm of technical grade chlordane for 18 months. There was no evidence of weight loss in any group, and food consumption did not differ between groups of animals. Survival rates at 18 months in males were 45% in controls, 57% in the 5 ppm group, 41% in the 25 ppm group, and 11% in the 50 ppm group. In females, survival rates were 54% in controls, 61% in the 5 ppm group, 44% in the 25 ppm group and 17% in the 50 ppm group.

The original analysis by the IRDC suggested a highly significant ($p<0.000000001$; Authors' Note: this is the p-value stated by the authors of this study) increase in liver hepatocellular nodules in males and females fed the diets containing 25 or 50 ppm chlordane (Epstein, 1976). However, there was no significant increase in the incidence of hepatocarcinomas in any treatment group. The re-evaluation of liver histopathology by a panel of the National

Academy of Science (NAS) pathologists [as reported in an IARC monograph on chlordane (IARC, 1991)] revealed that chlordane had increased the incidence of hepatocarcinomas in the males fed diets containing 25 ppm chlordane ($p=0.015$), and females fed the 25 or 50 ppm diets ($p < 0.001$ and 0.009, respectively). Male and female mice fed the diets containing 25 or 50 ppm of chlordane had increased incidence of combined hepatocellular carcinomas and nodules ($p<0.001$). This study has some limitations. The low rate of survival in the high-dose group of both sexes indicates that the 50-ppm dose exceeded the maximum tolerated dose. Therefore, there were too few animals left in the high-dose group to evaluate either a dose-related effect or the incidence of late-stage tumors.

In 1977, the National Cancer Institute (NCI) reported the results of a long-term cancer assay for chlordane in mice (NCI, 1977). Fifty B6C3F1 mice of each sex were administered analytical-grade chlordane (94.8% chlordane, 0.3% heptachlor, 0.6% nonachlor, 1.1% hexachloropentadiene, 0.25% chlordene isomers and other chlorinated compounds) in their diet beginning at 35 days of age for 80 weeks and then were observed for an additional 10 weeks. In male mice, doses were increased during the course of the study, while in female mice, doses were decreased during the study. Time-Weight Average (TWA) doses were 29.9 and 56.2 ppm, for low and high doses, respectively, in males, and 30.1 and 63.8 ppm, respectively, in females. Matched controls included 20 untreated mice for each sex; pooled controls consisted of matched controls combined with 70 untreated males and 80 untreated female mice from similar bioassays of other chemicals. It should be noted that pooled controls are no longer considered acceptable controls in cancer bioassays. Survival rates were over 60% and 80% for treated male and female mice, respectively, and over 90% for controls. Chlordane induced a dose-related increase ($p<0.0001$) in the incidence of hepatocellular carcinomas in male and female mice compared to matched or pooled controls. A review of the NCI study by a panel from the NAS as cited by IARC (IARC, 1991) revealed that there was a statistically significant increase, as assessed by linear trend, in hepatocarcinomas at the high dose in males ($p<0.031$) and females ($p<0.018$). The incidence of other tumors was comparable between treated and control mice. The statistical differences were maintained when "hepatocellular carcinoma" was combined with "hyperplastic nodules" ($p<0.003$ and $p<0.002$ in males and females, respectively (IARC, 1991).

The incidence of liver tumors was evaluated in a 104 week chlordane bioassay in mice. Eighty Charles River (ICR) specific pathogen free (SPF) mice of each sex were fed diets containing 0, 1.0, 5.0, or 12.5 mg/kg of technical chlordane starting at 5 weeks of age (Khasawinah and Grutsch, 1989a). An interim kill was conducted at 1 year of age; mortality rates did not differ between treated groups and controls at 52 or 104 weeks of age. The incidence of hepatocellular adenomas was significantly increased only in the male mice fed the 12.5 mg/kg dose (27/80) compared to controls (12/79) ($p<0.01$). Male mice in the control and treated groups (1.0 and 5.0 mg/kg) had similar incidences of hepatocellular adenomas. No significant increases in malignant tumors at any organ site were found in chlordane treated female mice (Khasawinah and Grutsch, 1989a).

The hepatocarcinogenicity of chlordane was also observed in a study that used C57BL/6N mice, a strain known to be resistant to spontaneous hepatocellular carcinomas (Becker and Sell, 1979). Male mice were fed chlordane (90% chlordane, 10% heptachlor) in the diet at 0, 25, and 50 ppm starting at 5 weeks of age for three years. Starting a six months, three animals per week per dietary treatment were sacrified and autopsied. Hepatocellular carcinomas occurred in 27% of the surviving chlordane-treated male mice (25 and 50 ppm groups combined). Since tumor incidence for the control and each of the treatment groups was not provided, it is not possible to determine if there was a dose-response relationship to the chlordane treatment and the incidence of liver tumors.

The hepatocarcinogenicity and ability of chlordane to stimulate liver and thyroid cell proliferation was assessed in 100 male C57B1/10J mice fed chlordane (purity not specified) in the diet at 50 ppm for 24 months (Barrass et al., 1993). However, because control mice were not run concurrently, this study is invalid, and can not be interpreted.

Hepatocarcinogenicity has also been reported in male and female mice fed other structurally similar organochlorine pesticides, including aldrin, dieldrin, heptachlor, and heptachlor epoxide (Reuber, 1978). This suggests a common mechanism of liver tumor induction in these structurally related compounds.

2. Rats:

In contrast to the studies of chlordane-induced liver cancer in mice, a long-term cancer bioassay conducted by NCI did not provide any evidence of hepatocarcinogenicity in rats. Osborne-Mendel rats (50 of each sex) were fed diets containing 800 ppm (high-dose) and 400 ppm (low-dose) of analytical-grade chlordane (NCI, 1977; NTP, 1977). These levels were reduced during the course of the study because signs of toxicity were observed in the treated animals. Time-Weighted Average (TWA) of chlordane for high and low dose groups were 203.5 and 407.0 ppm in males, and 120.8 and 241.5 ppm in females, respectively. Matched controls consisted of 10 untreated rats of each sex; pooled controls for each sex were obtained by combining the matched controls with 60 controls from similar bioassays of other chemicals. The treatment lasted 80 weeks and the rats were observed for an additional 29 weeks. After 109 weeks, the survival rate was approximately 50% in both treated and control males, and 60% in treated females and 90% in control females. In contrast to the NCI mouse bioassays, there was no increase in the incidence of liver tumors in rats treated with chlordane compared to controls.

However, the validity of this study is limited because of the high mortality rate (40 to 50%) and the shorter exposure time of 80 weeks instead of the 104 weeks for a standard long-term cancer bioassay (time frame used currently by the National Toxicology Program). A review panel of pathologists from the National Academy of Sciences (NAS) noted an increase in the incidence of follicular-cell thyroid neoplasms in the treated female group at the highest dose ($p<0.05$) of chlordane (IARC, 1991).

In a regulatory study conducted by Velsicol Chemical Co. to establish a no-observed-effect-level (NOEL) for chlordane, graded doses (0, 1, 5, and 25 ppm) of technical chlordane were fed in the diet to 80 Charles River Fischer 344 rats of each sex for 130 weeks (Khasawinah and Grutsch, 1989b). There was an increased incidence of hepatocellular adenomas only at the 25 ppm dose level in males ($p<0.08$). This effect was only observed after 130 weeks of exposure, and was not found in lower dose males or in any females at 104 weeks. The liver foci were not apparent upon gross examination, but were only discovered by microscopic examination of histological slides. A panel of six pathologists was convened by Velsicol to re-evaluate the liver pathology slides from this study. This Pathology Working Group (PWG) concluded that there was no statistically significant difference in the incidence of hepatocellular adenomas in the male rats (as summarized in IRIS, 1992; IRIS 1998; USDA, 1997). Based on the peer-review of the liver pathology, a NOEL of 25 ppm was assigned for chlordane for male rats. Liver lesions (hypertrophy) were observed in the female rats at 5 ppm, and therefore a NOEL of 1 ppm was established for female rats (IRIS, 1992; Khasawinah and Grutsch, 1989b).

