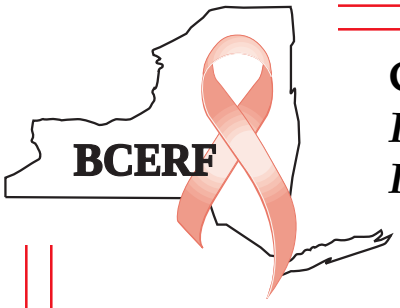

Cornell University
Program on Breast Cancer and Environmental Risk Factors in New York State (BCERF)*

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Critical Evaluation of Heptachlor and Heptachlor Epoxide's Breast Cancer Risk

by

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Critical Evaluation of Heptachlor and Heptachlor Epoxide's Breast Cancer Risk

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

I. Chemical Information:

A. Common Name: Heptachlor (IARC, 1991)

B. Chemical Name for Heptachlor: 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (IUPAC) (IARC, 1991)

C. Chemical Formula for Heptachlor: $C_{10}H_5Cl_7$ (Montgomery, 1993)

D. Trade Names for Heptachlor: Biarbinex®, Cupincida®, Fennotox® (Velsicol Chemical Co.); Vegfru Heptox® (Pesticides India); Drinox®; H-34, Heptamul®; Heptox® (Savriti Pesticides & Agrochem Ltd.) (Meister, 1996)

E. Chemical Structure of Heptachlor: (IARC, 1991)

F. CAS Registry Number for Heptachlor: 76-44-8 (replaced CAS registry numbers: 23720-59-4; 37229-06-4) (IARC, 1991)

G. Major Metabolite: Heptachlor epoxide

Heptachlor epoxide is the oxidation product of heptachlor (Worthing, 1991).

H. Chemical Name for Heptachlor Epoxide: 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene (IUPAC) (IARC, 1991)

I. Trade Name for Heptachlor Epoxide: Velsicol 53-CS-17 (Velsicol Chemical Co.) (ATSDR, 1993)

J. Chemical Formula for Heptachlor Epoxide : $C_{10}H_5Cl_7O$ (IARC, 1991)

K. CAS Registry Number for Heptachlor Epoxide 1024-57-3 (replaced CAS registry numbers: 4067-30-5; 24699-42-1;24717-72-4; 28044-82-8; 66240-71-9) (IARC, 1991)

L. Chemical Structure of Heptachlor Epoxide: (IARC, 1991)

II. History of Use , Usage and Nomenclature:

Heptachlor is a persistent, non-systemic, chlorinated hydrocarbon insecticide (ATSDR, 1993; Worthing, 1991). Heptachlor is a member of the cyclodiene class of insecticides which includes the structurally similar insecticides aldrin, isoaldrin, dieldrin, endrin, chlordane, isobenzan and [alpha] endosulfan (Smith, 1991c). Technical-grade heptachlor is a mixture of several chemicals, and contains about 72% heptachlor and 28% related compounds, including 20-22% gamma chlordane, and 4-8% gamma nonachlor. Heptachlor is also one of the components of technical-grade chlordane (approximately 10-20% heptachlor by

weight) (IARC, 1991). One of the predominant isomers in technical chlordane, *trans*-chlordane, can be metabolized to heptachlor. The major breakdown product of heptachlor is its oxidized form, heptachlor epoxide, a persistent environmental contaminant (ATSDR, 1993; IARC, 1991).

Heptachlor was introduced in the US in 1952, and was used to treat soil and to protect field corn, seed (for corn, wheat, oat, barley and sorghum), bulbs, citrus fruits and pineapple from insect pests. It was used as an insecticide to control ants, cutworms, maggots, termites, thrips (wingless insect pest), weevils, wireworms, grasshoppers and mosquitoes to prevent insect damage to agricultural crops. In the mid-1960s heptachlor was recommended as an insecticide to control grubs and ants in turfgrass at the rate of 2 pounds (lbs) and 5 lbs per acre, respectively (Weidhass et al., 1966). Heptachlor was also used to protect buildings, homes, lawns and gardens from soil insects and termites, and for control of fire ants in power transformers (ATSDR, 1993; Kutz et al., 1991a; Smith, 1991a; Worthing, 1991). It has also been used in the processing of termite-resistant plywood in Europe (Mussalo-Rauhamaa et al., 1988). In 1974, 2 million lbs of heptachlor was used in the US, with 75% used in agriculture, mostly for corn soil treatment (EPA, 1976). The distribution of heptachlor use in the 1974 was: 58% corn, 27% pest control, 13% to treat seeds, 2% for various uses, including fire ant control, and use on fruit crops (citrus and pineapples) (IARC, 1979). No current information could be located on the production, importation or exportation levels of heptachlor in the US.

Special note on heptachlor use in New York State.
In New York State (NYS), heptachlor use was restricted to incorporation into baits to control the alfalfa snout beetle. Heptachlor was not allowed for use as a termiticide in NYS. However, technical chlordane, which is 10-20% heptachlor (IARC, 1991) was used to control insects on turf and agricultural crops up to 1975, and was heavily used in downstate NYS as a termiticide until 1985 (NYSDEC, 1986).

III. Current Regulatory Status:

A. Regulatory Status:
1. EPA: In 1974, because of risk of carcinogenicity as determined from animal cancer bioassays; detection of heptachlor epoxide in dairy, meat, fish, and poultry products; and the persistence and bioaccumulation of heptachlor, the EPA recommended cancelling nearly all registered uses of this insecticide (ATSDR, 1993; USEPA, 1990). A letter from Russell E. Train, Administrator of the Velsicol Chemical Corp., the main manufacturer and registrant of heptachlor and chlordane, announced the "Intent to Cancel Registrations of Certain Pesticide Products Containing Heptachlor or Chlordane" in December of 1975. A series of documents clarifying the original letter was published in the Federal Register in February of 1976 (EPA, 1976). Some limited agricultural uses of heptachlor as a seed treatment, against the narcissus bulb fly, and on pineapple against mealybug wilt, was recommended in the 1976 documents (EPA, 1976). These agricultural uses of heptachlor were gradually phased out over a five year period ending on July 1, 1983 (ATSDR, 1993). In 1983, certain uses were exempted from EPA's 1983 cancellation, including use of heptachlor for control of termites in underground areas, fire ant control in buried cable closures, and dipping of roots or tops of nonfood plants. All other uses of heptachlor were either voluntarily cancelled by the manufacturer, Velsicol Chemical Co., or were suspended for failure to meet EPA requirements. Effective as of April 15, 1988, the sale, distribution and shipment of heptachlor products was prohibited in the US. Commercial use of existing stocks of heptachlor products was also prohibited, except for fire ant control in power transformers. Domestic use of existing stocks of chlordane and heptachlor products that are in the possession of homeowners is also permitted by the EPA (ATSDR, 1993; USEPA, 1990).

2. NYS DEC: All use of both heptachlor and chlordane in NYS was prohibited on March 13, 1985 by emergency action as mandated by the NYS DEC (NYSDEC, 1986).

B. Drinking Water Standards and Health Advisories:
The EPA has set a Maximum Contaminant Level (MCL) for heptachlor in drinking water at 0.0004 mg/L, and the MCL for heptachlor epoxide is 0.0002 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 heptachlor and heptachlor epoxide in drinking water as are follows (USEPA, 1996):

Heptachlor:
10 kg child:
- One day = 0.01 mg/L
- Longer-term = 0.0001 mg/L

70 kg adult
- Longer term = 0.0001 mg/L

Heptachlor epoxide:
10 kg child:
- One day = 0.01 mg/L
- Longer-term = 0.0001 mg/L

70 kg adult
- Longer term = 0.0001 mg/L

*The HAs are nonenforceable limits of the concentration of a chemical in drinking water that are not expected to cause any adverse noncarcinogenic health effects when consumed for up to the time period specified, with a margin of safety (USEPA, 1996).

C. Food Residue Tolerances and Action Levels:

The Food and Drug Administration (FDA) and the US Department of Agriculture (USDA) are responsible for monitoring the levels of heptachlor and heptachlor epoxide residues in domestic and imported foods, and animal feed. The EPA sets tolerances (the maximum amount of a residue that is permitted in or on the food). Because heptachlor and heptachlor epoxide are possible carcinogens, the tolerance for heptachlor and heptachlor epoxide residues in foods has been set at zero. Since heptachlor and heptachlor epoxide persist in soil, the FDA established action levels for unavoidable residues of these chemicals in raw agriculture products. These action levels represent limits at or above which the FDA can take legal action to remove these commodities from the market place. The action levels for heptachlor and heptachlor epoxide range from 0.01 to 0.02 ppm in raw vegetables, raw fruits, grains, eggs, nuts, and sugarcane; 0.01 ppm for processed animal fed, fodder and hay; 0.1 ppm for milk on a fat basis; 0.2 ppm in the fat portion of rabbits; and 0.3 ppm for the edible portion of fish (FDA,1994).