3. Summary, Experimental Animal Studies (non-breast sites):

In summary, administration of chlordane through the diet has been found to increase the incidence of hepatocellular carcinomas in both male and female CD-1 (Epstein, 1976), and B6C3F1 mice (NCI, 1977; NTP, 1977); hepatocellular adenomas in male Charles River (ICR) (SPF) mice (Khasawinah and Grutsch, 1989a); and hepatocellular adenomas and carcinomas in C5751/10J male mice (Barrass et al., 1993). Chlordane also increased the incidence of hepatocellular carcinomas in C57BL/6N male mice that are historically known to be resistant to spontaneous liver tumors (Becker and Sell, 1979). In contrast, administration of chlordane in the diet did not induce liver tumors in either sex in Fischer 344 rats (Khasawinah and Grutsch, 1989b), or in Osborne-Mendel rats (NCI, 1977; NTP, 1977). An increase in the incidence of follicular-cell thyroid neoplasms was noted in females rats that were fed the highest dose of chlordane in the NCI cancer bioassay (IARC, 1991; NCI, 1977; NTP, 1977).

C. Current Classification of Carcinogenicity by Other Agencies:

1. IARC Classification:

The International Agency for Research on Cancer (IARC) has determined that there is inadequate evidence in humans for the carcinogenicity of chlordane, but that there is sufficient evidence in experimental animals for the carcinogenicity of chlordane, based on liver neoplasms of mice and follicular-cell thyroid neoplasms in female rats treated with chlordane. The overall evaluation of IARC is that chlordane is "possibly carcinogenic to humans" and consequently assigned chlordane to Group 2B (IARC, 1991).

2. NTP Classification: Not classified

3. EPA Classification:

The EPA (IRIS, 1998; USEPA, 1988, USEPA, 1997) has used a weight-of-evidence approach to evaluate the carcinogenicity of chlordane. The agency has determined that the evidence for carcinogenicity of chlordane is "sufficient" in animals. This is based on studies that have demonstrated that chlordane induces benign and malignant liver tumors in both sexes of multiple strains of mice, and that chlordane is structurally similar to other rodent liver carcinogens (IRIS, 1998). However, the EPA has found that the evidence for chlordane carcinogenicity from human studies is "inadequate" (IRIS, 1998; USEPA, 1988). The agency estimated a potency factor (F) of 15.13 mg/kg/day, which places chlordane in the potency group 2 (USEPA, 1988). Chlordane has been classified by the EPA as a "probable human carcinogen, Group B2" based on the 1986 Guidelines for Carcinogen Risk Assessment (IRIS, 1992; IRIS, 1998; USEPA, 1988). Under EPA's 1996 proposed guidelines for carcinogenicity assessment, chlordane would be characterized as a "likely carcinogen" by all routes of exposure (IRIS, 1998).

V. Critical Evaluation of Breast Cancer Risk:

A. Experimental Animal Studies of Breast Carcinogenicity:

To the best of our knowledge, there have been no reports of increased mammary oncogenicity in chlordane-treated laboratory animals.

B. Human Studies on Breast Cancer Risk:

1. Human Tissue Levels:

There is little information on the metabolism of chlordane following acute exposure to this chemical. One case of chlordane poisoning in a two year child estimated that the half-life of chlordane in the serum was about 21 days. Adipose tissue levels of chlordane rose in the week following ingestion of a solution of technical chlordane, indicating tissues redistribution of this chemical. Levels peaked at 40 mg/kg eight days post-ingestion, and dropped slightly to 25 mg/kg 94 days after the poisoning (Curley et al., 1969). This was one of the first reports indicating that chlordane can be stored in the fatty tissues of the body.

Other studies have also shown that chlordane metabolites have a high affinity for lipids and can accumulate in fat tissues of humans. The National Human Adipose Tissue Survey (NHATS) has reported that levels of the chlordane metabolite oxychlordane in the general U.S. Population ranged from 0.09 to 0.12 ppm (lipid adjusted geometric means) for the period 1971-1982 (Kutz et al., 1991). Levels of the persistent component of technical chlordane, *trans*-nonachlor, ranged from 0.04 to > 0.1 ppm in human adipose tissue in the U.S. Population for the period 1973-1983. These chlordane compounds and metabolites are very persistent and can remain in the body for several years.

2. Human Breast Milk Levels:
Breast milk has a high lipid content, is an excretion route of chlordane, and therefore, a means of exposure for breast-fed infants.

Chlordane contamination of human milk samples has been reported in the U.S. A study of chlorinated hydrocarbon insecticide residues in 1,436 human milk samples obtained throughout the U.S. (Savage et al., 1981) reported that oxychlordane, the most prevalent metabolite of chlordane, was above the detection limits in 74% of the samples, with levels ranging from 75.5 ± 51.4 to 116.3 ± 156 ppb (mean ± SD). Levels of oxychlordane varied according to geographic region. The highest mean levels within the U.S. were reported in the Southeast (116.3 ppb) and the Southwest (109.4 ppb), while lower levels were reported in the Northwest (75.4 ppb), Midwest (80.6 ppb), and the Northeast (81.4 ppb) (Savage et al., 1981). These geographical differences may be due to different patterns of chlordane use as a termiticide, since termites are most prevalent in the warm regions of southern U.S. It should be noted that these figures are from human milk samples obtained in the late 1970s to the early 1980s, and more recent estimates of chlordane contamination in U.S. milk samples are not available. It has been estimated that the daily consumption of oxychlordane by a 5-kg breast-fed infant in the U.S. is 0.098 µg/kg body weight, well below the World Health Organization's "allowable daily intake" (ADI) of 0.5 µg chlordane /kg body weight (Rogan, 1996).

Chlordane was not widely used in Europe so the levels in human breast milk reported in the 1980s were low and usually below detection limits (Jensen, 1991; Mussalo-Rauhamaa et al., 1988). This is in contrast to widespread contamination of the chlordane metabolite oxychlordane in human breast milk in Victoria, Australia, with 79% of samples containing oxychlordane at a mean level of 0.13 ppb milk fat (Quinsey et al., 1995). Some of the highest levels of chlordane residues have been detected in the breast milk samples from other countries, including Mexico and Iraq (>2 ppm chlordane in milk fat) (Jensen, 1991).

Because of the persistence of chlordane and past reports of contaminated breast milk, researchers have evaluated if breast feeding an infant poses a future cancer risk. A study from the National Institute for Environmental Health Sciences (NIEHS) has compared lives saved in the postneonatal period by breast feeding, to the estimated excess cancer mortality attributable to contaminants in breast milk, and concluded that there is not sufficient evidence to advise against breast feeding (Rogan et al., 1991). This, and a subsequent study with commentary concluded that "in the vast majority of women, the benefits of breast-feeding appear to outweigh the risks..."(Rogan, 1996).

3. Human Case-Control Studies of Breast Carcinogenicity:
The ability of chlordane to bioaccumulate in fat tissues of the body and cause cancer in mice have urged some investigators to evaluate possible associations between breast cancer and mammary fat levels of chlordane components, and its metabolites. Only a few, very small case-control studies have been conducted to determine if there is a possible association between tissues levels of chlordane, and/or its metabolites, and the risk for breast cancer. These studies are not of sufficient size to have the statistical power to accurately assess whether there is or is not an association between body burdens of chlordane and breast cancer risk. They can only be considered to be pilot studies. Results of these studies are presented in Table 1, and are summarized below.

One study reported that the levels of heptachlor epoxide plus oxychlordane (both metabolites of technical chlordane) in the breast fat of 20 patients with breast cancer were slightly elevated, but were not statistically different from those of 20 control patients with benign breast disease (Table 1) (Falck et al., 1992). Nonsignificant differences were seen between the levels of *trans*-nonachlor in breast cancer patients and those in controls with benign breast tumors. It would have been more appropriate to have used surgical controls who were free of cancer and other breast diseases, rather than using controls with benign breast disease. Although patients and controls were matched for height, weight and smoking history, other confounding variables were not taken into consideration, including race, age of menarche, reproductive history, lactation history, menopausal status and socioeconomic status. It was also not determined if any of the breast cancer patients had had any recent weight loss. Weight loss can result in the mobilization of fat stores, and the temporary elevation of levels of organochlorines in the blood.