D. Acceptable Daily Intake:
The acceptable daily intake (ADI) of heptachlor and heptachlor epoxide from food set by the World Health Organization (WHO) is 0.1 μg/kg/day (WHO, 1993)

E. Workplace Regulations:
The Occupational Health and Safety Administration (OSHA) has set limits on workplace exposures to heptachlor. OSHA recommends that the maximum level of heptachlor in workplace air for an 8-hour day for a 40-hour work week not exceed 0.5 mg/m^3 (ATSDR, 1993).

IV. Summary of 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.

In one case report, a woman who drank cow's milk contaminated with heptachlor during her pregnancy gave birth to a boy diagnosed at seven weeks of age with a relatively rare brain tumor, a gliosarcoma. Chromosomal abnormalities were found in a tissue culture of the tumor, but the peripheral blood displayed normal karotyping. The authors of the case-report concluded that heptachlor could not be implicated as the causal factor in the development of the tumor, and the occurrence of the sarcoma was considered to be spontaneous (Chadduck et al., 1987).

Two reports have described cases of leukemia diagnoses after exposure to chlordane/heptachlor in domestic settings. In the first report, Infante et al. (1978) described three cases of leukemia, two of which were associated with exposure to chlordane or heptachlor and one in which exposures to other chemicals including 2,4-D and diazinon were also reported. Epstein and Ozonoff (1987) reported five other cases of leukemia associated with chlordane and heptachlor exposure. In three of these cases, the exposure was limited to chlordane and heptachlor, and in the other two cases, the patients were exposed to other chemicals as well, including Telodrin, and malathion.

These case-reports are based on self-reported exposures, lack a complete medical history of the individuals and do not provide the details on the formulation of chlordane/ heptachlor applied or the duration or extent of exposure. In addition, none of the studies provided an estimate of the expected number of leukemia cases in absence of exposure to chlordane and heptachlor. Consequently, these studies do not provide a good basis to assess the role of heptachlor in affecting the incidence of leukemia in humans.

2. Case-Control Studies:
One case-control study conducted on male agricultural workers in Iowa and Minnesota, has evaluated the risk of leukemia in workers who handled, mixed or applied heptachlor (Brown et al., 1990). Information on residential history, drinking water sources, nonfarm occupational history, smoking and alcohol use, family history of cancer, and farm activities were obtained by questionnaire. The risk of developing leukemia was not elevated (Odds Ratio [OR] = 0.9; 95% Confidence Interval [CI] 0.5-1.7) in 14 male agricultural workers who handled, mixed or applied heptachlor compared to 43 controls who had never worked as farmers. There also was no increased risk of leukemia in the agricultural workers who had handled chlordane (OR=0.7; 95% CI 0.3-1.16). There was no mention if nonagricultural usage of heptachlor was controlled for in cases and controls. The ORs were adjusted for confounding factors, including age, state, tobacco use, family history of lymphopoietic cancer, and exposure to substances (benzene, napththa, hair dyes) related to the risk of leukemia (Brown et al., 1990).

3. Cancer Mortality Occupational Cohort Studies:
a. Manufacturing Plants:
Three industry-funded cohort studies have examined the

association between occupational exposure to heptachlor and the risks of cancer mortality among workers employed at a heptachlor/endrin manufacturing plant. The findings of these studies are summarized below.

In a retrospective cohort study, Wang and MacMahon (1979) examined the causes of mortality in a cohort of 1,403 white male workers employed for at least three months at two plants manufacturing either chlordane (plant 1) or heptachlor and endrin (plant 2). A total of 835 workers in the heptachlor/endrin plant were followed from 1952 to 1976. Expected deaths were calculated on the basis of national rates. Standardized Mortality Ratios (SMR) were computed by dividing the observed deaths by the expected values. A small but nonsignificant excess of deaths from all cancers (SMR 1.21; 95% CI not given) was observed at plant 2. Excess deaths from lung cancer was higher at plant 2 (SMR 1.74) compared to the plant 1 (SMR=1.15), but was not statistically significant. The combined SMR for lung cancer mortality at both plants was much higher (7 observed, 2.6 expected, $p < 0.01$) for men who were under age 35 at the beginning of employment.

However, because of the limited number of individuals followed, it was not possible to determine whether the number of lung cancer deaths increased as a function of duration of follow-up or duration of employment. The validity of the study is further limited by the lack of complete occupational history for each worker and hence a history of previous chemical exposures, and lack of consideration of confounding factors, such as smoking history that may have affected the results of this study. In addition, workers at plant 2 were also exposed to other chemicals, including chlorine, vinyl chlorine, chlorendic anhydride and hexachlorocyclopentadiene. Therefore, exposure to these other chemicals may have played a role in the induction of employment-related cancers. Causes of mortality were not analyzed among the 68 female workers employed at both manufacturing plants.

A second cohort study reported by Ditraglia, et al. (1981) examined the mortality rates from cancer among 305 white males recruited between 1951 and 1964 who were employed for at least 6 months at the same plant 2 as described previously (Wang and MacMahon, 1979). This cohort was followed from 1951 or 1964 to 1976. There was no excess mortality from all cancers (SMR=0.91; 95% CI 0.3-1.98) and the authors commented that this low SMR may reflect a "healthy worker effect." There was a slight, but statistically nonsignificant increase for respiratory cancer deaths at plant 2 (SMR=1.22; 95% CI 0.25-3.58). The relationship between cancer mortality and numbers of years since date of first employment was also determined. There was a deficit of deaths from all cancers in those employed for 10 to 19 years (SMR= 0.91; 95% CI 0.18-2.66), while there was a higher risk of cancer deaths in those employed for ≥ 20 years (SMR=1.62; 95% CI 0.33-4.74) at the heptachlor/endrin plant. While neither observation was statistically significant, the size of the sample may not have been sufficiently large to detect statistical differences (Ditraglia et al., 1981) .

This cohort was followed at plant 2 for an additional 11 years by Brown (Brown, 1992). No excess cancer mortality was reported (SMR=1.0; 95% CI 0.6-1.59). There was a nonsignificant deficit of deaths from respiratory cancer (SMR=0.88; 95% CI 0.32-1.92) and intestinal cancer (SMR=0.63; 95% CI 0.02-3.53). Mortality from stomach cancer (SMR=2.84; 95% CI 0.43-10.27) was elevated, but it was not statistically significant, while mortality from bladder cancer was significantly elevated (SMR=7.12; 95% CI 1.47-20.84). The authors recognized that survival of bladder cancer has improved and consequently, a better estimate of the true risk would have been determined from incident cases rather than deaths. The strength of evidence of the carcinogenicity of heptachlor in both the original (Ditraglia et al., 1981) and the updated follow-up (Brown, 1992) studies is limited because death rates from specific types of cancer were not analyzed as a function of latency, and exposure data on heptachlor were not provided. Risk analyses in these studies also carry over the confounding effects of co-exposure to endrin, chlorine, vinyl chloride, chlorendic anhydride and hexachlorocyclopentadiene.

In summary, with the exception of bladder cancer, mortality from all neoplasms among workers employed at the heptachlor plant in Memphis, TN, was comparable to that of the general US population. A small but nonsignificant excess mortality from lung cancer was reported in three of the studies (Ditraglia et al., 1981; Wang and MacMahon, 1979; Brown, 1992). A significant excess of deaths from bladder cancer based on a small number of cases was reported by one investigator (Brown, 1992). All three studies were conducted at the same manufacturing plant and consequently, do not provide independent estimates of cancer risk. The validity of these studies was also limited because the number of individuals enrolled was small (835 for Wang and MacMahon, 1979; and 305 for Ditraglia et al., 1981 and Brown, 1992). Moreover, it is difficult to attribute specifically the reported cancer deaths to heptachlor because the workers were also exposed to many other chemicals. There is also an inherent difficulty in interpreting occupational exposures and cancer mortality data. Because of long latency periods from the exposures to development of neoplasms, cancer may not develop in those occupationally exposed to suspect chemicals until after retirement or a change in employment.

b. Pesticide Applicators:

The association between exposure to heptachlor and risk of cancer mortality has also been studied among pesticides applicators. An

18 year long follow-up cohort study of mortality causes among 16,124 urban pesticide applicators (MacMahon et al., 1988) found a significant excess of deaths from lung cancer among non-termite control operators (SMR=1.58; 95% CI 1.29-1.90) but not among the group of workers who had the higher probability of exposure to chlordane and heptachlor (SMR=0.97; no 95% CI given). This study did not provide individual exposure data and the pesticide applicators were exposed to varying amounts of other chemicals as well. This study did not control for tobacco use among the pesticide applicators, and patterns of tobacco use may have contributed to the incidence of lung cancer deaths in this cohort.