In another case-control study, a supercritical fluid extraction method was used to measure the levels of several chlorinated pesticides and polychlorinated biphenyls (PCBs) in breast adipose tissue from five breast cancer patients and five women without breast cancer (Table 1) (Djordjevic et al., 1994). The levels of the chlordane metabolite, oxychlordane and *trans*-nonachlor, a persistent component of technical chlordane, were higher in samples from breast cancer patients compared to hospital controls.

This study has severe limitations because of weaknesses in the design of the study. The study was based on very small sample sizes (n=5 in both groups) and there was a high variability in levels

within each group. No information was available on patient characteristics in breast cancer and control groups, and no attempt was made to control for confounding variables.

Dewailly et al. (1994) conducted a case-control study comparing breast fat levels of organochlorine residues between nine patients with estrogen receptor (ER)-positive breast carcinomas, nine patients with ER-negative breast carcinomas, and 17 patients with benign breast disease (controls). The ages and parity of the breast cancer patients and controls were similar. However, this study also failed to control for other breast cancer risk factors such as reproductive history, menopausal status, smoking, or socioeconomic status. The levels of oxychlordane and *trans*-nonachlor were higher in the breast fat of patients with ER-positive carcinomas compared to those of control patients, though the differences were not statistically significant (Table 1). In contrast, levels of chlordane metabolites in ER-negative breast cancer patients were actually lower than control subjects, although these differences were also not significantly different. The relationship to ER status in breast cancer patients, and their body burdens of chlordane should be further explored in larger case-control studies that uses control subjects free of breast disease.

In conclusion, there is inadequate evidence in the available human studies for a causal relationship between chlordane exposure and breast carcinogenicity. All three of these studies were based on a small number of individuals, less than 25 per group, which limits the power of the statistical tests. In several of these studies, control subjects were not always free of breast disease, nor were they matched for other established breast cancer risk factors, and other confounding factors. Larger population-based case-control studies will be required to further investigate whether higher body burdens of chlordane metabolites increase the risk of developing breast cancer.

Table 1. Breast adipose tissue concentrations (ng/g) of organochlorine residues in women with breast cancer (cases) or benign breast disease (controls)

Authors	**Chemical**	**Controls***	**Cases***	**p**
Falck et al.,	Oxychlordane [a]	97±49 (20)	116±50 (20)	0.22
1992	*Trans*-nonachlor	96±80 (20)	87±37 (20)	0.65
Djordjevic	Oxychlordane	16.8±16.6 (5)	32.7±23.7 (5)	NA
et al., 1994	*Trans*-nonachlor	22.4±22.5 (5)	29.7±16.1 (5)	NA
Dewailly	Oxychlordane	31.1±12.4 (17)	26.8±7.4[b] (9)	0.59
et al., 1994			38.9±13.8[c] (9)	0.12
	Trans-nonachlor	42.5±17.8 (17)	34.8±8.3[b] (9)	0.37
			50.8±11.1[c] (9)	0.07

[a] oxychlordane + heptachlor epoxide
[b] estrogen receptor-negative
[c] estrogen receptor-positive
NA = Not available
*Mean ± SD (n = number of subjects)

C. Other Relevant Data on Breast Cancer Risk:

1. Oncogene Activation:

Studies were conducted to determine if the induction of chlordane-dependent hepatocellular carcinomas in male mice was dependent on the activation of *ras* oncogenes (Malarkey et al., 1995). Technical grade chlordane was fed continuously to B6C3F1, and B6D2F1 male mice at 55 ppm starting at 9 weeks of age. Untreated male mice served as controls. Animals were selected randomly for sacrifice at 15 to 50 day intervals, starting at 408 days of age, and were examined for the presence of tumors. By the end of the study, the incidence of the chlordane-induced liver tumors approached 100% in both strains of mice. DNA was isolated from the hepatocellular tumors of 30 chlordane treated, 10 control B6C3F1 mice, and 20 chlordane treated B6D2F1 mice (spontaneous liver tumors were not detected in the B6D2F1 mice). The samples were used for DNA amplification, and subsequent screening of H-*ras* and K-*ras* mutations. *Ras* mutations were not detected in any of the chlordane-induced liver tumors in treated mice of either strain. The authors concluded that the development of hepatic tumors in chlordane-treated animals is independent of *ras* oncogene activation (Malarkey et al., 1995).

2. Mutagenicity:

Chlordane was found to be genotoxic in yeast (Chambers and Dutta, 1976; Gentile et al., 1982) and human fibroblasts (Ahmed et al., 1977). Results of numerous mutagenicity tests of chlordane in bacteria, including *Salmonella typhimurium* and *escherichia coli*, have been summarized by IARC; chlordane was not found to be mutagenic in these tests (IARC, 1991). Chlordane was found to be non-genotoxic to cultured rat liver and human cells (Ruch et al., 1990). Chlordane was not mutagenic in Chinese hamster lung cells (Tsushimoto et al., 1983) and did not induce dominant lethal changes in male albino mice (Arnold et al., 1977). Moreover, in Syrian hamster embryo (SHE) cells, chlordane inhibited intercellular communication and potentiated the transforming effects of 12-O-Tetradecanoylphorbol-13-acetate (TPA) at concentrations that did not induce DNA adduct formation (Bessi et al., 1995).

3. Evidence of Tumor Promotion:

B6C3F1 male mice were treated with 20 ppm diethylnitrosamine (DEN), a known liver carcinogen, in drinking water for 14 weeks, and then fed a diet containing 25 or 50 ppm technical grade chlordane for 25 weeks. Approximately 80% of the chlordane treated mice developed liver tumors, compared to 40% liver tumor incidence in mice that received a control diet after DEN-pretreatment (Williams and Numoto, 1984). These results suggest that chlordane is a promoter of liver tumors in male B6C3F1 mice.

Chlordane has also been found to stimulate the activity of protein kinase C (PKC) in the brain, epidermal, and liver cells of mice *in vitro* (Moser and Smart, 1989). PKC may play a critical role in tumor promotion. PKC is thought to phosphorylyate serine and threonine residues of critical target proteins which regulate expression of genes associated with tumor promotion. More studies are needed to determine if chlordane or its metabolites could act as breast tumor promoters in the presence of known mammary carcinogens.

4. Signal Transduction and Intercellular Communication:

Gap junctional intercellular communication (GJIC) plays an important role in the regulation of cell proliferation and differentiation. Chlordane has been found to inhibit GJIC in mouse and rat hepatocytes (Ruch et al., 1990). It is possible that the inhibition of GJIC represents one possible mechanism by which chlordane promotes the formation of tumors in the mouse liver (Williams and Numoto, 1984). Many non-genotoxic chemical carcinogens and tumor promoters inhibit GJIC *in vitro* and *in vivo* (Ruch et al., 1990). A recent study has shown that heptachlor, a constituent of technical chlordane, and its metabolite heptachlor epoxide also inhibit GJIC in normal human breast epithelial cells at noncytotoxic concentrations (Nomata et al., 1996).

5. Oxidative Stress:

Studies in adult male rats indicate that oral administration of chlordane (120 mg/kg) can induce significant increases in hepatic lipid peroxidation and DNA single strand breaks. This suggests that chlordane is capable of inducing oxidative tissue damage, perhaps through the generation of free radicals or reactive oxygen species. Further work is needed to determine if these oxidative stresses play a role in the chlordane-induced liver carcinogenesis (Hassoun et al., 1993).

6. Summary of Genotoxic and Promotion Effects:

Although there are conflicting reports on the genotoxicity of chlordane, the evidence points to an epigenetic mechanism of action including interruption of cell-cell communication or through cytotoxic effects which stimulate compensatory cellular proliferation. In most of these mechanisms chlordane is more likely to be promoting rather than initiating tumors (IARC, 1991). Chlordane has been found to be a liver tumor promoter (Williams and Numoto, 1984), and there is evidence that it has the ability to stimulate the activity of PKC, which is known to play a role in the promotion of certain tumors (Moser and Smart, 1989). An analysis of chlordane-induced hepatocellular carcinomas in mice showed no indication of activated H-*ras* or K-*ras* mutations in these tumors, and it is likely that chlordane-dependent tumors develop independently of *ras* oncogene activation (Malarkey et al., 1995). There is some evidence that chlordane can induce hepatic lipid peroxidation and DNA single strand breaks. This suggests that free radicals or other pathways that involve the generation of reactive oxygen species may be important in chlordane-mediated carcinogenesis.

7. Disruption of the Endocrine System:
A number of studies have described the ability of technical chlordane and its metabolites to disrupt endocrine pathways. Disturbance of the endocrine system may occur through changes in the activity of liver microsomal enzymes which are important in the metabolism and degradation of ovarian steroids and thyroid hormones. Endocrine disruption may also occur at the level of the target tissues. The significance of these studies are discussed in more detail below.

a. Hepatic Microsomal Hydroxylases and Steroid Metabolism:
Studies conducted in the late 1960s and early 1970s investigated the ability of chlordane to affect the metabolism of steroids in the liver. When chlordane was administered to immature male rats for 10 days by intraperitoneal injection (i.p) (25 mg chlordane/ 2X/day), this treatment stimulated the microsomal hydroxylation of testosterone, estradiol-17β, progesterone, and deoxycorticosterone to more 'polar metabolites.' Additional experiments indicated that chlordane treatment stimulated the 16-alpha hydroxylation of testosterone (Welch et al., 1967). Pretreatment of immature, or ovariectomized female rats with chlordane also has been reported to increase levels of hepatic microsomal enzymes that metabolize estradiol-17β, resulting in an increased formation of polar metabolites (Levin et al., 1968; Welch et al., 1971). Other studies have indicated that progesterone metabolism in young male rats may be altered by a chlordane-induced stimulation of liver microsomal enzymes (Conney et al., 1966).

These studies suggest that chlordane can affect the metabolism of a variety of steroids, included estradiol-17β and progesterone, both of which are important in stimulating breast cell proliferation in the mature breast (Imagawa et al., 1994). Long-term exposure to estradiol and other ovarian steroids has been associated with increased breast cancer risk in women (Dorgan et al., 1997; Harris et al., 1992; Pike et al., 1993). It would be important to determine how the chlordane-induction of steroid hydroxylases affects both circulating levels of estradiol available to the breast, as well as determining the estrogen metabolites generated during the hydroxylation, and if these forms of estrogen can affect breast cancer risk.

In regard to circulating levels of estradiol, while the early studies of Levin and Welch did show an increased clearance of estradiol in the liver with chlordane pretreatment in rats, none of these studies measured circulating levels of estradiol in response to chlordane treatment (Levin et al., 1968; Welch et al., 1967; Welch et al., 1971). Some of these studies did evaluate the uterotropic response (a classic test of estrogenicity) to injected radiolabled estradiol after chlordane-pretreatment in female rats. This resulted in a decreased uterine weight and decreased uptake of labeled estradiol into the uterus (Levin et al., 1968; Welch et al., 1971). Whether this was due to the metabolism of estradiol to more polar, less estrogenic metabolites, or whether chlordane interfered with the ability of the radiolabled estradiol to bind to its receptor in the uterus, is not known. Future studies should determine: (a) if chlordane treatment can result in long-term effects on circulating estradiol levels, and (b) the effect of this pesticide on estradiol metabolism, and ligand-receptor binding.

Also, there has been a recent interest in whether estrogen 'polar metabolites' have the capacity to affect breast cancer risk. Since then several researchers have hypothesized, and have offered preliminary evidence, that the stimulation of p-450 microsomal hydroxylation pathways by some organochlorine pesticides yield estrogen metabolites that may increase breast cancer risk, while other hydroxylation pathways yield metabolites that may decrease breast cancer risk (Bradlow et al., 1995; Davis et al., 1997). The estrogen-related polar metabolite associated with an increased breast cancer risk is called 16-alpha hydroxyestrone (16-OHE1), and the hydroxylated metabolite associated with possible decreased breast cancer risk is called 2-hydroxyestrone (2-OHE1). Some studies have suggested that 16-OHE1 can enhance breast cell growth, increase unscheduled DNA synthesis, and increase anchorage independent growth, while the 2-OHE1 does not have any of these properties, is a very weak estrogen, and may even be protective against breast cancer (Davis et al., 1997; Suto et al., 1993; Telang et al., 1992a; Telang et al., 1992b; Telang, 1996; Tiwari et al., 1994). Whether chlordane can affect 16-OHE1 and 2-OHE1 metabolism in humans, and subsequently affect breast cancer risk is currently under investigation as a part of the Long Island Breast Cancer study. This study will determine serum levels of chlordane and chlordane metabolites, and the urinary levels of estrogen metabolites, in Long Island women with and without breast cancer (see Section IX. for a summary of this study which is currently in progress).

Studies with the MCF-7 breast tumor cell line have shown that some pesticides, including DDT and atrazine, decreased the amount of 2-OHE1 formed by these cells while increasing the levels of 16-OHE1 formed (Bradlow et al., 1995). Studies have not determined the effect of chlordane administration on the formation of these hydroxylation products *in vivo* or *in vitro*. Therefore, further studies are needed to determine which p-450 dependent hydroxylation pathways are induced by chlordane, and if the estrogen metabolites generated are potentially genotoxic to breast cells.

b. Other Evidence of Endocrine Disruption at Target Sites:
The brain is also a target tissue for the endocrine disruptive action of chlordane. Exposure of rats to chlordane during pre- and postnatal development, when the brain is undergoing sexual dimorphic organization, has been shown to masculinize sexual dimorphic functions and behaviors (Cassidy et al., 1994).

Gonadal steroids are not the only hormones with altered metabolism in response to chlordane. Chlordane-stimulated activity of liver microsomal enzymes has also been associated with an increase in peripheral metabolism of thyroid hormones. This results in a subsequent increased release of thyroid stimulating hormone (TSH) from the pituitary, which may increase the risk of thyroid gland tumors by a secondary mechanism (Capen, 1992).

c. Evidence of Estrogenicity:
Chlordane does not appear to be estrogenic, as evidenced by its inability to stimulate proliferation in MCF-7 cells. These cells are a estrogen-dependent breast tumor cell line used in the E-SCREEN assay to test estrogenicity of xenobiotics (Soto et al., 1995). The inability of chlordane to stimulate cell proliferation in MCF-7 breast tumor cells was later confirmed by Verma and associates (Verma et al., 1997). Other evidence of chlordane's lack of estrogenicity includes its inability to displace tritiated 17β–estradiol from human or alligator-derived estrogen receptor (Arnold et al., 1997).

While initial reports indicated that chlordane and other organochlorines may synergize the estrogenicity of combinations of organochlorine pesticides in a yeast estrogen system transfected with human estrogen receptor (Arnold et al. 1996), these findings were called into question by several researchers (Asby et al., 1997; Ramamoorthy, 1997a; Ramamoorthy, 1997b). Citing the difficulties in reproducing the synergistic estrogenic effects of mixtures of pesticides by their own lab, and others, the original report published in *Science* by Arnold et al. (1996) was formally retracted (McLachlan, 1997). Therefore, there is currently no evidence that chlordane can synergize the estrogenicity of other organochlorine pesticides.

There is little information on the estrogenicity of other components of technical chlordane, or chlordane metabolities. One preliminary report has found that *trans*-nonachlor, a persistent environmental contaminant, and component of technical chlordane, does weakly inhibit the binding of tritiated 17β-estradiol to the alligator estrogen receptor (aER) (Vonier et al., 1996). This experiment was conducted with aER because there was concern that environmental contaminants in Florida lakes, such as *trans*-nonachlor, may be responsible for observed reproductive problems in alligators. These results need to be confirmed in both *in vivo* uterotropic estrogenicity tests, and in *in vitro* cell lines that have been transfected with human estrogen receptor.

8. Immunological Effects:
A compromised immune system may affect host defenses against cancer. There is some evidence, as discussed below, suggesting that exposure to chlordane adversely affects immune response in animals, especially in the developing fetus.