A study of mortality causes among 3,827 white male pesticide applicators in Florida (Blair et al., 1983) reported a nonsignificant excess of deaths from lung cancer (SMR=1.35; 95% CI not given). A moderate, but nonsignificant excess in mortality from lung cancer (SMR=1.35) and brain cancer (SMR=2.00) was observed among workers employed by firms licensed for termite treatment. The risk of mortality from lung cancer rose with the number of years licensed with an SMR of 1.01 among those licensed for less than 10 years, compared with a SMR of 2.89 ($p<0.05$) for those licensed for more than 20 years. Unfortunately, since use of tobacco products was not controlled for in this study, conclusions can not be made about the relationship between estimates of exposure to pesticides and lung cancer mortality. The termite applicators were also licensed to use other organochlorine pesticides such as aldrin, DDT, chlordane, propuxur and chlorpyrifos, hence co-exposures to these chemicals were likely. There were only 11 deaths observed among the 1,638 white women pesticide applicators, and the authors stated that there were too few deaths for statistical analysis to determine risk of cancer mortality.

4. Summary, Human Studies (non-breast sites):
An isolated case report of a gliosarcoma has been reported in a child born to a mother who ingested heptachlor-contaminated milk during her pregnancy, but no causal relationship could be established (Chadduck et al., 1987). While there have been several case-reports of leukemia in chlordane / heptachlor exposed individuals (Epstein and Ozonoff, 1987; Infante et al., 1978), a small case-control comparison of agricultural workers exposed to heptachlor or chlordane failed to find an increased risk of leukemia (Brown et al., 1990). A significant increase in risk of death from lung cancer was reported among termite control applicators who had been licensed for at least 20 years (Blair et al., 1983). However, this study did not control for the use of tobacco products, and the evidence that occupational exposure to heptachlor increases the risk of death from lung cancer is not supported by the results of other studies (Brown, 1992; MacMahon et al., 1988; Ditraglia et al., 1981; Wang and MacMahon, 1979a). A significant increase in deaths from bladder cancer was reported among workers at the heptachlor manufacturing plant (Brown, 1992) but the excess mortality from this type of cancer was not statistically significant among termite applicators (Blair et al., 1983). It is difficult to specifically attribute any excess of cancer deaths to heptachlor because in all these studies workers and pesticide applicators were exposed to other chemicals as well. Other confounding factors, such as smoking history, were not controlled for in these studies. Consequently, these cohort studies provide insufficient evidence to determine whether or not exposure to heptachlor imparts a significant risk for cancer in humans.

Finally, these studies have not examined mortality causes among women employed by the heptachlor manufacturing plant or firms licensed for pesticide application. Therefore, there are no reported data available on occupational exposure to heptachlor in women and cancer mortality. Wives of heptachlor manufacturing workers and applicators may have been exposed to heptachlor and heptachlor epoxide by handling contaminated clothing during laundering of spouse's work clothes, but there have been no studies that have evaluated cancer incidence or mortality in wives of workers who may have been exposed to heptachlor.

B. Experimental Animal Studies:
Selected histological materials from previous unpublished and published long-term studies on heptachlor's oncogenicity in rodents have been reviewed by a panel of pathologists from the National Academy of Science (NAS) (IARC, 1991). Some of these studies are reported here.

1. Mice:
Epstein (1976) reported on a 1965 unpublished FDA study in which 100 C3H mice of each sex were fed 0 or 10 ppm of heptachlor or heptachlor epoxide for 24 months. A review panel of pathologists from the NAS concluded that there was a significant increase in the incidence of hepatocellular carcinomas in females but not in males given heptachlor, whereas heptachlor epoxide caused liver carcinomas in both male and female mice (IARC, 1991). The study is of limited value because the mortality rate was markedly elevated in heptachlor (70%) and heptachlor epoxide (90.5%) treated groups and consequently, few animals survived for the evaluation of the treatment effects. Epstein, in his review of the FDA study, noted that premature death was particularly high in animals fed heptachlor epoxide, but no data were available on time of unscheduled deaths or tumor detection in either control or treated groups (Epstein, 1976). The high mortality rate among the controls (66%) raises the possibility that the mice were also dying from causes unrelated to heptachlor or heptachlor epoxide. In addition, the purity of the heptachlor and heptachlor epoxide preparations was not specified, therefore, the actual concentration of these insecticides, and level of other

contaminates or metabolites in the diets, is uncertain. This study also only administered one treatment dose to the animals. Multiple doses should have been administered so a dose-response effect could have been evaluated.

Epstein also reported the results of an unpublished cancer bioassay conducted by the International Research and Development Corporation (IRDC) for the Velsicol Chemical Corporation in 1973 (Epstein, 1976). Charles River CD-1 mice, 100 of each sex, were fed a mixture of 75% heptachlor epoxide and 25% heptachlor (purity not specified) at 0, 1.0, 5.0 and 10.0 ppm starting at 7 weeks of age for 18 months. At six months, 10 animals from each test and control group were killed for the preparation of histology samples from the liver. Mortality rates for the remaining animals by 18 months were high, ranging from 34 to 49% in the 1 and 5 ppm groups, and 71% and 70% in the female and male high dose 10 ppm groups, respectively. The usefulness of the results of this study is severely limited, because of the high mortality rate, and in addition, a large numbers of tissues from animals that died during the study were lost to autolysis, and could not be evaluated histopathogically. Epstein notes that this autolysis probably resulted in an underestimate of the incidence of liver tumors. Also, there was no evidence that the organs of the animals sacrificed at 18 months and that died during the study were examined or evaluated "blindly "(Epstein, 1976).

Since the original IRCD report was vague as to the carcinogenicity or non-carcinogenicity of the heptachlor/heptachlor epoxide mixture, the histological material from the IRDC study was subsequently reevaluated by a NAS panel in 1977. The results of the NAS panel review has been summarized by others (IARC, 1991). The NAS panel concluded that there was a significant ($p<0.01$) increase in the combined incidence of hepatocellular carcinomas and nodules in the male and female mice in the mid-dose 5 ppm and high-dose 10 ppm groups as compared to controls (IARC, 1991).

In a study conducted by the National Cancer Institute (NCI) (NCI, 1977; NTP, 1977), 50 $B6C3F_1$ mice of each sex, five weeks of age, were fed technical-grade heptachlor (72% heptachlor, 18% *trans*-chlordane, 2% *cis*-chlordane, 2% nonachlor, 1% chlordene, 0.2% hexachlorobutadiene) for 80 weeks, and then observed for 10 weeks. Matched controls, 20 males and 10 females, were combined with matched controls from other bioassays to generate pooled controls consisting of 100 males and 80 females (It should be noted that the use of pooled controls is no longer acceptable in animal bioassays). Because of toxic effects, the initial dietary concentrations of 10 and 20 mg/kg heptachlor were reduced several times during the course of the study. In males, time-weighted average (TWA) concentrations of heptachlor in the diet were 6 ppm (low-dose group) and 14 ppm (high-dose group), whereas in females TWAs were 9 ppm (low-dose group) and 18 ppm (high-dose group). Body weights of mice given either low or high doses of heptachlor showed little or no difference compared to those of control mice. In females survival rates were > 60% in the high-dose and ≥ 80% in the control and low-dose groups. In males, survival rates were high (≥ 70%) in all treated and control groups. The review panel of toxicologists from the NAS concluded that there was a significant increase in the combined incidence of hepatocarcinomas and nodules in the males ($p<0.042$) and females ($p<0.022$) fed the highest dose of heptachlor. The nature of the "nodules" was not specified. There were no significant differences between treated animals and controls in either sex when the statistical analysis was based only on the incidence of hepatocellular carcinomas (IARC, 1991).

Liver tumors have also been reported in male and female mice fed other structurally similar organochlorine pesticides, including aldrin, dieldrin or chlordane (Reuber, 1978). This suggests there may be a common mechanism of liver tumor induction for these structurally related cyclodiene compounds.

2. Rats:

In a 1955 study conducted by the Kettering Laboratory for Velsicol Chemical Co., and reported by Epstein (1976), 20 CF rats of each sex received 0, 1.5, 3.0, 5.0, 7.0, or 10.0 ppm of heptachlor in the diet (purity unspecified) for 110 weeks, beginning at 10 weeks of age. Mortality rates in all test groups were high but were not dose related. Mortality ranged from 40 to 75% in the males, and from 35 to 55% in female rats. No weight loss was noted in female groups, whereas in males small decreases in weight were only seen in the 10 ppm group. A review panel of pathologists from the NAS concluded that heptachlor did not induce liver tumors in rats in this study (IARC, 1991). However, this study is of limited value because of the inadequate number of animals per dose group, especially in view of the high mortality rates (35 to 75%). In addition, a full necropsy was not performed on the rats that died during the experiment and there was no mention of the number of animals examined histologically in each group.

In an 1972 Italian study by Cabral and colleagues (Cabral et al., 1972 as cited in IARC, 1991), 10 mg heptachlor (97% purity) in corn oil was given by gavage to 95 sucking Wistar rats of each sex five times at two-day intervals; controls (19 males and 27 females) received corn oil alone. The total number of tumors in treated and control groups were comparable. No information was available on the tumor incidence by organ site, except that two renal tumors were detected in female rats that received heptachlor (dose level not specified). The usefulness of this study for determining the oncogenic potential of heptachlor is limited because of the short duration of treatment, the small number of controls, limited information on tumor incidence by organ site, and the use of only one treatment dose which prevented an evaluation of a dose-response effect.

Another a heptachlor bioassay was conducted by NCI on 50 Osborne-Mendel rats of each sex, starting at five weeks of age (NTP, 1977). The initial levels of technical heptachlor in the diet were 80 and 160 ppm in the males, and 40 and 80 ppm in the females, but because of toxic effects these doses were changed several times during the course of the study. Matched controls consisted of 10 rats of each sex and pooled controls consisted of 60 rats of each sex. In males TWAs dietary concentrations of heptachlor were 38.9 and 77.9 ppm for the low- and high-doses, respectively; in females, TWAs were 25.7 and 51.3 ppm for the low- and high-dose groups, respectively. Average body weights of male rats receiving the high but not the low dose of heptachlor were consistently lower than those of untreated controls. At 110 days 60 to 75% of all treated and control rats had survived. In contrast to mice (NCI, 1977; NTP, 1977), heptachlor did not induce hepatic carcinomas in rats in this study. There was a significant increase in the number of thyroid follicular-cell neoplasms in female (3/58 control; 14/38 high-dose, $p<0.01$) and male rats (4/51 controls; 9/38 low-dose, $p<0.05$). However, the incidence of such thyroid follicular cell neoplasms in the male high-dose group was only 3/38, indicating the absence of a dose-related effect. The absence of a significant increase in thyroid tumors in the high-dose group could reflect a reduction in food consumption. The body weights in the high-dose group were consistently lower than in the control group, indicating that the high-dose of heptachlor was toxic to the rats. The validity of this study is also limited because the exposure time was only 80 weeks instead of 104 weeks currently used for a long-term rodent cancer bioassay by the National Toxicology Program. This study also used inappropriate pooled controls.

3. Summary, Animal Studies (non-mammary sites):
In summary, the incidence of liver tumors was significantly increased following dietary administration of heptachlor epoxide in both male and female C3H mice (Epstein, 1976). Dietary administration of heptachlor also induced liver tumors in male C3H mice (Epstein, 1976) and B6C3F1 mice in both sexes (NCI, 1977; NTP, 1977). Diets containing a mixture of heptachlor and heptachlor epoxide induced a significant increase in the combined incidence of hepatocellular carcinomas and nodules in male and female CD-1 mice (Epstein, 1976; IARC, 1991). In contrast to mice, dietary administration of heptachlor did not induce liver tumors in CF (Epstein, 1976), Wistar (Cabral et al., 1972) or Osborne-Mendel rats (NCI, 1977; NTP, 1977). However, in the NCI/NTP bioassay, heptachlor fed in the diet significantly increased the incidence of thyroid follicular-cell neoplasms in both male ($p<0.05$) and female ($p<0.01$) Osborne-Mendel rats.

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 heptachlor. This agency has also assessed that there is sufficient evidence in experimental animals for the carcinogenicity of heptachlor, based on studies demonstrating increased incidence of liver hepatocellular neoplasms in mice of each sex, and thyroid follicular-cell neoplasms in rats of each sex. Overall, the IARC has determined that heptachlor is "possibly carcinogenic to humans" and consequently assigned heptachlor to Group 2B (IARC, 1991).

2. NTP Classification: Not classified

3. EPA Classification:
The EPA has classified heptachlor in Group B2 as a "probable human carcinogen" (IRIS, 1993; IRIS 1997). Though the EPA concluded that there is inadequate evidence of carcinogenicity from human data, sufficient evidence exists for the carcinogenicity of heptachlor and heptachlor epoxide in animals. This is based on the observation of benign and malignant tumors in both sexes of multiple strains of mice treated with these chemicals. Included in their carcinogenicity assessment was a review of the long-term carcinogenicity bioassays of heptachlor in male and female CH3 mice [unpublished FDA study reported in Epstein (1976), and in male and female B6C3F1 mice (NCI, 1977; NTP, 1977)]. The EPA has concluded that heptachlor caused an increase in the incidence of liver carcinomas in mice (IRIS, 1997). The EPA also observed that in these studies, adequate numbers of animals were observed for the majority of the expected lifetime and that the incidence of liver carcinomas was increased in all data sets. Supporting data of carinogenicity included that other structurally related compounds have produced liver tumors in mice (IRIS, 1997). (IRIS, 1993; IRIS 1997).

V. Critical Evaluation of Breast Carcinogenicity:

A. Human Studies:

1. Adipose Tissue Levels:
Heptachlor is oxidized by microsomal enzymes to heptachlor epoxide in animals and humans (Kutz et al., 1991a). Because it is lipophilic and has a very long half-life, heptachlor epoxide concentrates in the food chain, and bioaccumulates and persists in adipose tissues of humans (Frank et al., 1988; Kutz et al., 1991a; Levine, 1991; Teufel et al., 1990; Wassermann et al., 1972). In the US population, adipose tissue levels of heptachlor epoxide were found to be higher in those aged ≥ 45 years (0.098 ppm) than in the 15 to 44 year (0.073 ppm) and 0 to 14 year (0.047 ppm) age groups (Levine, 1991). Similar increases in the concentration of heptachlor epoxide over the course of a lifetime have been observed in other countries (Frank et al., 1988; Levine, 1991; Wassermann et al., 1972).

Most of the reported values for heptachlor epoxide in the adipose tissue of adults in North America has been reported to range between 0.027 ppm (Hawaii) to 0.24 ppm (Monroe County, LA), respectively, in samples obtained in the 1970s and 1980s (Adeshina and Todd, 1990; Kutz et al., 1991b). Results from the National Human Adipose Tissue Survey summarized by Kutz et al., (1991b) showed that the average levels of heptachlor epoxide in the US population have been remarkably stable. Average levels of heptachlor epoxide were 0.09 ppm in 1970 (n=1412); 0.08 ppm in 1976 (n=682) ; and 0.09 ppm in 1983 (n=407). A more recent study has reported similar average levels of heptachlor epoxide (0.086 ppm) in 35 adipose samples obtained from autopsies specimens of residents of North Texas in 1987-88 (Adeshina and Todd, 1990).

A small study that examined organochlorine levels in postmortem adipose tissue levels in 19 males and six females from El Paso, Texas, reported mean levels of 0.12 ppm heptachlor, and 0.01 ppm heptachlor epoxide (Redetzke et al., 1993). An interesting finding was that the women in this study had significantly higher levels of heptachlor at 0.31 ppm compared to the mean value of 0.06 ppm heptachlor in the men. The authors suggest that the higher level of heptachlor in the women may have indicated that the women were exposed to a domestic source of heptachlor, such heptachlor used in residential termite control. However, air samples were not taken from the homes or workplaces of the subjects to confirm this hypothesis.

2. Cow's Milk and Human Breast Milk Levels:

Human milk contains 3 to 5% of lipids, and heptachlor has been widely reported as a breast milk contaminant (Levine, 1991). (Note on units: studies report chemical concentrations in human breast milk as either the level in whole milk, or the concentration of the chemical per gram of milk fat). A review of lactation studies from Europe, Africa, Asia, and America reported that heptachlor epoxide present in human milk fat ranged between 0.015 ppm (Canada) to 0.72 ppm (Israel) (Jensen, 1991). Analysis of human milk fat samples obtained in the early 1980s from 1,436 women residing in the US has revealed that heptachlor epoxide was present above detection limits in 63.1% of the samples and averaged 91.4 ppb (Savage et al., 1981). Savage et al. (1981) reported that in the US the highest levels of heptachlor epoxide in human milk fat were found in the Southeast (128 ppb, n=221), followed by the Midwest (90.6 ppb, n=272), Southwest (75.8 ppb, n=178), Northeast (71.8 ppb, n=144), and Northwest (66.1 ppb, n=91).

There are reports of agricultural misuse of heptachlor that resulted in elevated levels of heptachlor epoxide in cow's milk and human breast milk in Hawaii (Allen et al., 1997; Rogan et al., 1991; Smith, 1982). The Hawaiian State Department of Health detected unexpected high amounts of heptachlor epoxide in cow's milk in January of 1982 that were traced to "green chop" fed to dairy cows over a 15 month period during 1981 and 1982. The green chop included heptachlor contaminated pineapple leaves (Rogan et al., 1991; Smith, 1982). Heptachlor was used on the pineapples to prevent mealybug wilt, a condition caused by toxins released into the pineapple by the parasitic mealybug. Heptachlor was used to kill ants that influenced the survival of the mealybug population (Smith, 1982). It was found that 18 out of the 19 dairy farms on Oahu used pineapple green chop for feeding dairy cattle. All of the milk from the dairy farms was pooled for processing at two facilities on Oahu. Virtually all of the heptachlor contaminated cow's milk was sold on the island of Oahu, and with few exceptions, was not exported to other Hawaiian islands or to the mainland (Baker et al, 1991). Levels of heptachlor in cow's milk were reported to be as high as 1.2 to 2.7 ppm (Smith, 1982; Baker et al., 1991). The FDA action level for heptachlor epoxide was 0.3 ppm in 1982 (Smith, 1982).