Male and female offspring of pregnant mice that were fed 8 mg analytical grade chlordane/kg/day throughout gestation were found to have a significant reduction in cell-mediated immune response ($p < 0.01$), measured as the contact hypersensitivity to oxazolone (Spyker-Cranmer et al., 1982). In a more recent study, a gender-specific sensitivity to prenatal chlordane exposure has been observed (Blyler et al., 1994). Bone marrow cells isolated from 100 day old female offspring of mice that had received 8 mg per day chlordane for 18 days during gestation showed a significant reduction (65 ± 1.2 %) in their granulocyte-macrophage colony-forming unit (GM-CFU) response to mitogen stimulation. Bone marrow cells from female progeny of unexposed mice and prenatally exposed males were found to be unaffected (Blyler et al., 1994). In another study using the same experimental animal model and exposure route, prenatally exposed female mice were shown to have depressed macrophage cytokine production in response to tumor cells (tumor type not specified) (Lau et al., 1990). In a tumoricidal induction assay, macrophages from mice that were exposed to chlordane *in utero* showed a delay in cytotoxicity against P815 mastocytoma target cells (Theus et al., 1990).

These studies in mice indicate that prenatal exposures to chlordane could impair the developing immune system of the fetus, particularly the development of the macrophage lineage of cells. Since macrophages provide immune surveillance against cancer cells for the body (Tizard, 1995), suppression of this surveillance system could compromise the body's defense systems against breast and other cancers. Future animal studies need to determine if chlordane exposure *in utero* can affect the tumorigenic response of the progeny to challenges with transplants of mammary tumor cells in multiple animal species, including the rat and mouse.

While altered immune function has been reported consistently in mice to prenatal-exposures to chlordane (Blaylock and Mehendale, 1995; Blyler et al., 1994; Lau et al., 1990; Menna et al., 1985; Theus et al., 1990), the immunotoxic effects reported for exposed adult animals were more variable. Immune responses in chlordane-exposed adult mice were unaffected (Johnson et al., 1986), but immune responses were suppressed in lymphocytes that were isolated from adult rhesus monkeys and then exposed to chlordane (Chuang et al., 1992). The developing fetal immune system may be particularly sensitive to chlordane exposures. More extensive studies are needed to determine if adult exposures to this chemical can alter immune response and susceptibility to cancer.

Whether chlordane exposure affects immune response in humans, and subsequent susceptibility of humans to cancer, is not known. There is some data available on the effect of chlordane exposure and immune response in humans. One study reported that 11 out of 12 individuals exposed to chlordane showed some level of increased titer of autoimmune antibodies, suggesting that chlordane mediated a deregulation of the immune system. This study had severe limitations, since exposures were variable and self-reported; the study included individuals exposed to chlordane by accidental

ingestion, dermal soaking, and inhalation of chlordane vapors from treated buildings (McConnachie and Zahalsky, 1992). Chlordane-exposed populations should be followed, not only for actual cancer incidences, but also for their immune responsiveness to cancer, to better determine if this pesticide may affect cancer risk by compromising the immune surveillance system.

VI. Other Relevant Information:

A. Environmental Fate and Potential for Human Exposure:
Chlordane is extremely persistent in the environment. It has been estimated that 70,000 tons of chlordane were produced since 1946, and 25 to 50% is thought to exist unaltered in the environment (ATSDR, 1994).

1. Soils and Sediments:
Chlordane has a half-life in soil of 4 years (USEPA, 1987). It strongly adsorbs to particles in the upper layers of soils, and has been detected in run-off, both from urban termiticide use, and from its past use on agricultural crops (ATSDR, 1994). A recent report on the concentrations of chlordane and chlordane metabolites in river and stream bed sediments from the Hudson River Basin of NYS found a higher frequency of detectable residues in samples from urban watersheds (75%) compared to non-urban watersheds (less than 5%) (Phillips et al., 1997). This would be expected, since chlordane's major use from the mid-1970s to 1988 was as a termiticide in urban areas. Because of contamination of waterways, and lake and river sediments, ingestion of chlordane-contaminated sea food constitutes a route of exposure to humans.

2. Fish:
Several studies in the mid-1980s when chlordane was still in use as a termiticide, have determined the concentration of chlordane and chlordane metabolites in fish. DeVault (1985) reported on gamma-chlordane, and oxychlordane concentrations from fish caught in ten rivers in the Great Lakes area. With the exception of fish from the Shebogan River in Wisconsin that had levels in the 0.2 to 0.47 mg/kg range, fish from all other rivers had levels less than 0.002 mg/kg (the detection limit). Levels of oxychlordane in the fish in these waterways were also very low, and ranged from 0.05 to 0.47 mg/kg. The National Biomonitoring Program monitored residues of chlordane in U.S. freshwater fish from 112 stations in major rives and lakes (Schmitt et al., 1990). A report on organochlorine residues in fish caught during the time period of 1976 to 1984 included data on *cis*-chlordane, *trans*-chlordane, *trans*-nonachlor, and oxychlordane residue levels. During this time period, the percentage of stations with positive detections declined from 92.5% to 84.8% for *cis*-chlordane, the most abundant component of technical chlordane, and also declined for *trans*-chlordane (84% to 68.8%), but increased for *trans*-nonachlor (70.8% to 89.3%), one of the most persistent components of technical chlordane. Oxychlordane figures were not available for 1976-77, but 44.6% of the stations had positive detections of this persistent chlordane metabolite in 1984. However, despite the widespread contamination, the levels of chlordane and associated chemicals were low. Mean levels of *cis*-chlordane were 0.06 mg/kg in 1976-77, and declined to 0.03 mg/kg in 1984. Mean levels of *trans*-chlordane were 0.02 mg/kg, and oxychlordane levels averaged 0.01 mg from 1976 to 1984. *Trans*-nonachlor levels were variable, and mean levels ranged from 0.03 to 0.05 mg/kg. Relatively high individual values for one or more chlordane compounds was reported in the Great Lakes, watersheds of the Ohio River, Missouri River, the Delaware and Raritan Rivers, and in the Northeast. The highest level of chlordane-related compounds was at Monoa Stream in Hawaii which drains a suburban watershed. The authors noted that these high levels may have been indicative of termite control efforts in Hawaii (Schmitt et al., 1990).

3. Residential Exposures:
Chlordane can remain in some soils for over 20 years and has been detected in the indoor air of homes 15 years after treatment. EPA has estimated that about 52 million people have lived in homes treated with chlordane. In addition, the vapor phase of chlordane that forms in water or that results from soil surface treatment readily evaporates and travels in the atmosphere, contaminates and bioaccumulates in species far from its source (ATSDR, 1994). One of the first requests for a risk-benefit analysis of chlordane by the EPA was initiated because of evidence that chlordane was migrating from the soil under slab construction houses at military bases at Wright-Patterson and Scott Air Force bases in Dayton, Ohio, and Bellview, Illinois into air ducts for the heating and cooling of these homes (1981). No follow-up studies of cancer incidence in military personnel or their families who lived in these homes could be located.

Among non-work related exposures, the populations with an increased risk of exposure to chlordane include those who have lived in buildings treated with chlordane for termite control, and those who have dug around foundations of chlordane-treated buildings. Consequently, activities such as gardening close to the foundation, or children playing with soil near foundations of chlordane treated homes, can still result in exposure to this insecticide. The majority of buildings treated with chlordane are located in the deep South of the U.S., though moderate to heavy use of chlordane has been documented in Pennsylvania, the lower New England States, to the west to Colorado, and up to Northern California (ATSDR, 1994).

There are some cases where chlordane was used extensively to protect structures in the Northeast. Areas that used termiticides to treat structures in NYS included the Pine Bush area of Albany, parts of Saratoga County, and Sulffolk and Nassau counties of Long Island, NY. In 1982, a misapplication of the termiticide aldrin occurred at a residence on Long Island. Subsequent publicity of health problems of occupants that may have been caused by the

misapplication, resulted in the NYS Department of Environmental Conservation (DEC) opening a telephone hot-line to allow the public to report suspected misapplications of termiticides. At that time in NYS, over 95% of the termiticide applications involved the use of chlordane (NYSDEC, 1986). Studies were undertaken to investigate suspected termiticide misapplications by the NYS DEC and the NYS Department of Health (DOH).

In the first study (NYSDOH, 1985), concentrations of termiticides in the air were determined in living and nonliving spaces (i.e., unfinished basements, crawlspaces, laundry rooms and attics) of 515 structures from 17 NYS counties. Most of the 1284 air samples were obtained from Long Island counties; 198 samples from Nassau County and 770 from Suffolk County. It was found that 6% of the air samples taken from living spaces exceeded the National Academy of Science (NAS) suggested guideline of 5.0 μg chlordane/m^3, while 32% of the samples from nonliving spaces exceeded the NAS guideline. The second study obtained hexane swab samples of areas of suspected misapplications inside the homes (93% of the samples), as well as some soil samples. Of the 1248 swab samples taken, 58% had chlordane samples less than 1 μg, while 42% exceeded this level. The maximum level reported per swab was 62,000 μg (NYSDEC, 1986).

A study was undertaken to follow air levels of chlordane over time in chlordane-treated homes. Residents of treated homes were exposed to the highest concentrations of chlordane in the first 24 hours after termiticide application. The levels plateaued seven days after application. Thirty to 180 days after chlordane application, chlordane levels in the air were 4 to 17 fold lower than ambient air levels suggested by the National Research Council (NRC). Of concern were air levels of heptachlor, a termiticide that is also present in technical chlordane. Air levels of heptachlor were consistently higher than levels of chlordane, and these levels were similar to or higher than quantities allowed by the NRC guidelines (Kamble et al., 1992).

The EPA has initiated a series of studies to assess and improve the methodology used to measure exposure to pesticides in nonoccupational settings. Whitmore et al. (1994) determined the exposure to chlordane and other pesticides via inhalation and dietary routes in residences in area of relatively low pesticide use, Chicopee/Springfield, MA, compared to a city with relatively high domestic use of pesticides, Jacksonville, FL. To estimate seasonal variations in exposure, samples were taken in the summer of 1986, the spring of 1987, and the winter of 1988. Forty-nine to 72 persons participated per site, per season. Inhalation exposure was measured by analyzing 24-hour indoor, personal and outdoor air samples. Dietary exposure to pesticides also was estimated through the use of a 24-hour dietary recall questionnaire, the values from the USDA Total Diet Survey's levels of pesticide residues on raw agricultural commodities, and by analyzing tap water samples. Mean air concentrations where higher in Jacksonville residences compared to Chicopee/Springfield residences. Seasonal variations indicated that in Jacksonville, the highest average levels of chlordane were detected in indoor air in the summer (324 ng/m^3, followed by 245.5 ng/m^3 in the spring, and 220 ng/m^3 in the winter. Outdoor air samples were 10 to 20 fold lower than indoor air samples in Jacksonville, FL. Mean air exposure to chlordane in Jacksonville was 3,942 ng/day while estimated dietary exposure was 180 ng/day in 1987. This study suggested that inhalation of indoor air , rather than diet, may be the major route of chlordane exposure in areas in the Southern U.S.

A pilot study conducted by the EPA evaluated methods to measure exposure of small children to pesticides in a residential environment (Lewis et al., 1994). This included use of a high-volume carpet dust sampler, hand rinses, bare-hand prints, and analyzed soil samples from play areas, walkways and entryways. Chlordane was detected in the carpet dust of five out of nine of the homes, and was up to 10 fold higher (mean 1.8 μg/g) than levels found in outdoor soils (0.15 to 0.17 μg/g).

4. Occupational Exposures:

There have been some estimates of exposure to chlordane and its metabolites in pesticide applicators (ATSDR, 1994; Kamble et al., 1992; Saito et al., 1986).

In a study of chlordane exposure in pesticide applicators, exposure to chlordane included dermal exposure, especially through the hands, forearms, and head, and through penetration of the pesticide through clothing (Kamble et al., 1992). Approximately 25% of the insecticide on the exterior surface penetrated through the clothing fabric.

The relationship between spraying conditions and concentrations of chlordane and its metabolites has been evaluated in 70 male pest control operators (Saito et al., 1986). The operators included 51 men who had been employed as pest control operators for 0.5 to 21 years (average 6.1 years), and 19 men with no previous experience spraying chlordane. No information was provided on the time elapsed between last exposure to chlordane and when blood samples were obtained from subjects, though there were some attempt to quantify exposure by calculating the number of spraying days in the last 12 months.

Among the 51 chlordane exposed workers, *trans*-nonachlor was detected in 37% of the men (mean=0.55 ppb); oxychlordane in 22% (mean=0.29 ppb); and heptachlor epoxide in 20% (mean=0.29 ppb). The authors commented that although *cis*-and *trans*-chlordane are the major components of technical chlordane, they were below the detectable levels because they were metabolized to oxychlordane. The concentration of chlordane and its metabolites were all below detectable limits in the 19 unexposed workers.

Further analysis indicated that the concentration of total chlordane in the blood (*trans*-nonachlor + oxychlordane + heptachlor epoxide) was significantly positively correlated to the number of spraying days in the last year ($r=0.78$, $p< 0.001$). However, the analysis of the data was biased, since only the 19 operators that had detectable levels of chlordane in their blood were included in the calculation of the correlation coefficient. Ironically, the study found the chlordane was detected more frequently in those pest control operators who always wore respirators compared to those who did not wear respirators. The 19 chlordane-exposed pesticide applicators that did have detectable levels of chlordane in their blood used significantly more of a concentrated chlordane preparation (40% emulsified concentrate) over the course of the year (average use of 172 L per year), compared to the 32 exposed applicators who did not have detectable chlordane in their blood (average use of 45 L per year).

5. Other Potential Exposures:
Individuals living near waste disposal sites for chlordane also may have been exposed to this chemical. Pesticide applicators, chlordane manufacturing workers, lawn care workers, and farmers who handed chlordane prior to when it was banned in 1988 may have been exposed to elevated levels of chlordane or its metabolites (ATSDR, 1994).

VII. Summary and Recommendation for Breast Cancer Risk Classification:

There is inadequate evidence to classify chlordane as a "human breast carcinogen. We propose that chlordane should be classified in Group 3, *not classifiable as to its breast carcinogenicity*" (see Appendix B for BCERF Breast Cancer Risk classification scheme). We base this conclusion on the following evidence:

• **Human studies are inadequate to conclude that chlordane exposure directly causes breast cancer in women.** Case-control studies on the breast carcinogenicity of chlordane in humans are inadequate because they were based on very few cases (less than 25 per group) limiting the statistical power. Results were inconsistent, did not provide exposure data, some used inappropriate controls, and did not adequately control for confounding breast cancer risk factors (Dewailly et al., 1994; Djordjevic et al., 1994; Falck et al., 1992).

• **There is no evidence that chlordane is a mammary carcinogen in long-term animal cancer bioassays.**

• **There is limited related evidence of the potential and mechanisms by which chlordane may affect breast cancer risk.** These other data include, but are not limited to, chlordane's persistency in the environment and the potential for continued exposure to human populations (ATSDR, 1994); the ability of chlordane to promote tumors (Williams and Numoto, 1984); interrupt gap junctional communications between cells (Bessi et al., 1995); increase the activity of liver microsomal enzymes; affect the metabolism of steroid hormones, including estrogens (Levin et al., 1968; Welch et al., 1967; Welch et al., 1971); and compromise immune response in animals prenatally exposed to this chemical (Blyler et al., 1994; Lau et al., 1990; Spyker-Cranmer et al., 1982). While this limited evidence can be used to generate testable hypotheses, more research needs to be conducted to see if chlordane can affect breast cancer risk by any of these mechanisms.

VIII. Identification of Research Gaps, and Other Recommendations:

• Larger case-control studies are needed to determine if women with breast cancer have higher levels of chlordane or chlordane metabolites in their blood and/or breast fat, than women without the disease (Some of these studies are in progress; please see Section IX.).