The heptachlor contamination in Hawaii appears to have influenced levels of heptachlor epoxide in human breast milk. The levels of heptachlor epoxide in 50 human breast milk samples obtained from nursing women in Hawaii in 1977 were reported to be relatively low, averaging 35 ppb in milk fat (0.035 ppm) in women 18-24 years of age, and 27-44 ppb in women in women 30 to 37 years of age (Takahashi et al., 1981). Levels in the early 1980s during the episode of the heptachlor contamination, were reported to be threefold higher at 0.1 ppm [unpublished study cited in (Rogan et al., 1991)], which was similar to the range of heptachlor epoxide levels reported in human breast milk on the US mainland during a similar time period (Savage et al., 1981). Other researchers have reported that the levels of heptachlor epoxide in 102 human milk samples obtained from Hawaiian lactating women in the 1980s (0.036 ± 0.013 ppm, lipid basis) were comparable to levels of heptachlor epoxide found in US mainland human milk samples (0.055 ± 0.039 ppm) (Takei et al., 1986). In contrast, others (Allen et al., 1997) have cited unpublished reports from the Heptachlor Research and Education Foundation which reported heptachlor epoxide levels as high as 1.2 ppm in breast milk fat during 1981 to 1982 (Baker, 1994). Ten years later in 1993, the levels of heptachlor epoxide in breast milk and in the serum of adults and children on Oahu, was higher than levels on neighboring islands and the US mainland [unpublished study (Baker, 1994) as cited by (Allen et al., 1997)]. It has been suggested that studies be undertaken in Hawaii to determine if exposure to heptachlor, and to many other organochlorine pesticides that were used extensively on some of the islands, may play a role the rising incidence rates of breast cancer reported in Hawaii over the last several decades (Allen et al., 1997).

Another case of contamination of commercial cow's milk occurred in Van Buren, Arkansas in 1986. Heptachlor metabolites, components of technical chlordane, and chlordane metabolites (heptachlor epoxide, *trans*-nonachlor and oxychlordane, respectively) were found in the raw milk from 33 dairy farms (Stehr-Green et al., 1986). The source of the contamination was discarded seed that had been treated with heptachlor and other pesticides to make alcohol-based fuel, and the left over mash had been sold as animal feed to the dairy farmers (Farley, 1988). Heptachlor epoxide levels in raw milk were found to be as high as 89.2 ppm. Thousands of gallons of milk and dairy products were subsequently removed from store shelves, and laboratory analyses found the processed milk had heptachlor epoxide levels as high as 12.6 ppm (fat basis) (Stehr-Green et al., 1986). Subsequently, researchers attempted to evaluate the magnitude of the exposure, and the presence of acute health effects in group with the highest risk of exposure; the dairy farm families that had consumed the raw, heptachlor contaminated milk (Stehr-Green et al., 1986; Stehr-Green et al., 1988). Serum samples were collected from 45 individuals from 13 families for the determination of heptachlor epoxide, oxychlordane, and *trans*-nonalchor levels. Serum levels from heptachlor exposed families were compared to serum samples from participants in the National Health and Nutrition Examination Survey (NHANES) from neighboring states who were known not be exposed to the heptachlor contaminated milk. Adjustments were made, using covariance, for age and sex differences. Exposed farm family members had statistically significantly ($p<0.01$) higher mean levels of serum heptachlor epoxide (0.81 ± 0.94 ppb), oxychlordane (0.7 ± 0.75 ppb) and *trans*-nonachlor (0.79 ± 0.60 ppb) compared with unexposed individuals from the NHANES study (control values not provided). This study also assessed whether liver function was affected in the dairy farm families that had consumed heptachlor contaminated milk, compared to 85 individuals from dairy farm families who had not consumed contaminated milk. There were no differences in either levels of liver function enzymes or the induction of liver microsomal enzymes in the exposed and non-exposed dairy farm families (Stehr-Green et al., 1986; Stehr-Green et al., 1988). Data was not available on long-term follow of this population in regard to chronic disease states, including breast cancer.

A study has examined the relationship between occupational exposure to heptachlor and levels of heptachlor epoxide in the breast milk of Finnish women (Mussalo-Rauhamaa et al., 1988). Over 50 to 60 metric tons of heptachlor was used in the manufacture of plywood during the mid-1980s in Finland. Scrap wood was destroyed by burning, potentially releasing heptachlor into the environment. These investigators did not find that the residues of heptachlor or heptachlor epoxide differed in 22 lactating women who worked in the plywood industry compared to those who lived in plywood burning areas, or in "other" mothers.

Because of the widespread use of persistent organochlorine pesticides in the US during the 1950s through the 1980s, and the bioaccumulation in the breast adipose tissue of women and its transfer to human milk, researchers have struggled with the question of whether mothers should breast feed their infants. Researchers at the National Institute of Environmental Health Sciences (NIEHS) have conducted risk analyses comparing the lives saved in the postneonatal period by breast feeding to the estimated excess cancer deaths attributable to a variety of organochlorine compounds in human breast milk. These researchers 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. Case-Control Studies:

The ability of heptachlor epoxide to bioaccumulate in fat tissues of the body has led some investigators to evaluate possible associations between breast cancer and mammary fat levels of heptachlor epoxide. Only a few, very small case-control studies have been conducted to determine if there is a possible association between heptachlor/heptachlor epoxide residues in breast tissue and the risk for breast cancer. Although 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 heptachlor epoxide and cancer risk, we have presented these studies because they are the only studies available on heptachlor and breast cancer risk. The results of these studies are presented in Table 1, and are summarized below.

In a small pilot study, Falck et al. (1992) determined the levels of organochlorine residues in the breast fat from 20 patients with malignant breast tumors and 20 patients with benign breast disease. The levels of heptachlor epoxide plus oxychlordane in patients with breast cancer were not statistically different from those of control patients with benign breast disease. This study had several limitations. Levels of heptachlor epoxide and oxychlordane were not reported separately. Cases and controls were not matched for age, nor were other breast cancer risk factors such as parity, reproductive history or menopausal status controlled for in this study. Tissue samples were obtained from patients with benign breast disease; it would have been more appropriate to obtain samples from non-cancer surgical controls who were free of breast disease.

A study conducted by Mussalo-Rauhamaa et al. (1990) determined the levels of heptachlor epoxide in breast fat samples from 44 breast cancer patients compared with postmortem samples obtained from 33 cancer-free Finnish women of similar weight, height and age. Background information on age weight, height, occupation, residence, fish consumption, parity and lactation

Table 1. Breast adipose tissue concentrations (ppm) of heptachlor epoxide residues in women with breast cancer (cases) or women without breast cancer (controls)

Authors	Cases	Controls	p value
Falck et al., 1992	0.136 ± 0.053 [ab] (20)	0.121 ± 0.053[ac] (20)	0.36 (NS)
Mussalo-Rauhamaa et al., 1990	0.03 ± 0.02 [a] (12)	0.02 ± 0.02[ad] (12)	0.62 (NS)
Wassermann et al., 1976	0.274 (9)	0.044[d] (5)	none specified

[a] Mean ± SD

[b] heptachlor epoxide + oxychlordane

[c] controls had benign breast disease

[d] samples obtained from deceased patients free of breast cancer

Parentheses: number of subjects

NS = not significant at $p<0.05$

history was obtained by questionnaire from the breast cancer patients; similar background information was obtained from relatives of most of the deceased controls. More women with breast cancer were nulliparous (14/44) compared to the women without breast cancer (4/33), but these differences were not statistically different (p=0.16). While the levels of heptachlor epoxide were higher in the women with breast cancer compared to the women without breast cancer, there was high variability within the values for each group, and the mean levels of heptachlor epoxide were not statistically different between cases and controls (Mussalo-Rauhamaa et al., 1990). This study is of limited value because of the very small sample size, use of postmortem controls, and because background information on controls could only be obtained by proxy.

In a third epidemiological study, there were higher levels of heptachlor epoxide in breast adipose tissues from nine patients with mammary carcinomas compared to five controls (Wasserman et al., 1976). It should be noted that this was an extremely poor quality study, both in terms of the design of the study and the statistical analysis of the results. The sample size was extremely small, and there was no attempt to collect background information on the cases and controls. There was no attempt made to control for confounding breast cancer risk factors such as reproductive history, age, weight, height or parity. The statistical analysis of the data was minimal; only means and ranges were available, standard deviations were not computed, and no statistical comparisons were made between the levels of organochlorine residues in the adipose tissues of breast cancer patients as compared to controls. This study is only cited here for the sake of completeness and to point out its severe limitations.

These studies do not provide sufficient evidence of a causal relationship between heptachlor exposure and breast carcinogenicity. None of the three studies found a statistically significantly higher level of heptachlor epoxide in breast cancer patients compared to controls without the disease. These studies also have experimental design limitations. None of the studies attempted to characterize the exposure to heptachlor or chlordane (i.e., diet, occupational, residential termiticide treatment). All

three studies were based on a very small number of individuals, less than 25 per group, which limits the power of the statistical tests. In one study, control subjects were not free of breast disease (Falck, et al. 1992). Two of the studies used autopsy specimens to obtain control tissue, and limited information could be obtained by proxy on the background characteristics and confounding breast cancer risk factors from deceased cases and controls (Mussalo-Rauhamaa, et al. 1990; Wassermann, et al. 1976). None of the studies carefully controlled for confounding breast cancer risk factors. Larger population-based case-control studies will be required to determine whether higher body burdens of heptachlor metabolites do or do not affect the risk of developing breast cancer.

B. Animal Studies:
To the best of our knowledge, there have been no reports of increased incidence of mammary tumors in oncogenicity studies of heptachlor or heptachlor epoxide-treated laboratory animals.

C. Other Relevant Data on Breast Cancer Risk:
1. Immunological Effects:
A compromised immune system may affect host defenses against cancer. There is some evidence from studies in mice that *in utero* exposures to chlordane can adversely affect the developing immune system of the fetus [see BCERF Critical Evaluation on chlordane] (Blaylock and Mehendale, 1995; Blyler et al., 1994; Lau et al., 1990; Menna et al., 1985; Spyker-Cranmer et al., 1982). No *in vivo* animal studies were found that evaluated the possible immunotoxic effects of heptachlor *in utero* or in adult animals. However, one of the predominant isomers of chlordane, *trans*-chlordane can be metabolized to heptachlor (IARC, 1991).

Heptachlor has been shown to induce differentiation of human myeloblastic leukemia cells (Chuang et al., 1993; Chuang et al., 1991), proliferation of rhesus monkey lymphocytes (Chuang et al., 1992) and stimulation of superoxide generation by pig leukocytes (Suzaki et al., 1988). However, all the above studies on heptachlor have looked at the response of immune-competent cells in culture, and such assays have been shown by other researchers to be of limited value in predicting the *in vivo* effect of pesticides on the immune system (Johnson et al., 1987). Hence, *in vivo* animal studies are needed to determine if exposures to heptachlor can alter host immune response and susceptibility to cancer.

Whether heptachlor exposure affects immune response in humans and subsequent susceptibility of humans to breast cancer is not known. We were not able to locate studies in the scientific literature that have evaluated heptachlor exposure and immune response in humans. One small study reported some level of increased titer of autoimmune antibodies in 11 out of 12 individuals exposed to technical chlordane in the home or in a work environment (McConnachie and Zahalsky, 1992). Since technical chlordane is approximately 20% heptachlor, further studies are needed to determine if heptachlor or heptachlor epoxide affects immune function and subsequent cancer risk in humans.

2. Mutagenicity:
Heptachlor is not mutagenic in bacteria (Probst et al., 1981) or mammalian liver cells (Telang et al., 1982). Heptachlor was also shown to be non-genotoxic in rodent hepatocytes (Maslansky, 1981; Probst et al., 1981) and a SOS microplate assay (Venkat et al., 1995). Dominant lethal assays were also negative for heptachlor and its epoxide (Arnold et al., 1977). These studies support the conclusion that heptachlor and heptachlor epoxide are not genotoxic.

3. Evidence of Tumor Promotion:
B6C3F1 male mice were pretreated with the carcinogen diethylnitrosamine (DEN) (20 ppm in the drinking water) for 14 weeks, and after a 4-week period were fed 5 or 10 ppm technical grade heptachlor in the diet for 25 weeks. Approximately 80% of the heptachlor-treated mice developed liver tumors, compared to 40% in mice that received a control diet without heptachlor after DEN-pretreatment (Williams and Numoto, 1984). A similar incidence of liver tumors was reported in mice pretreated with 25 or 50 ppm technical chlordane. These results suggest that both technical heptachlor and chlordane are promoters of liver tumors in male B6C3F1 mice.

4. Signal Transduction and Intercellular Communication:
Gap junctional intercellular communication (GJIC) plays an important role in the regulation of cell proliferation and differentiation, and agents that affect GJIC may affect cancer risk. Many non-genotoxic chemical carcinogens and tumor promoters inhibit GJIC *in vitro* and *in vivo* (Ruch et al., 1990). Heptachlor has been shown to inhibit GJIC in mouse and rat hepatocytes (Matesic et al., 1994; Ruch et al., 1990). Heptachlor is capable of promoting hepatocarcinomas in mice (Williams and Numoto, 1984). The inhibition of GJIC may represent one possible mechanism by which heptachlor and heptachlor epoxide promote the formation of tumors in the mouse liver. Heptachlor and heptachlor epoxide also inhibit GJIC in normal human breast epithelial cells at non-cytotoxic concentrations (Nomata et al., 1996). Therefore, animals studies are needed to determine if these insecticides could act as mammary tumor promoters in the presence of known mammary carcinogens such as dimethylbenz[*a*]anthracene (DMBA) or *N*-nitroso-*N*-methylurea (NMU).

5. Disruption of the Endocrine System:
A number of studies have described the ability of technical heptachlor 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. Endocrine disruption may also occur at the level of the target tissues. The significance of these studies are discussed below.

a. Effects on Hepatic Microsomal Hydroxylases:
Studies have investigated the ability of heptachlor to affect the metabolism of steroids in the livers of laboratory animals. In a study reported by Haake et al. (1987) three week old male Long Evans rats received an intraperitoneal injection of 250 mmol/kg heptachlor (purity unspecified) in corn oil and were sacrificed five days later. Heptachlor induced an increase in the activity of several liver microsomal monoxygenases, including the P-450 isoenzymes involved in the 16α– and 16β-hydroxylation of testosterone and induced benzo[*a*]pyrene hydroxylase.

In another study, Welch et al. (1971) showed that intraperitoneal (i.p.) treatment of immature female rats with 10 mg/kg/day heptachlor for seven days induced a 2.5-fold increase in the metabolism of estrone by liver microsomal enzymes resulting in increases in the secretion of estrone 'polar metabolites.' The exact nature of the polar estrone metabolites was not specified.

Both of these studies suggest that heptachlor may affect hydroxylation pathways of steroids, including estrogen (Haake et al., 1987; Welch et al., 1971). There has been increased interest in the role polar metabolites of estrogen may play in breast cancer risk. Several researchers have hypothesized, and have offered preliminary evidence, that the stimulation of P-450 microsomal hydroxylation pathways by some organochlorine pesticides yields estrogen metabolites that may increase breast cancer risk, while other hydroxylation pathways yields metabolites that may decrease breast cancer risk (Bradlow et al., 1995; Davis et al., 1997). The polar estrone metabolite associated with an increased breast cancer risk is called 16-alpha hydroxyestrone (16-OHE1), and the 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. In contrast, while the 2-OHE1 does not have any of these properties, it 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; Tiwari et al., 1994).

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 yet tested the effect of heptachlor or heptachlor epoxide administration on the formation of these hydroxylation products *in vivo* or *in vitro*. Therefore, further studies are needed to determine if P-450 dependent estrogen hydroxylation pathways are induced by heptachlor and heptachlor epoxide, and if the metabolites generated are potentially genotoxic or non-genotoxic to breast cells.

b. Evidence of Estrogenicity:
Increased exposure to estrogen has been associated with increased breast cancer risk (Dorgan et al., 1997; Harris et al., 1992; Pike et al., 1993). Whether or not heptachlor can mimic estrogen effects would help define its potential to affect breast cancer risk. Soto et al. (1995) have developed an E-SCREEN assay designed to test the "estrogenicity" of xenobiotics based on their ability to stimulate the proliferation of estrogen-dependent human breast MCF-7 cells relative to estradiol-17β (100% potency induced by 10-100 pM estradiol-17β). These authors state that the MCF-7 proliferation test is biologically equivalent to the classic test of estrogen-induced increase in mitotic activity in immature rodent uteri. Heptachlor did not induce MCF-7 proliferation in the E-SCREEN assay (Soto et al., 1995). These results suggest that heptachlor is not estrogenic and should be confirmed by other *in vivo* and *in vitro* screening tests for estrogenicity.

c. Effects on Reproduction:
Studies have found that technical heptachlor is a reproductive toxin in rats (Rani and Krishnakumari, 1995). Technical heptachlor was fed to 30 CFT-Wistar rats of each sex at 0, 45.25, and 90.5 mg/kg for males, and 0, 25 and 50 mg/kg for females. Females and males were treated for 14 and 70 days prior to mating, respectively. The low dose of 25 mg/kg heptachlor significantly decreased serum levels of both estradiol and progesterone in females ($p<0.05$). In contrast, diets containing the high 50 mg/kg dose of heptachlor had no effect on the rats' secretion of estrogen and progesterone. Both doses of heptachlor significantly reduced the percentage of pregnancies ($p<0.01$). In males, heptachlor induced a dose-dependent decrease in sperm count (18.5 and 67.2%, respectively). This study demonstrates that heptachlor can disrupt the secretion of steroid hormones and interfere with fertility in both sexes.

No studies have been conducted to test the effects of heptachlor on the female rodent estrous cycle or effects on circulating levels of estrogen or progesterone.

A lack of reproductive success has been reported in female mink fed technical grade heptachlor at 6.25, 12.5, and 25 ppm (mg/kg) prior to and throughout the reproductive period (181 days) (Crum et al., 1993). However, the mortality rates of the female dams (67% of the 12.5 ppm group and 100% in the 25 ppm group) were so high that dose-response relationships could not be effectively evaluated. Significant weight loss was also reported

in the 12.5 and 25 ppm females during the first 12 weeks of the study. Consumption of the 6.25 ppm heptachlor diet by the female mink prior to breeding and before gestation had no effect on pup birth weight or pup survival. This study did, however, provide evidence of placental transfer of heptachlor/heptachlor epoxide to the pups, as evidenced by increased body burdens of heptachlor epoxide at birth in pups born to dams fed 6.25 ppm (mean 0.86 μg heptachlor epoxide/g birth wt) or 12.5 ppm heptachlor (mean 3.08 μg/g birth wt) compared to controls (Crum et al., 1993).

In summary, these studies indicate that heptachlor disrupts the endocrine system by increasing the activity of liver microsomal enzymes which are involved in the metabolism of testosterone and estrogens (Haake et al., 1987; Welch et al., 1971). Further studies are needed to determine if heptachlor can affect the P-450 mediated hydroxylation pathways of estrone, and affect levels of estrone metabolites associated with genotoxic effects in breast cells. Heptachlor is a reproductive toxin in rats (Rani and Krishnakumari, 1995). Mink pups born to dams fed heptachlor retain heptachlor epoxide in their tissues (Crum et al., 1993). Heptachlor does not appear to be estrogenic as determined by the E-SCREEN assay (Soto et al., 1995).

VI. Other Relevant Information

A. Environmental Fate and Potential for Human Exposure

Heptachlor is readily oxidized to heptachlor epoxide in the environment (ATSDR, 1993; IARC, 1987). In animals and humans, the principal metabolites of heptachlor are heptachlor epoxide and 1-*exo*-hydroxychlordene epoxide. Heptachlor epoxide does accumulate in body tissues and is excreted in the feces and urine. The 1-*exo*-hydroxychlordene epoxide is excreted in the urine (Tomlin, 1994). The half-life of heptachlor epoxide is longer than heptachlor, and hence, heptachlor epoxide is an extremely persistent metabolite and can bioaccumulate in fat tissue (See Section VII. A on Human Tissues Levels of Heptachlor and Heptachlor Epoxide). Because heptachlor and heptachlor epoxide are very lipophilic and not very water soluble, drinking water does not constitute a major route of exposure (ATSDR, 1993). The 90th percentile levels of heptachlor epoxide in the groundwater of New Jersey in the early 1980s has been reported to be 0.1 ppb, which is half of the MCL for drinking water at 0.0002 mg/L (equal to 0.2 ppb) (Page, 1981). Heptachlor epoxide was rarely found in water samples taken from the Grand, Saugeen, and Thames Rivers in Ontario, Canada (Frank et al., 1991). In samples of 466 water samples taken from these rivers from 1986-90, only a single sample in the Grand and Saugeen River and none in the Thames River had detectable levels of heptachlor epoxide.

Both heptachlor and heptachlor epoxide adsorb strongly to soils and lake/river bed sediments. The half-life of heptachlor in soil has been reported to range between 0.75 and 2 years (IARC, 1991). Heptachlor and heptachlor epoxide have the potential of bioconcentrating in the food chain in aquatic and terrestrial animals. This is why one of the major routes of exposure is through the diet, primarily in milk and dairy products, root vegetables, meat, fish, and poultry (ATSDR, 1993).

The contamination of cow's milk in Hawaiian dairy cows fed "green chop", leaves from pineapples treated with heptachlor, during the early 1980s has been previously discussed in this Critical Evaluation (Smith, 1982). BCERF could not locate reports documenting adverse health effects in the individuals who drank the heptachlor-contaminated cow's milk, or in the offspring of women exposed during their pregnancy, or in infants fed breast milk with elevated levels of heptachlor/heptachlor epoxide (Stehr-Green et al., 1986; Le Marchand et al., 1986). Researchers have recommended a surveillance program to assess the incidence of adverse health effects, including the incidence of breast cancer (Allen et al., 1997).

The National Contaminant Biomonitoring Program has surveyed the presence of organochlorine chemicals in freshwater fish in the US (including Hawaii), and reported on trends in the levels of heptachlor and heptachlor epoxide levels in fish from 1976 to 1984. Since heptachlor is converted to heptachlor epoxide by many organisms, including fish, relatively little unmetabolized heptachlor was detected in fish. The concentrations of heptachlor epoxide in fresh water fish in 1984 were highest in Hawaii (mean 0.20 μg/g wet wt), followed by the Midwest, especially in fish from Lake Michigan (0.015 to 0.3 μg/g wet wt), and in the rivers of Mississippi, Missouri, Ohio, and Illinois (0.01 μg/g wet wt) (Schmitt et al., 1990). A 1985 study of contaminants in fish from Midwestern rivers reported levels of heptachlor epoxide as high as 0.48 mg/kg in fish obtained from the Sheboygan River in Wisconsin, compared to levels of 0.02 mg/kg in fish from the Milwaukee River (De Vault, 1985).

There are relatively few recent reports on the levels of heptachlor epoxide in the diet of Americans. One study (MacIntosh et al., 1996) has assessed dietary exposure to heptachlor epoxide by matching food consumption patterns as collected from two large epidemiological studies, the Nurse's Health Study, and the Health Professional's Follow-up Study, with the pesticide residue levels reported for 236 foods in the Food and Drug Administration (FDA) 1986-1991 Total Diet Study. The strength of this study is that it estimated dietary exposure to pesticide residues in a large cohort; 78,882 females and 38,075 males. They estimated the mean dietary exposure to heptachlor epoxide to be 0.3 μg per day, with a maximum concentration of 1.0 μg (MacIntosh et al., 1996). The World Health Organization's (WHO) Acceptable Daily Intake (ADI) for heptachlor and heptachlor epoxide (combined) is 0.0001

mg/kg (WHO, 1993); which for a 60 kg female would be 6 μg. Therefore the estimated levels of heptachlor epoxide in the diets of most adult Americans appear to be below the ADI.

Besides diet, another primary route of exposure is through inhalation. Both heptachlor and heptachlor epoxide have low vapor pressures and can volatilize from soil particles into the air. Kamble et al. (1992) reported that in homes treated with technical chlordane (heptachlor makes up to 20% of 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 National Research Council (NRC) guidelines (2 μg/ m^3) (Kamble et al., 1992). In this study, air was sampled for four hours from the basement, kitchen and one bedroom of each home (n=19), but only mean values per residence were reported. Levels of heptachlor were highest during treatment (5 μg/m^3), and were between 3 and 2 μg / m^3 in the air 180 days posttreatment.

Since a higher proportion of homes in the Southeastern US were treated in the past for termites with chlordane and heptachlor compared to other areas in the US, these southern homes have the greatest potential for having detectable levels of heptachlor in indoor air (ATSDR, 1993). Those persons who live in heptachlor or chlordane treated homes and spend most of their time indoors (i.e., limited mobility, retired persons, infants, young children) may have the potential for exposure to heptachlor or heptachlor epoxide via indoor air (ATSDR, 1993).

Pilot studies have been conducted to develop methods for sampling the indoor air and household dust for pesticides residues. This includes the development of the HVS3 dust sampler to determine dislodgable pesticide residues in carpets (Lewis et al. 1994). This study also estimated childhood hand-to-mouth exposures to pesticides using bare-hand prints, and solvent hand rinses. Nine homes were included in this pilot study. Mean heptachlor levels were: 0.62 μg /m^3 in dislogable carpet dust; 0.02 μg/m^3 by hand press; 0.04 μg in child hand rinses, 0.07μg /m^3 in the household air, and 0.01 mg /g in play area soil. Therefore, carpet dust had more heptachlor than outdoor play soil. No indication was given as to where the soil sample was obtained. While more studies are needed to improve methods to predict how household dust may contribute to the ingestion of heptachlor, these preliminary results do indicate that residues are present in the household dusts of some homes, and may contribute to heptachlor exposure.

Because heptachlor and heptachlor epoxide can be in soils around foundations treated with chlordane or heptachlor, inhalation can take place when digging or handling the contaminated soil. Therefore, activities such as gardening close (2-3 feet) to foundations of homes or buildings treated with chlordane or heptachlor should be discouraged. Individuals living near hazardous waste sites contaminated with heptachlor or chlordane could also be exposed to heptachlor or heptachlor epoxide in air or soil (ATSDR, 1993).

An EPA sponsored study (Whitmore et al., 1994) has attempted to determine the nonoccupational exposure to heptachlor via inhalation and dietary routes. The study was conducted in an area of relatively low pesticide use, Springfield/Chicopee, MA and 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. Samples of 49 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 and the values from the USDA Total Diet Survey's levels of pesticide residues on raw agricultural commodities, and by analyzing tap water samples. Indoor and personal air levels of heptachlor tended to be higher indoors than outdoor, and higher in Jacksonville than in Springfield/Chicopee. Heptachlor levels in the air tended to be highest in summer, lower in spring, and lowest in winter. The highest levels reported were in the summer in Jacksonville with a mean indoor air concentration of 163 ng/m^3. Mean air concentrations for heptachlor were 2,312 ng/day in Jacksonville and 544 ng/day for Springfield. These levels were higher than estimated dietary exposures, with 155 ng heptachlor/day in Jacksonville and a comparable 150 ng heptachlor/day in Springfield. This study would suggest that inhalation is the major route of exposure to heptachlor. However, it should be noted that dust samples were only obtained in nine homes and because of methodological problems, values were not reported, so it is not known to what extent household dust would have contributed to nonoccupational heptachlor exposure in this study.

Those who have been occupationally exposed to heptachlor or that applied heptachlor in their homes may have been exposed to heptachlor by inhalation and/or by dermal contact if protective clothing were not worn. Occupations with potential for exposure to heptachlor include: 1) agricultural workers who used, mixed, or applied heptachlor or chlordane (technical chlordane contains heptachlor), 2) pesticide applicators, 3) lawn care workers, 4) those employed in manufacturing operations, such as plywood manufacturing, or those who were or are currently employed at heptachlor or chlordane manufacturing plants. Another potentially exposed population would include those who handled or laundered work clothing contaminated with heptachlor. Virtually all of the studies that have followed occupational exposures to heptachlor and the incidence of cancer deaths has been done on male workers

employed in a heptachlor manufacturing plant or male pesticide applicators (see section IV. of this Critical Evaluation). No occupational studies were located on women exposed to heptachlor and cancer risk.

VII. Summary and Recommendation for Breast Cancer Risk Classification:

A. Breast Cancer Risk Classification:
There is inadequate evidence to classify heptachlor as a "human breast carcinogen" (see Appendix B for BCERF Breast Cancer Risk Classification scheme). Because of the insufficient evidence of human breast carcinogenicity, no evidence of mammary carcinogenicity in experimental animals, and limited evidence of mechanisms by which heptachlor and heptachlor epoxide may affect breast cancer risk, we conclude that these chemicals should be classified in Group 3, *"inadequate evidence for classification of breast cancer risk."* These conclusions are based on the following evidence:

• **The available human studies provide insufficient evidence to conclude that heptachlor or heptachlor epoxide exposure causes breast cancer in women.** Case-control studies on the breast carcinogenicity of heptachlor in humans are inadequate because: 1. they were based on very few cases (less than 25 per group) limiting their statistical power; 2. studies did not provide or characterize exposure to heptachlor or heptachlor epoxide; 3. some of these studies used inappropriate controls, including women with a history of benign breast disease; and 4. none of the studies adequately controlled for confounding breast cancer risk factors (Falck et al., 1992; Mussalo-Rauhamaa et al., 1990; Wassermann et al., 1976).

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

• **There is limited evidence of various mechanisms by which heptachlor may affect breast cancer risk.** These data include: heptachlor's persistency in the environment; the potential for continued exposure to human populations (ATSDR, 1993); the ability to affect the metabolism of estrone by increasing the activity of hepatic microsomal steroid hydroxylases (Haake et al., 1987; Welch et al., 1971); the ability of heptachlor to promote liver tumors (Williams and Numoto, 1984); the ability to affect the differentiation (Chuang et al., 1993; Chuang et al., 1991) and mitogenic response (Chuang et al., 1992) in immune competent cells ; and the ability to interrupt gap junctional communications between cells (Haake et al., 1987; Welch et al., 1971). However, further research will need to be conducted to determine if heptachlor or heptachlor epoxide affects breast cancer risk by any of these mechanisms.

VIII. Research Gaps and Other Recommendations:

• Large-scale, case-control studies are needed to determine if women with breast cancer have higher levels of heptachlor or heptachlor metabolites in their blood and/or breast fat than women without the disease.

• Animal studies are needed to evaluate heptachlor and heptachlor epoxide'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).

• Populations of women with known exposures to heptachlor/ heptachlor epoxide should be identified and monitored to determine if past exposures influence health-related effects, including the incidence of breast cancer. This includes: 1) women exposed to technical heptachlor or technical chlordane in manufacturing facilities; 2) female agricultural workers who worked in fields treated with chlordane or heptachlor; 3) female spouses of men exposed to chlordane occupationally (agricultural workers, pesticide applicators, lawn care workers) who may have been exposed by handling or laundering contaminated clothing; and 4) Hawaiian and Arkansas women and offspring of the women exposed to heptachlor/heptachlor epoxide contaminated cow's milk or breast milk.

• Studies are needed to determine the effect of heptachlor/ heptachlor epoxide exposure on estrogen metabolism in animal models. This includes the short- and long-term effects of heptachlor on: circulating blood levels of estradiol-17β, effects on binding of ligands to the estrogen receptor; and if P-450 dependent hydroxylation pathways are induced by heptachlor/ heptachlor epoxide, and whether the hydroxylated estrone metabolites generated are potentially genotoxic to breast cells.

• Further studies are needed to determine if heptachlor/heptachlor epoxide can compromise the immune system in ways that will affect the body's defense mechanisms against breast cancer. Studies should be conducted to determine if animals exposed to heptachlor/heptachlor epoxide *in utero*, or as adults, and subsequently exposed to transplantable mammary tumors cells, develop higher incidences of mammary tumors. Studies following human populations exposed to heptachlor should include an assessment of immune function, as well as breast cancer incidence, to determine if heptachlor or heptachlor epoxide may affect breast cancer risk by compromising immune function.

IX. Summary of Human Studies Currently Being Conducted:

We have recommended the need for more epidemiological studies on breast cancer incidence rates in women with exposure to heptachlor /heptachlor epoxide. 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 web <http://eos12.dcrt.nih.gov:8002/crisp_pilot/owa/crisp.main> or by Gopher Menu <gopher://gopher.nih.gov:70/11/res/crisp>.

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 among New York City and Long Island researchers 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 5-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 and pesticides 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, as well as self-reports of occupational and residential exposure.

Organochlorine Residue Levels and Risk of Breast Cancer (PI: L. Bernstein, University 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 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 heptachlor epoxide, oxychlordane, *trans*-nonachlor. Serum residue levels will be examined in relation to odds of breast cancer in multivariate unconditional logistic regression models.

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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	Cornell Program on Breast Cancer and Environmental Risk Factors in New York State
bwt	body weight
C	carbon
CAS	Chemical Abstract Service
CASRN	Chemical Abstract Service Registry Number
CfE	Cornell University Center for the Environment
CI	Confidence Interval
Cl	chlorine
CRISP	Computer Retrieval of Information on Scientific Projects; database of scientific intra- and extramural projects supported by the Dept. of Health and Human Services (i.e., NIH, EPA, USDA)
DEC	Department of Environmental Conservation
DEN	diethylnitrosamine; a liver carcinogen
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
CIJC	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	Cornell 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.
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 Contaminant Level; enforceable limit set by the EPA which sets the maximum level of a contaminate in a public drinking water supply
MM	multiple myeloma
nmol	millimole(s)
n	number of subjects/animals in the group
ng	nanogram
NA	Not available
NAS	National Academy of Science
NHANES	National Health and Examination Survey
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
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
pM	picomole(s)
ppm	parts per million
ppb	parts per billion
ppt	parts per trillion
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
US	United States
USC	University of Southern California
USDA	United States Department of Agriculture
WHO	World Health Organization
2-OHE1	2-hydroxyestrone
16-OHE1	16-alpha hydroxyestrone

Symbols:

α	alpha
β	beta
γ	gamma
μ	micro
<	less than
>	greater than
≥	greater than or equal to
%	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. 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. Critical Evaluations of Breast Cancer Carcinogenicity

BCERF Breast Carcinogenicity 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 Carcinogenicity 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.