• Populations of women that were exposed to chlordane should be identified and monitored to determine if past exposures result in an increase in health-related effects, including increased incidence of breast cancer. This includes: 1) women exposed to chlordane in manufacturing facilities; 2) female spouses of men exposed to chlordane occupationally (agricultural workers, pesticide applicators, lawn care workers); and 3) female spouses and daughters of military personnel with known exposures to chlordane from volatilization of chlordane in soil beneath the slabs of treated homes that migrated to air ventilation systems of military housing.

• Animal studies are needed to evaluate chlordane's potential to be a co-carcinogen and or a tumor promoter of known mammary gland carcinogens such as 7, 12-dimethylbenz[*a*]anthracene (DMBA) and *N*- nitroso-*N* -methylurea (NMU).

• The disruptive effects of chlordane on estrogen metabolism and action warrants studies to determine the role of this termiticide in the etiology of breast cancer as well as other estrogen-sensitive tumors. Studies are needed to determine the effect of chlordane exposure on steroid metabolism in animal models. This includes evaluating the short-term, and long term effects of chlordane on: circulating blood levels of estradiol-17β, progesterone, thyroid hormone; the effects on binding of ligands to steroid receptors; and which P-450 dependent hydroxylation pathways are induced by chlordane, and if the hydroxylated estrone metabolites generated are potentially genotoxic to breast cells.

• Further studies are needed to determine if chlordane can compromise the immune system in ways that will affect the body's

defense mechanisms against cancer. It should be determined if animals exposed to chlordane *in utero*, or as adults, and subsequently exposed to transplantable mammary tumors cells, develop a higher incidences of mammary tumors. Studies following human populations exposed to chlordane should include an assessment of immune function, as well as cancer incidence, to determine if chlordane may affect cancer risk by compromising the immune system.

• More testing will be needed to determine if chlordane, in combination with other pesticides or environmental contaminants, has a synergistic effect or antagonistic effect on the estrogenicity of these mixtures. If synergistic combinations are identified, further studies will be needed to test the effect of the combined exposures on mammary carcinogenesis in long-term rodent studies. Further studies are also needed to test the estrogenicity of persistent components of technical chlordane, including *trans*-nonachlor, and chlordane metabolites, such as oxychlordane.

IX. Summary of New Human Studies Currently Being Conducted:

We have pointed out earlier the paucity and inadequacy of case-control studies on environmental exposure to chlordane and breast cancer risk in humans.

There are several studies that are currently being conducted to address this research need. The summaries of these studies provided below are adapted from abstracts in the 1997 and 1998 Computer Retrieval of Information on Scientific Projects Database (CRISP). CRISP is a searchable database of federally funded biomedical research projects conducted at universities, hospitals, and other research institutions that can be accessed via the World Wide Web at: <http://eos12.dcrt.nih.gov:8002/crisp_pilot/owa/crisp.main> or by Gopher <gopher://gopher.nih.gov:70/11/res/crisp>.

A. Breast Cancer and the Environment on Long Island (PI: M.D. Gammon, Columbia University School of Public Health, New York, NY)

The goal of this collaborative project is to determine whether environmental contaminants, including organochlorine pesticides, increase the risk of breast cancer among women on Long Island, New York. This investigation is a five-year, population-based case-control study. All new cases of breast cancer diagnosed during a 12-month period in residents from Nassau and Suffolk County, Long Island, NY will be included in this study. Population based controls will be matched to cases by five year age groups. Completed in-home interviews are expected for 80% of eligible subjects (1,623 cases and 1,623 controls). About 60% of all respondents are expected to provide biologic specimens (urine and blood). Laboratory analyses include determination of organochlorine compounds, including chlordane and chlordane metabolites, in the blood, and urinary markers of estrogen metabolism. Home samples of water, soil, and dust will be collected among women who have resided in their homes for 15 years or longer, and will be analyzed for organochlorine pesticides. For all respondents, historic environmental exposure to these compounds will also be calculated using geographic modeling techniques, and self-reports of occupational and residential exposure.

B. Organochlorine Residue Levels and Risk of Breast Cancer (PI: L. Bernstein, Univ. of Southern California School of Medicine, Los Angeles, CA)

This project is a case-control study to determine if there is an association between the levels of organochlorine residues in serum and increased risk of breast cancer among African-American women. The study will be added on to an ongoing study funded by the National Institute of Child Health and Development. Blood will be obtained from 300 African-American breast cancer cases and 300 controls in the Los Angeles area. These blood samples will be analyzed for serum organochlorine pesticide residues, including oxychlordane, and *trans*-nonachlor. Serum residue levels will be examined in relation to odds of breast cancer in a multivariate unconditional logistic regression model.

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XI. Appendix A. Common Abbreviations, Acronyms and Symbols:

ADI	Allowable Daily Intake, set by the World Health Organization
ATSDR	Agency for Toxic Substances Disease Registry
BCERF	Program on Breast Cancer and Environmental Risk Factors in New York State, based the Cornell's Center for the Environment, Institute of Comparative and Environmental Toxicology
bwt	body weight
C	carbon
CAS	Chemical Abstract Service
CfE	Cornell University's Center for the Environment
CI	Confidence Interval
Cl	chlorine
CRISP	Computer Retrieval of Information on Scientific Projects; database of scientific intra- and extra mural projects supported by the Dept. of Health and Human Services (i.e., NIH, EPA, USDA)
DEN	diethylnitrosamine; a carcinogen in the liver
DMBA	7,12-dimethylbenz[*a*]anthracene; known mammary carcinogen
DNA	deoxyribonucleic acid
EPA	United States Environmental Protection Agency
ER	estrogen receptor
E-SCREEN	screening assay for estrogenicity that measures proliferative response in estrogen-dependent breast tumor cells
FDA	Food and Drug Administration
GIJC	gap junctional intercellular communication
GM-CFU	granulocyte-macrophage colony-forming unit
HA	The health advisories are nonenforceable limits of the concentration of the chemical in the drinking water that is not expected to cause any adverse noncarcinogenic health effects when consumed for no more than the time period specified, with a margin of safety
IARC	International Agency for Research on Cancer, headquartered in Lyon, France
ICET	Institute for Comparative and Environmental Toxicology
i.p.	interperitoneal
IRDC	International Research and Development Corporation
IRIS	Integrated Risk Information System; database maintained by the EPA available through the National Library of Medicine MEDLARS system and on the World Wide Web.
kg	kilogram
L	liter
LI	Long Island, New York
µg	microgram
mg	milligram
MCF-7	Michigan Cancer Foundation; cells derived from human breast tumor
MCL	Maximum Contamination Level; enforceable limit set by the EPA which sets the maximum level of a contaminate in a public drinking water supply
n	number of subjects/animals in the group
NA	Not available
NAS	National Academy of Science
NHATS	National Human Adipose Tissue Survey
NHL	non-Hodgkin's lymphoma
NIOSH	National Institute of Occupational Safety and Health
NCI	National Cancer Institute
NIEHS	National Institute of Environmental Health and Safety
NIH	National Institutes of Health
NMU	*N*-nitroso-*N*-methyurea; mammary carcinogen
NOEL	No observed effect level
NOPES	The Non-Occupational Pesticide Exposure Study
NRC	National Research Council
NS	Not statistically significant
NTIS	National Technical Information Service; repository for federal agency technical reports
NTP	National Toxicology Program
NY	New York
NYS	New York State
OR	Odds Ratio
OSHA	Occupational Safety and Health Administration
PKC	protein kinase c
ppm	parts per million
ppb	parts per billion
ppt	parts per trillion
PWG	Pathology Working Group
RR	Relative Risk
RfD	Reference Dose
SD	Standard Deviation
SHE	Syrian hamster embryo
SMR	Standardized Mortality Ratio; SMR= the ratio of "observed" to expected" deaths
TMA	Time-weighted average
TSH	thyroid stimulating hormone
U.S.	United States
U.S.C.	University of Southern California
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency

WHO	World Health Organization
YES	yeast estrogen system; estrogenicity screening assay which uses yeast cells transfected with human estrogen receptor and reporter gene
2-OHE1	2-hydroxyestrone
16-OHE1	16-alpha hydroxyestrone

Symbols:

α	alpha
β	beta
γ	gamma
μg	microgram
<	less than
>	greater than
%	percent
p	p value
±	plus or minus
=	equal
®	registered trademark

XII. Appendix B. BCERF Critical Evaluations of Breast Cancer Risk

This includes an overview of the Critical Evaluations and explanation of the BCERF Breast Cancer Risk Classification Scheme

The Process:

Starting Point - Existing Critical Evaluations on Evidence of Carcinogenicity
IARC Monographs (**I**nternational **A**gency for **R**esearch on **C**ancer)
NTP ARC (**N**ational **T**oxicology **P**rogram, **A**nnual **R**eport on **C**arcinogens)
ATDSR (**A**gency for **T**oxic **D**isease **S**ubstance **R**egistry of the CDC)

Conduct **Literature Searches** using databases to obtain historical and the most recent information; i.e. Toxline, Medline, Biosis, Cancerlit
-Peer-reviewed scientific literature-available through Cornell libraries and interlibrary loans.
-Technical Reports-NTIS-National Technical Information Service
-TOXNET databases—USEPA's IRIS database source of oncogenicity and regulatory status information
-Grey literature—Studies submitted to U.S. Environmental Protection Agency that are not published—i.e., industry generated oncogenicity studies
-Some abstracts of cancer bioassays are on line (IRIS database)
-Request reports from industry
-Request reports from USEPA through Freedom of Information Act

The critical evaluation includes some general background information, including chemical name, CAS#, trade name, history of use, and current regulatory status.

Evidence of cancer in other (non-breast) organ systems is provided in synopsis form with some critical commentary, along with the current overall carcinogenicity classification by international (IARC) and U.S. Federal Agencies (NTP, USEPA).

Human epidemiological studies, animal studies, and other relevant studies on possible mechanisms of carcinogenesis are critically evaluated for evidence of exposure to agent and breast cancer risk based on "strength of evidence" approach, according to a modification of IARC criteria as listed in the IARC Preamble. (See attached sheets for a more detailed explanation of the BCERF Cancer Risk classification scheme)

The **emphasis of the document** is a critical evaluation of the evidence for breast cancer risk, classification of the agent's breast cancer risk, identification of research gaps, and recommendations for future studies. A section is devoted to brief summaries of new research studies that are in progress. A bibliography with all cited literature is included in each critical evaluation. Major international, federal and state agencies will be provided with copies of our report.

General Outline of BCERF Critical Evaluations

I. Chemical Information
   - A. Common Name
   - B. Chemical Name(s)
   - C. Chemical Formula(s)
   - D. Trade Name(s)
   - E. Chemical Structure
   - F. CAS # (Chemical Abstract Subject Number)
   - G. Major Metabolite(s)

II. History of Use, Usage and Nomenclature
   - A. Date of first registration
   - B. Uses
   - C. Past Usage / If available, current usage levels in US and NYS

III. Current Regulatory Status
   - A. Current Regulatory Status, EPA
   - B. Drinking Water Standards and Health Advisories
   - C. Food Residue Tolerances and Action Levels
   - D. Workplace Regulations (when applicable)

IV. Summary of Evidence of Overall Carcinogenicity (non-breast sites)
   - A. Human Studies
   - B. Experimental Animal Studies
   - C. Current Classification of Carcinogenicity by other Agencies
     1. IARC (International Agency for Research on Cancer)
     2. NTP (National Toxicology Program)
     3. USEPA (Environmental Protection Agency)

V. Critical Evaluation of the Scientific Evidence for Breast Cancer Risk
   - A. Humans Studies will include:
     1. Case-Studies
     2. Human Epidemiological Cohort Studies
     3. Human Epidemiological Case-Control Studies
     4. When available will summarize information on detection/accumulation in human tissues/and validation of biomarkers
   - B. Experimental Animal Studies
   - C. Other Relevant Information, including mechanisms by which exposure may affect breast cancer risk (examples: co-carcinogenicity, tumor promotion estrogenicity, endocrine disruption, reproductive toxicology, mutagenicity, cell proliferation, oncogene/tumor suppressor gene expression, immune function, etc.)

VI. Other Relevant Information
   - A. Specific for the pesticide; (i.e. may include information on environmental fate, potential for human exposure)

VII. Summary, Conclusions, Recommendation for Breast Cancer Risk Classification

VIII. Identification of Research Gaps, and Other Recommendations

IX. Brief Summaries of New Human Studies Currently Being Conducted

X. Bibliography

XI. Appendix A. Common Abbreviations, Acronyms and Symbols

XII. Appendix B. BCERF Critical Evaluations of Breast Cancer Risk

BCERF Breast Cancer Risk Classification Scheme-revised 12/97 sms
(adapted from the IARC Preamble by S.M.Snedeker)

Group 1: **Human breast carcinogen;** *sufficient evidence* of carcinogenicity to humans is necessary. *Sufficient evidence* is considered to be evidence that a **causal** relationship has been established between exposure to the agent and human breast cancer.

Group 2A: **Probable breast carcinogen**; this category generally includes agents for which there is *1) limited evidence* of breast carcinogenicity in humans and *sufficient evidence* of mammary carcinogenicity in experimental animals. The classification may also be used when there is *2) limited evidence* of breast carcinogenicity in humans and strong supporting evidence from other relevant data, or when there is 3) *sufficient evidence* of mammary carcinogenicity in experimental animals and strong supporting evidence from other relevant data.

Group 2B: **Possible breast carcinogen;** this category generally includes agents for which there is 1) *limited evidence* in humans in the absence of *sufficient evidence* in experimental animals; 2) *inadequate evidence* of carcinogenicity in humans or when human data is nonexistent but there is *sufficient evidence* of carcinogenicity in experimental animals, 3) *inadequate evidence* or no data in humans but with *limited evidence* of carcinogenicity in experimental animals together with supporting evidence from other relevant data.

Group 2C: **Potential to affect breast cancer risk**; this category includes agents for which there is *inadequate or nonexistent human and animal data*, but there is *supporting evidence from other relevant data* that identifies a mechanism by which the agent may affect breast cancer risk. Examples are, but are not limited to: evidence of agent's estrogenicity, disruption of estrogen metabolism resulting in potential to affect exposure to estrogen; evidence of breast tumor promotion, progression or co-carcinogenicity; increased expression of proto-oncogenes or oncogenes; evidence of inactivation of tumor suppressor gene associated with breast cancer; evidence of adverse effect on immune function; or evidence of a structural similarity to a known breast carcinogen (structure-activity relationship).

Group 3: **Not classifiable** as to its breast carcinogenicity to humans. Agents are placed in this category when they do not fall into any other group.

Group 4: **Probably not a breast carcinogen in humans**: This category is used for agents for which there is evidence suggesting a lack of breast carcinogenicity in human studies and in animal studies, together with a lack of related evidence which may predict breast cancer risk. The absence of studies does **not** constitute evidence for a lack of breast carcinogenicity.

BCERF Breast Cancer Risk Classification Scheme, continued

Brief Definitions of Sufficient, Limited, and Inadequate Evidence: (adapted from the IARC Preamble by S.M. Snedeker)

Human Studies

Sufficient evidence of carcinogenicity in humans: Must have established evidence between exposure to the agent and human breast cancer. Case-reports are given the least weight in considering carcinogenicity data in humans—they are suggestive of a relationship, but by themselves cannot demonstrate causality. Consistent, case-control studies which have controlled for confounding factors and have found high relative risks of developing breast cancer in relation to an identified exposure are given the most weight in determining a causal relationship.

Limited evidence of breast carcinogenicity in humans: A positive association has been observed between exposure to the agent and breast cancer, but chance, bias or confounding factors could not be ruled out.

Inadequate evidence of breast carcinogenicity in humans: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association.

Experimental Animal Studies:

Sufficient evidence of breast carcinogenicity in animals: Evidence of malignant tumors or combination of benign and malignant tumors in (a) two or more species of animals, (b) or two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

Limited evidence of breast carcinogenicity in animals: The studies suggest a carcinogenic effect, but are limited for making a definitive evaluation because: (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (c) the agent increases the incidence of only benign neoplasms of lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains of animals.

Inadequate evidence of breast carcinogenicity in animals: The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations.