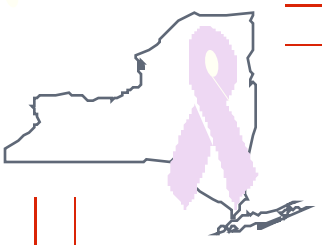

Cornell University

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

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Critical Evaluation of Lindane's Breast Cancer Risk

by

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Author's Note: The reader is encouraged to read the attached document, Appendix B, which includes an explanation of the BCERF Breast Carcinogen Classification System, before reading this Critical Evaluation.

I. Chemical Information

A. Common Names: Lindane (except in the United Kingdom), *gamma* HCH, *gamma* 1-1 BHC (Worthing and Hance, 1991)

B. Chemical Name: gamma isomer of 1α, 2 α, 3β, 4 α, 5 α, 6β–Hexachlorocyclohexane (IUPAC) (Worthing and Hance, 1991)

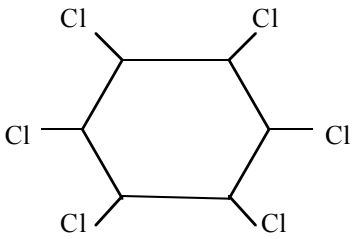
C. Chemical Formula: $C_6H_6Cl_6$ (Montgomery, 1993)

D. Formulators' Trade Names: Acitox® (Agro Chemicals Industries Ltd.); Hammer® (Agsin Pte. Ltd.); Etan® (Diachem S.P.A.); Wireworm FS® (DowElanco Ltd.); Lindasun® (Gupta Chemicals Pvt. Ltd.); Germate® Plus, Lindane 30, Lindane 40% (Gustafson, Inc.); Gamma-Mean 400®, Gamma-Mean L.O.®, Gamma Mean Seed®, Gamma-Up®, (Oregon-California Chemicals, Inc.); Kwell® (Reed and Carnrick), Sulbenz® (Sulphur Mills, Ltd.); Quellada® (Stafford-Miller Ltd); Lindagam®, Novigam®, Silvanol® (Meister, 1997).

E. Discontinued Trade Names: Isotox® (Chevron Chemical Co.); Chimac L200®, Lidax® (Chimac-Agriphar S. A.); Agrox® S (+ captan), Agrox® 3-Way (+ captan + diazinon) (Chipman Chemicals); Gammexane®, Gammasan®, Gammalin® 20, Didigam® (+DDT) (ICI Agrochemicals); Noita-koisumu® (+ DDT) (Kemira Oy); Lacco Hi Lin®, Lacco Lin-O-Mulsion® and Lin-O-Sol® (Los Angeles Chemical Co.); Cloroble Fort® (+ copper oxide + endosulfan) (Pechiney Progil); Lindol® (Rhone-Poulenc); Kotol®, Lindacol®, (Shell Chemicals, UK Ltd.); Agronexit®, Inexit®, Nexit® (Shell International Chemical Co. Ltd.); Ceregram® (+methoxyethylmercury silicate), Sopragam® (+ parathion) (SOPRA) (Meister, 1997).

F. CAS Registry Number: 58-89-9 (Montgomery, 1993)

G. Chemical Structure: hexachlorocyclohexane



II. History of Use, Usage and Nomenclature:

A. History of Use:
Hexachlorocyclohexane (HCH) was first prepared by Michael Faraday in 1825 by adding chlorine to benzene in sunlight (IARC, 1979). It was used as a smoke bomb during World War I. Its insecticidal properties were described independently by Dupire and Raucourt in 1943 (Smith, 1991). **Technical-grade HCH** is a mixture of 65 to 70% alpha-HCH, 7 to 10% beta-HCH, 14 to 15% gamma-HCH and 10% of other isomers and compounds (IPCS, 1991). The pure gamma-HCH (γ–HCH) isomer is called lindane, but may also be referred to as gamma-benzene hexachloride (γ-BHC). In this document, **lindane** is used to denote the purified gamma-isomer of HCH and **technical-grade HCH** is used to denote the mixture of HCH isomers. **HCH** is used when all isomers of hexachlorocyclohexane are present, but the proportions of the different isomers was not defined.

Slade showed in 1945, that the insecticidal properties of technical-grade HCH were due to the gamma-isomer, or pure lindane (Smith, 1991). Lindane is a contact poison since it is absorbed directly into the parasite and its eggs, as well as a stomach poison with some fumigant action (PDR, 1998; Worthing and Hance, 1991). Lindane is effective against a broad range of insects including cutworms and wireworms that eat leaves, insects that live in the soil, human and animal parasites such as fleas, ticks, lice and scabies, and in the treatment of mange (USEPA, 1990).

Commercial production of lindane in the U.S. was first reported in 1950 (IARC, 1979). Lindane was used as a spray for foliage, in soil applications, for seed treatment, and in baits for rodent control (Worthing and Hance, 1991). Registrations for lindane included its use as an insecticide for the treatment of a variety of fruits, seed grains and vegetable crops (including greenhouse vegetables and tobacco), in forestry (including Christmas tree treatment), for poultry and livestock, on animal farms and cultivated land, for household and treatment of uncultivated land (ATSDR, 1994; IARC, 1979). The major non-agricultural use for lindane has been for wood and timber protection (IPCS, 1991). In human medicine, lindane is still used at 1% levels for treatments of head lice, usually as lotions, creams, and shampoos (IARC, 1979; Smith, 1991; PDR, 1998).

B. Nomenclature: There is extensive literature on technical-grade lindane and the alpha- and beta-HCH isomers. Discussions in this evaluation focus on lindane, the gamma-HCH isomer. A recent study reports no significant interconversion of lindane into other isomers of HCH during its

metabolism (Waliszewski, 1993). An earlier study indicated the presence of small amounts of hexachlorobenzene (HCB) as a metabolite of lindane (Smith, 1991), but no conversion of lindane to hexachlorobenzene was detected in several other studies on lindane metabolism (IPCS, 1991).

C. Usage:
1. Agricultural Use:
The amount of active ingredient lindane used for fruits and vegetable cultivation per year during 1990-1993, was estimated at 61,189 lbs (Gianessi and Anderson, 1995). It ranked 51st in insecticide usage on agricultural crops, and was among the ten least used insecticides in the U.S. during this period (Gianessi and Anderson, 1995). Estimates of its use in New York State were not available.

2. Non-Cropland Use:
Less than 1000 kg of lindane was used in the U.S. in human medicine in 1971 (IARC, 1979). More recent estimates of its use were not found.

III. Current Regulatory Status:

A. Regulatory Status:
Concern over carcinogenicity, delayed toxic effects and acute toxicity risks related to hazards in aquatic wildlife, prompted EPA to issue a notice of Rebuttable Presumption Against Registration (RPAR), and continued registration of pesticide products containing lindane in 1977 (IARC, 1979). RPAR was the term used for the Special Review Process prior to 1986 (USEPA, 1996b). [Note: "The Special Review process" is set in motion when EPA has reason to believe that the use of a pesticide may result in adverse effects to people or the environment" (USEPA, 1996b)]. Following the initiation of RPAR, use of HCH isomers other than the gamma-isomer was voluntarily canceled. In October 1983, a notice of intent to cancel pesticide products containing lindane was issued by EPA (USEPA, 1990), but it was successfully challenged by registrants. In 1985, lindane was classified as a Restricted Use Pesticide, to be applied only by a certified applicator using protective equipment (USEPA, 1990). Its use for aquatic application and in vaporizers was canceled. New regulations were issued requiring specific warning statements on the labels of all lindane products. The use of existing stocks of lindane products by end users is permitted until the supply is exhausted. Any existing stock of products within the possession of the registrant, distributors or retailers must be disposed of in accordance with the Resource Conservation and Recovery Act (USEPA, 1990). EPA requires that spills or accidental discharges into the environment of 1 lb or more must be reported.

B. Drinking Water Standards and Health Advisories:
1. Maximum Contaminant Level:
The EPA has set the Maximum Contaminant Level (MCL) in drinking water for lindane at 0.0002 mg/L (USEPA, 1996a). This is an enforceable limit on the maximum allowable concentration in public water supplies.

2. Health Advisory:
Health advisory (HA) levels for drinking water as are follows (USEPA, 1996a):

10 kg child

- One day = 1 mg/L
- Ten day = 1 mg/L

70 kg adult

- Long term = 0.1 mg/L
- Lifetime = 0.0002 mg/L

The HA are non-enforceable 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 (USEPA, 1996a).

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

D. Food Tolerances:
The amount of pesticide permitted to occur on the edible portion of raw agricultural commodities and in processed foods, called tolerances, are set by EPA. Residue tolerances for lindane are: 0.1 mg/kg for pecans, 1 mg/kg for fruits and vegetables, 3 mg/kg for squashes, lettuce, melons and mushrooms, 4 mg/kg for pork and 7 mg/kg for other meat-fat (USEPA, 1998).

IV. Summary of Evidence of Overall Carcinogenicity

A. Human Studies
1. Case Reports:
Case reports alone are inadequate to infer a causal relationship for carcinogenicity of chemicals. They may indicate a need for further studies of a chemical and associated health risks in larger case-control studies.

Three case reports have linked lindane exposures with leukemia. A 66 year old male patient diagnosed with acute myeloblastic leukemia had used aerosol mixtures containing lindane, piperonyl butoxide and pyrethrum in different organic solvents (kerosene, xylene and toluene) (Sidi et al., 1983). Another case-report describes two cousins, both male, with no family history of cancer who were diagnosed with paramyeloblastic leukemia after occupational exposure to Gammexane®, a lindane containing insecticide (Jedlicka et al., 1958). Exposure to a lindane and toxaphene mixture was implicated in a case of myelomonocytic leukemia, secondary to aplastic anemia (IARC, 1979). These are reports of rare incidences of leukemia among people who were exposed to many chemicals including lindane.

While providing insufficient evidence of a causal relationship, they do suggest the need for evaluating the incidence of leukemia among populations exposed to lindane.

2. Population-Based Case-Control Studies

Pesticide use was evaluated in the families of 45 children who were diagnosed with brain cancer (Missouri Cancer Registry) by the age of ten years. Among the families that participated in the study, 97.8% recalled having used pesticides between when the child was of seven months of age and the diagnosis of cancer (Davis et al., 1992). A significant association was observed between the use of a lindane containing treatment against head lice, Kwell® (1% lindane) and the odds ratio (OR) for childhood brain cancer (OR = 4.6; 95% CI 1.0-21.3; p values not stated) in comparison with 85 cancer-free friends of the same age and sex as controls (Davis et al., 1993). Comparison with a control group of 108 other childhood cancers revealed a modest but not significant increase in the risk for brain cancer (OR = 1.9; 95% CI 0.6-6.9; p values not stated). The small sample size, lack of detailed exposure verification, potential for recall bias, and of multiple product use are limiting factors of this study. However, since anti-lice treatments are frequently used on children, this observation warrants further investigation.

In another case-control study of 266 cases and 547 controls among male farmers in Iowa and Minnesota, the risk for non-Hodgkin's lymphoma (NHL) in those who had ever handled lindane was significantly elevated. The increase in risk for those who used it as an animal insecticide (Group 1) was OR =1.4 (95% CI 1.0-2.1; p values not stated) and for those who used it as a crop insecticide (Group 2) was OR = 2.0 (95% CI 1.0-3.7; p values not stated). The risk for NHL was further increased if protective equipment was not used while handling lindane to OR = 1.6 (95% CI 1.0-2.4) for Group 1, and OR = 2.6 (95% CI 1.2-5.5) for Group 2 (Cantor et al., 1992). The OR for NHL were higher if lindane had been used prior to 1965, OR = 1.7 (95% CI 1.1-2.7) for Group 1, and 2.2 (95% CI 1.0-4.7) for Group 2 (Cantor et al., 1992). This study suggests an association between use of lindane and an increased risk for NHL, and the risk is increased with latency and the lack of protective equipment. However, in this population, increased risk for NHL was also observed in association with carbaryl, chlordane, DDT, dieldrin and malathion exposures. It was not possible to determine to what extent lindane exposures may have contributed to the increase in incidence of NHL. A parallel case-control study of male farmers in these two states, (578 cases and 1,245 controls) reported a non-significant elevation in the incidence of leukemia (OR = 1.1) among those who had ever handled lindane (Brown et al., 1990). In another study of male farmers from Iowa, in five cases of multiple myeloma and 19 population-based controls matched for age and vital status, there was no significant increase in the risk for multiple myeloma (OR = 1.2), in association with lindane exposures from mixing, handling or applying the pesticide (Brown et al., 1993).

In a later study, data from 987 cases of NHL and 2,895 population-based controls among male farmers from four Midwestern states (Kansas, Nebraska, Iowa and Minnesota) was pooled and evaluated for relationships between exposure to different pesticides and the risk for NHL. Controls were matched for age, sex, race, state of residence and vital status. The larger numbers in this study allowed for the computation of the OR for lindane with and without adjusting for the use of other pesticides. The OR for NHL associated with lindane use was significantly elevated (OR = 1.5; 95% CI 1.1-2.0); adjustment for use of organophosphates, inorganics, diazinon and 2,4-D reduced the OR observed for lindane use. The authors concluded that lindane did not appear to play a major role in increasing the risk of non-Hodgkin's lymphoma, but a small role could not be ruled out (Blair et al., 1998).

In another case-control study of 169 male NHL cases and 338 controls in Sweden, the exposure frequency to lindane was too low to determine an association (Eriksson et al., 1981; Hardell et al., 1981).

Studies with Exposure to Technical-Grade HCH and Other Isomers of HCH:

Studies summarized below are of limited value for determining the carcinogenic potential of lindane since they report on associations with technical-grade HCH or the most persistent beta-isomer of HCH, but not pure lindane.

Only one of the studies reviewed suggested a significant association between exposure to HCH and lung cancer. Male German agricultural workers (1,658) who had the potential for exposure to HCH (isomer not specified) and many other pesticides were observed to have a significant increase in lung cancer incidence ($p < 0.05$). The smoking habits of the farmers were stated to be no different than the general male population (Barthel, 1981). Although frequently cited, this study does not provide adequate evidence for a causal relationship between HCH exposures and lung cancer. While it lists all the chemicals that were available to the German farmers, exposures could only be deduced from the length of employment. There were many chemicals in use during the time of the study, including arsenic, asbestos, chlorinated dibenzodioxins and DDT.

Other studies have not indicated any significant associations between exposure to technical-grade HCH and cancer. Median total HCH levels were increased (37%) in the blood marrow of six male and seven female cases of leukemia and lymphoma in comparison with 16 healthy controls, but the increase was not statistically significant (Scheele and Niessen, 1996). Caldwell et al. (1981) found that serum levels of beta-HCH in childhood colorectal carcinoma cases (six males and four females) were not significantly higher than the levels in healthy siblings and parents. Another study found no association between higher levels of HCH isomers in fat tissues from autopsy cases and mortality from cancer (sex was not specified) (Hoffman et al., 1967). In a study of adipose tissue from 271 autopsy cases, higher levels of the beta-HCH were associated with an increased incidence of primary hepatomas, but only four cases were analyzed. A statistical analysis was not available for the association (Radomski et al., 1968).

3. Cohort Studies:
In a nested case-control study of 32 NHL cases and 158 controls from an international cohort of pesticide manufacturing workers and applicators, exposure to lindane was associated with a small, but not significant increase in the risk for NHL (OR = 1.6; 95% CI 0.3-8.8) (Kogevinas et al., 1995). These workers had exposures to many other chemicals, making the study limited in value for establishing an association between lindane and cancer incidences. Another study evaluated the health effects of 115 men and three women who were exposed to lindane, other HCH isomers and organic solvents through their work in a lindane production plant (average length of service of ten years). A clinical examination of the liver, kidneys, nervous system and blood producing organs of these workers did not reveal any persistent health problems associated with their occupation (Herbst, 1976).

Many other cohorts that were occupationally-exposed to technical-grade lindane have been studied for clinical symptoms, but not cancer incidence (Czegledi-Janko and Avar, 1970; Duffard and Duffard, 1996; Fleming et al., 1994; Munk and Nantel, 1977; Nigam et al., 1993; Rayner et al., 1972; Samuels and Milby, 1971; Srivastava et al., 1995). We recommend that these cohorts be followed for cancer incidences as well. A study of 26 farm workers exposed to technical-grade HCH reported no exposure-related changes in morbidities, but did not provide the mortality rates from cancer (Srivastava et al., 1995).

4. Summary, Human Studies:
Some case reports have implicated lindane exposures with leukemia. However, only a non-significant elevation in the incidence of leukemia was observed in a case-control study of farmers who had ever handled lindane (Brown et al., 1990). One study has reported an increased risk of childhood brain cancer associated with the family use of lindane containing delousing agents (Davis et al., 1993). This association needs to be evaluated further using larger sample sizes. One study has reported a significant increase in the incidence of NHL in association with lindane exposures, but the potential for confounding effects different pesticides were not adjusted (Cantor et al., 1992). A later study that pooled the data from four Midwestern states found that lindane exposures did not play a major role in the increase of NHL incidences in this population (Blair et al., 1998). Two other studies have not found a significantly increased risk for NHL associated with exposure to lindane (Kogevinas et al., 1995; Herbst, 1976).

B. Animal Experimental Studies
Most of the studies that have been done to evaluate the carcinogenicity of lindane would not be considered valid cancer bioassays due to the small number of animals evaluated (Fitzhugh et al., 1950; Truhaut, 1954, as cited in IPCS, 1991), the short durations of treatment (Hanada et al., 1973), or the low survival rates (Hanada et al., 1973). Many of the studies that were conducted from 24 to 26 weeks did not show any associations, but the short treatment periods may not have allowed time for tumors to develop (Nagasaki et al., 1971; Nagasaki et al., 1972). For example, in one study hyperplastic nodules were observed at the end of 26 weeks in mice treated with lindane, which may have progressed to tumors had the treatments continued (Goto et al., 1972, as translated by Vesselinovitch and Carlborg, 1983). Most of the studies that were done over longer time periods have shown associations with tumor development. The studies of animals that were treated for longer periods of time with lindane are summarized below.

1. Mice
The results of studies that have evaluated the liver carcinogenicity of lindane in mice are inconsistent. Some studies have observed an increase in liver tumor incidences in mice exposed to lindane. In one study, B6C3F1 mice (50/sex) were fed either a low (80 ppm), or a high (160 ppm) dose of lindane in diet for 80 weeks and were observed for an additional 10 to 11 weeks (NCI, 1977). The matched controls of 10 untreated mice/sex were combined together to form the pooled controls. Mice that survived were killed at 90 to 91 weeks. The incidence of hepatocellular carcinomas was significantly higher in the males fed the low dose of lindane (19/49, $p = 0.001$) compared to the pooled controls (5/49). The incidence of hepatocellular carcinomas in the males fed the high dose (9/46) was not statistically different from the matched controls (2/10). This indicates a lack of a dose-response relationship. No treatment-related response for hepatocellular carcinomas was observed for female mice. The survival rates were 88% for males and 80% for female mice in this study.

The strength of this study was the evaluation for lesions in all the mice, including unscheduled deaths during the experimental period. The limitations of this study included the use of pooled controls from overlapping studies on the carcinogenicity of other chemicals at the same facility. The body weights of the high dose group were not affected, indicating that the highest dose was lower than the maximum tolerated dose (MTD). Only two dose levels were used, which did not permit an evaluation of a dose-response effect. The authors concluded that lindane was not carcinogenic in B6C3F1 mice. Since one out of the two doses of lindane caused an increase in the number of liver tumors in treated animals, it is not possible to predict what the outcome might have been if more and / or higher doses had been used. A review panel from the IARC (IARC, 1979) criticized the very low doses used and the small number of animals in the control group (IARC, 1979).

Thorpe and Walker conducted an experiment in which 29 CF1 mice of each sex received a diet containing 400 ppm of lindane (a much higher dose than the NCI study's 160 ppm), starting at one week of age for 109 weeks. Based on histological analysis, liver tumors were classified as type a or type b. Type a tumors were described as focal hyperplasia. More progressed lesions, with evidence of portal-tract involvement were recorded as type b. Lindane-treated male mice had a significantly increased ($p < 0.01$) incidence of liver tumors (55% type b; 38% type a; 93% combined) compared to the controls (23% type a). Among females, 69% of treated mice had liver tumors (34% type b and 34% type a; 68% combined) compared to controls (23% type a;

$p < 0.01$) (Thorpe and Walker, 1973). This study has limitations since it used only one treatment dose and small numbers of mice per treatment group.

In contrast, there was no increase in incidence of hepatocellular carcinomas reported by a study in NMRI mice (50/sex/dose) that were fed 0, 12.5, 25 or 50 ppm of lindane for 80 weeks (Herbst and Bodenstein, 1974; Weisse and Herbst, 1977). This study may not have observed a treatment related effect since it used very low dose levels of lindane.

Details of a study that has been cited (IARC, 1979; PDR, 1998) as demonstrating the lack of association between lindane treatments and development of skin tumors were not available (Orr, 1948).

Studies That Used Technical-Grade HCH:
Technical-grade HCH (13% lindane) has been found to be hepatocarcinogenic in Swiss, BALB/c mice and dd mice by many investigators (Hanada et al., 1973; Kandarkar et al., 1983; Kashyap et al., 1979; Munir et al., 1983; Nagasaki et al., 1971; Nagasaki et al., 1972; Nigam et al., 1984). The bulk of technical-grade HCH is comprised of the alpha-isomer which has been more frequently associated with hepatocarcinogenicity. Hence, it is critical that the carcinogenicity of lindane be evaluated separately. These studies do not provide evidence for a causal relationship between lindane and hepatocarcinogenicity and have not been evaluated here.

2. Rats
Most studies on carcinogenicity of lindane in rats have not found any associations in response to lindane treatments. However, all the studies that were done would not be considered valid cancer bioassays. The limitations of the different studies included short treatment periods (Ito et al., 1975), the use of only one dose (Ito et al., 1975), small sample sizes (Truhaut, 1954, as cited in IPCS, 1991; Ito et al., 1975; Fitzhugh et al., 1950); and low survival rates (NCI, 1977).

One study did observe a treatment-related association in surviving animals, providing limited evidence that lindane causes thyroid cancer in female rats (NCI, 1977). Groups of 50 Osborne-Mendel rats of each sex were administered a low or a high dose of lindane for 80 weeks, and then observed for 29 to 30 weeks. Time-weighted average (TWA) dietary concentrations of lindane were 236 or 472 ppm in males, and 135 or 270 ppm in females respectively. In males, the survival rate was 60% for control, 50% for the low-dose and 48% for the high-dose group. In females, the survival rate was 40% for the controls, and at least 60% for the low and high-dose groups. The incidence of liver neoplastic lesions was not significantly increased by lindane. There was a significant increase ($p < 0.05$) in the incidence of thyroid neoplasms (C-cell adenomas) in the low-dose female group (4/44) compared to the pooled controls (0/48). A non-significant increase in the incidence of thyroid neoplasms was observed in the high-dose female group (3/42). The very low survival rates and the use of only two dose levels limits the value of this study. The animals may not have lived long enough to develop late-stage tumors.

3. Dogs
Beagle dogs (4 / sex) fed 25, 50 and 100 ppm /104 weeks, and 200 ppm/32 weeks of technical lindane in diet had a toxic effect, but did not cause tumors (Rivett et al., 1978). This study was too small to be considered adequate to demonstrate a negative association.

4. Summary, Animal Studies:
The results on hepatocarcinogenicity in mice in response to lindane treatments are inconsistent. While some studies have not found any hepatocarcinogenic effect in response in lindane treated mice (Nagasaki et al., 1971; Nagasaki et al., 1972; Ito et al., 1973c; Goto et al., 1972; Herbst and Bodenstein, 1974; Weisse and Herbst, 1977), these studies were of limited value as cancer bioassays because of the small numbers of animals and insufficient durations of treatments. Other studies have observed an increased incidence of liver tumors in lindane-treated animals (NCI, 1977; Hanada et al., 1973; Thorpe and Walker, 1973). Lindane was found to induce the incidence of liver tumors in mice, but not in rats. Lindane caused an increase in thyroid tumors in female rats in one study (NCI, 1977). Three previous studies in rats had not observed any increase in tumor incidences, but these studies had used inadequate number of animals per treatment group (Truhaut, 1954, as cited in IPCS, 1991; Ito et al., 1975; Fitzhugh et al., 1950). Cancer bioassays that meet current guidelines are needed to address the possible association of lindane with hepatic carcinomas in mice and thyroid tumors in female rats.

C. Current Classification of Carcinogenicity by Other Agencies

1. IARC Classification:
Lindane has been classified in Group 2B, possibly carcinogenic to humans (IARC, 1987). The evidence for carcinogenicity in humans has been considered to be inadequate. The evidence for carcinogenicity to animals is considered limited for lindane, but sufficient for technical-grade HCH (IARC, 1987).

2. NTP Classification:
Lindane has been classified as reasonably anticipated to be a carcinogen (NTIS, 1994).

3. EPA Classification:
Lindane is being evaluated for its carcinogenicity in humans (NTIS, 1994). Based on the weight-of-evidence, EPA assigned lindane a classification of B2/C (probable human carcinogen) and a hazard ranking of 'medium' in 1988 (NTIS, 1994).

V. Critical Evaluation on Breast Carcinogenicity

A. Human Studies
1. Human Tissue Levels
Two case-control studies have evaluated adipose levels of lindane in breast cancer patients. Neither of these studies

indicated a significant association between tissue levels of lindane and breast cancer. However, these studies had many limitations that are discussed below, and are inadequate for use in evaluation of a causal relationship.

In a hospital-based study, a small, but not significant elevation was observed in the mean levels of lindane in the lipids extracted from nine women with breast cancer (0. 61 ppm in malignant tissue, and 0.57 ppm in adjacent breast tissue) when compared to the five presumably healthy women whose deaths were accident-related (0.39 ppm in breast tissue, and 0.26 ppm in adjacent adipose tissue) (Wassermann et al., 1976). It should be noted that lindane was one of several compounds that were detectable in the tissues of the cancer and control groups. The very small size of this study prevents a meaningful statistical analysis. The study was also of limited value since it did not include any information on the age or lactation history of the women, factors that could affect the levels of organochlorine residues in breast tissue.

Adipose tissue from the breasts of five breast cancer patients and five hospital controls were analyzed for HCH levels (all isomers) in a second hospital-based study. While higher levels of total organochlorinated compounds (OCC) were observed in breast cancer cases, no differences were reported for lindane specifically; the average levels of another HCH isomer, beta-HCH were slightly, but not significantly higher in the breast cancer patients when compared to controls (Djordjevic et al., 1994). This is a report of a pilot study to show that such a comparison is feasible using their analytical methods. It included matching the case-controls for age and the type of breast cancer with respect to the presence of the estrogen receptor (ER). The small number of samples analyzed and the lack of details on lindane levels limits the value of the study. The large-scale study planned by the authors would be very useful, especially if it also controls for other risk factors associated with breast cancer risk such as the reproductive history, weight, height, dietary and tobacco use habits of the women.

Studies on Adipose Levels of Isomers of HCH Other Than lindane:

Two small case-control studies have evaluated another HCH isomer, beta-HCH. While not being very useful in determining the carcinogenicity of lindane, they are mentioned briefly here to indicate the need for further studies on the possible relationship between beta-HCH and breast cancer risk. A hospital-based study from Finland has presented the analysis of beta-HCH levels in the adipose tissues of 24 women with breast cancer in comparison with adipose tissues from 16 cancer-free women that had died accidental deaths. More women with breast cancer were nulliparous (child-less) compared to women without breast cancer. After adjusting for age and parity using stepwise logistic regression, women with > 0.1 mg/kg of beta-HCH in breast fat were reported to have a significantly increased risk for breast cancer (OR 10.51, 95% CI 2.00-55.26, p values not stated) (Mussalo-Rauhamaa et al., 1990).

In another case-control hospital-based study in Quebec City, beta-HCH levels in the breast adipose tissue from nine estrogen receptor (ER)-negative breast cancer cases and nine ER-positive breast cancer cases were compared to breast adipose tissue from 17 women with benign breast disease (Dewailly et al., 1994). The average concentration of beta-HCH was the same in the controls and the ER-positive breast cancer cases (39.7 μg/kg) and slightly lower in the ER-negative breast cancer cases (34.7 μg/kg). This study used women with benign breast disease as controls and failed to control for breast cancer risk factors other than age and parity. The above two studies are inadequate to draw any conclusions regarding the breast carcinogenicity of beta-HCH, but indicate the need for further studies including larger groups of women.

B. Animal Studies:

Animal studies done so far do not indicate a breast carcinogenic potential for lindane, but most of the studies were of too short duration and are inadequate to evaluate the breast carcinogenic potential of lindane.

1. Mice:

Groups of 10 to 11 dd mice of each sex were fed 0, 100 ppm, 300 ppm or 600 ppm lindane in diet for 32 weeks. A low incidence of mammary carcinomas was reported in females that were fed the alpha- and beta-isomers (1/8 and 2/8 respectively), but no mammary carcinomas were reported in the groups of animals fed lindane. The short duration of treatment may not have allowed sufficient time for the development of mammary tumors. The small number of animals used further limit the value of this study (Hanada et al., 1973). Larger life-time exposure studies are needed on lindane-treated mice.

2. Rats:

Groups of Osborne-Mendel female rats (50/group) were fed 0, 135 ppm and 270 ppm TWA doses of lindane for 103 weeks (NCI, 1977). The low dose group was evaluated after 44 weeks, and the high dose group after 61 weeks; no increase in mammary gland neoplasms was reported (NCI, 1977). The low survival rates (40% in controls and 60 % in experimental groups) weaken any conclusions that can be made from this study. The animals may not have lived long enough to develop mammary or other tumors.

3. Summary, Critical Evaluation on Breast Carcinogenicity:

Two human case-control studies have not reported a significant increase in risk for breast cancer in women with higher levels of lindane in their breast adipose tissue (Wassermann et al., 1976; Djordjevic et al., 1994). Both these studies were very small and are not adequate to assess the breast carcinogenic potential of lindane to humans.

Animal studies have not reported an increased incidence of mammary tumors in response to lindane treatments, but the studies conducted so far were of limited value due to the low survival rates (NCI, 1977), or short duration of lindane treatments (Hanada et al., 1973).

C. Other Relevant Data on Breast Cancer Risk:

1. Evidence of Endocrine Disruption

a) Female Sex Hormone Levels:

In adult female mice, lindane treatments had an adverse effect on the cholesterol side chain cleavage in the ovaries, a rate limiting step in steroidogenesis (Sircar and Lahiri, 1990) and suppressed adrenocortical function (Lahiri and Sircar, 1991). In rainbow trout hepatocytes, lindane induced the accumulation of estrogen receptor (ER) and vitellogenin mRNAs, but lindane did not compete for the ER (Flouriot et al., 1995). Catfish that were treated with HCH (purity not specified), had significantly reduced ($p < 0.001$) plasma levels of sex hormones such as testosterone, estradiol and estrone (Singh and Singh, 1987). These studies suggest that lindane disrupts steroidogenesis in animals.

b) Interference in Estrogen-Mediated Events:

Lindane has been reported by studies in rats, mice, birds and *in vitro* assays to interfere with estrogen-mediated events. While the evidence strongly suggests that lindane causes an interference in estrogen-mediated events, the mechanism for this action is not clear. Most studies have found that lindane does not bind to the ER. There is some evidence that lindane acts as an anti-estrogen. These studies are discussed below.

i) *In Vivo* Studies:

Female Fischer rats (eight/group) were gavaged with 0, 5, 10, 20 or 40 mg/kg/day lindane (Cooper et al., 1989). There was a significant delay in the vaginal opening in females that were treated with 10 and 40 mg/kg lindane ($p < 0.05$) and significant delays in the appearance of regular estrous cycles in almost all the treated groups ($p < 0.02$). The estrous cycles in the two low-dose groups became regular after 90 days and in the two high dose groups, after 110 days. During the periods of irregular cycles, both persistent estrus and diestrus were observed. The pituitary and serum luteinizing hormone (LH) and the serum prolactin concentrations were significantly lower ($p < 0.05$) in females that had received the two higher doses of lindane, but pituitary follicle stimulating hormone (FSH) levels were higher. The serum estrogen levels of lindane-treated animals were significantly increased ($p < 0.05$) in the group that was fed 10 mg/kg and significantly decreased ($p < 0.05$) in groups that were fed 40 mg/kg, and not different in groups that received 5 or 20 mg/kg lindane compared to controls (Cooper et al., 1989). In a second experiment, prepubertal female rats were gavaged with 30 mg/kg lindane and treated with 10 µg of estradiol benzoate (EB). The lindane treated females showed a significantly decreased uterine weight gain ($p < 0.05$) in comparison with those that were treated with EB alone. Serum levels of LH and prolactin were decreased, but pituitary levels of these hormones was increased. Serum estradiol concentration levels in lindane treated rats were not different from rats that were given EB alone (Chadwick et al., 1988; Cooper et al., 1989). The results from both these experiments suggest an anti-estrogen effect of lindane and a possible interference in the estrogen-induced release of pituitary hormones (Cooper et al., 1989).

Lindane administration (0.5 mg/kg) had been reported to cause a significant ($p < 0.05$) increase in the length of the estrous cycle and a reduction in the number of cycles per month in an earlier study (Naishtein and Leibovich, 1969). Another study observed that female Fischer rats treated intraperitoneally (i.p.) with 25, 33, 50 or 75 mg/kg lindane on the morning of proestrus had significantly reduced ($p< 0.05$) sexual receptivity in the evening for females given a dose 33 mg/kg or higher (Uphouse, 1987). Diestrus treatments (i.p. or p.o.) caused an increase in the length of estrous cycles and a decrease in the sexual receptivity. Lindane did not compete with tritiated-estradiol for its receptor, ER. This suggests that the anti-estrogenic action of lindane is not due to an interference with estrogen binding its receptor (Uphouse and Williams, 1989).

In a study involving sexually immature female rats and ovariectomized estradiol-primed Long-Evans rats, lindane treatments did not change the serum estradiol concentrations, induce progesterone receptor, effect a redistribution of ER, or disrupt the binding of estrogen to its receptor, but did significantly inhibit ($p < 0.05$) the estrogen-dependent uterine weight gain (Laws et al., 1994). This provides further evidence that lindane is not estrogenic, and may have some anti-estrogenic effects.

Lindane interfered with implantation during early pregnancy in Swiss mice, an effect that could be corrected with co-administration of estrogen (Sircar and Lahiri, 1989). Intragastric treatments of lindane during mid-pregnancy caused a total resorption of fetuses, and during late pregnancy, caused reduced body weights and survival of pups. Suppressed gonadal steroidogenesis or increased estrogen metabolism by lindane were suggested as possible mechanisms for this anti-estrogenic effect (Sircar and Lahiri, 1989).

There is further evidence of lindane's inhibition of estrogen-effects from experiments in avian species. One day-old chicks were fed on a diet containing 100 mg /kg lindane for 14 days, followed by an intramuscular injection of estradiol. The animals were sacrificed after 24 hours and the oviduct weights and plasma calcium levels were measured. Lindane caused a significant inhibition ($p = 0.01$) of the estrogen-stimulated gain in oviduct weights and the estrogen-stimulated rise in plasma calcium levels (Adamec et al., 1974). In another avian study, daily administration of 20 mg/kg lindane to ducks caused a delay in the maturation of vitellogenic follicles and ovulation, and a reduction of the clutch size, indicating an interference with estrogen-mediated pathways. These effects could be reversed by an injection of stilbesterol (Chakravarty et al., 1986).

Hybrid rabbits that were given 0.8 mg/kg lindane by gavage for 12 weeks were found to have a significantly reduced ($p <0.05$) ovulation rate, suggesting an interference in endocrine pathways. This effect was not seen if lindane treatments were combined with a 3 mg/kg dose of DDT (Lindenau et al., 1994). Some forms of DDT are weakly estrogenic. Inhibition of ovarian growth and impaired ovulation with lindane treatment has also been reported in catfish (Singh et al., 1993). In another study,

lindane did not to compete for the ER, but did significantly inhibit ($p = 0.05$) DNA synthesis in cultured bovine oviductal and uterine cells (Tiemann et al., 1996). In an artificial transactivation system in yeast, > 1 μM lindane was found to significantly inhibit ($p < 0.05$) the transactivation of a progesterone-sensitive reporter. This effect was not through a direct interaction with the progesterone receptor itself (Jin et al., 1997).

In contrast, one study has reported a weak estrogenic effect. Lindane fed ovariectomized albino rats had significantly increased glycogen ($p < 0.05$) in their uterus, cervix and vagina, but no histopathological changes or increase in organ weight (Raizada et al., 1980). The authors describe this as a weak estrogenic effect but, this conclusion is based on an increase in organ glycogen content alone and adequate tests of estrogenicity were not conducted to confirm this observation.

ii) *In Vitro* Assays for Estrogenicity:
Lindane does not appear to be estrogenic, as determined by its inability to stimulate proliferation of MCF-7 cells (an estrogen-dependent breast tumor cell line) in the E-SCREEN assay for estrogenicity (Soto et al., 1995). Lindane was neither estrogenic or anti-estrogenic in an *in vitro* assay for the induction of the estrogen-responsive gene apolipoprotein II. This study is only evaluated one of several estrogen-mediated responses (Ratnasabapathy et al., 1996; Ratnasabapathy et al., 1997). A major metabolite of lindane from rat microsomes, 2,4,6-trichlorophenol (TCP), did not mimic estrodiol. In contrast, another HCH isomer, beta-HCH (1mM) did induce progesterone receptors and caused redistribution of ERs in MCF-7 cells (Coosen and van Velsen, 1989). Since beta-HCH is not considered to be a major metabolite of lindane, this result is not relevant for lindane.

c) Male sex hormone levels:
Exposure to lindane among 60 male workers at a chemical factory was associated with significantly higher ($p < 0.01$) luteinizing hormone (LH) levels in their serum, but testosterone levels were not affected (Tomczak et al., 1981). In male rats, lindane significantly inhibited ($p < 0.001$) the formation of 5α-dihydrotestosterone-receptor complex in the prostrate cytosol (Simic et al., 1991). This effect was completely reversible in a week after the treatment was stopped. These studies indicate that lindane may also affect steroid-mediated pathways in males.

d) Summary, Endocrine Disruption:
Studies in rats, birds, rabbits and catfish suggest that lindane disrupts the reproductive cycles (Cooper et al., 1989; Chadwick et al., 1988; Naishtein and Leibovich, 1969; Uphouse, 1987; Uphouse and Williams, 1989), interferes with estrogen-mediated effects such as uterine-weight gain (Cooper et al., 1989; Chadwick et al., 1988; Laws et al., 1994), ovulation (Adamec et al., 1974; Chakravarty et al., 1986; Lindenau et al., 1994; Singh et al., 1993) and implantation during early pregnancy (Sircar and Lahiri, 1989). Lindane treatments caused delays in vaginal opening (Cooper et al., 1989) and inhibited the release of pituitary gonadotropins (Cooper et al., 1989; Chadwick et al., 1988). In *in vitro* assays, lindane interfered with the induction of a progesterone-sensitive receptor (Jin et al., 1997) and with the estrogen-mediated induction of DNA synthesis in bovine oviductal and uterine cells (Tiemann et al., 1996). A lack of an estrogenic effect is supported by three studies on lindane (Soto et al., 1995; Ratnasabapathy et al., 1996; Ratnasabapathy et al., 1997) and one study that evaluated a lindane metabolite (Coosen and van Velsen, 1989) These studies support the conclusion that lindane is not estrogenic and may have some anti-estrogenic action. The only evidence suggesting a weak estrogenic effect for lindane came from a study that showed that it increased the uptake of glycogen in an estrogen-responsive reproductive tissue (Raizada et al., 1980).

The mechanism by which lindane exerts its anti-estrogenic effects is not clear. Several studies have reported that lindane can disrupt steroidogenesis (Tomczak et al., 1981; Sircar and Lahiri, 1989; Cooper et al., 1989; Sircar and Lahiri, 1990; Lahiri and Sircar, 1991; (Singh and Singh, 1987). Lindane's anti-estrogenic effects do not involve its binding to the ER. Several studies have indicated that lindane does not compete with tritiated-estradiol for its receptor (Uphouse and Williams, 1989; Laws et al., 1994; Flouriot et al., 1995; Tiemann et al., 1996). However, one study has reported an interference in estrogen-ER binding by lindane (Tezak et al., 1992). There has been a recent report on the identification of a new estrogen receptor called the beta-receptor (Kuiper et al., 1996). Whether lindane interacts with this receptor needs to be determined. While it appears that lindane is an endocrine disruptor, the disuptions caused by lindane do not appear to have an estrogenic effect in animals, and are not the kind that have been linked with an increase in breast cancer risk.

2. Reproductive and Teratogenic Effects:
A study of women exposed to pesticides, including lindane, did not report any adverse effects on pregnancy outcomes (Willis et al., 1993). Adipose tissues of still born infants and those that died soon after birth ($n = 12$), and cord blood samples from normal full term pregnancies ($n = 30$) did not differ significantly in the levels of lindane (Curley et al., 1969). No teratological effects were observed in mice that were treated with a mixture of thimet/pthorate and lindane (oral dose, 1.40 mg/kg) (Savkovic et al., 1985). Lindane administrations (5, 10 and 15 mg/kg body weight) to CFY rats did not cause any teratogenic effects (Palmer et al., 1978). Benesan® (50% lindane), administered to pregnant rats (oral/day, 6.25, 12.5 or 25 mg/kg during 6th-15th day of pregnancy), did not affect fetal development adversely (Khera et al., 1979a; Khera et al., 1979b). Lindane was not teratogenic or affect the litter size in dogs. However, it did increase the percentage of still born pups. A statistical analysis was not presented (Earl et al., 1973).

A study of Fischer rabbits ($n = 5$) treated with 0.8 mg/kg lindane observed that lindane did accumulate in the genital tract tissues of the treated animals and interfered with their ovulation. The fertilization rates were not different in treated animals compared to controls (Lindenau et al., 1994; Seiler et al., 1994). While most studies have not observed that lindane has

any teratogenic or developmental effects, its interference with ovulation in rabbits further supports its ability to disrupt endocrine pathways.

In spite of all the above negative results for teratogenic effects in animal studies, because of the ability of lindane to cross the placenta, physicians prefer to use precipitated sulphur instead of lindane in topical treatments of the mucosal areas of pregnant women against ectoparasites. Since small amounts of lindane can be secreted in breast milk after topical treatments, physicians recommend that lactating women use alternate methods to feed their infant for two days after topical treatments with lindane (Stockton and Paller, 1990).

3. Tests of Mutagenicity:
The results from studies on the mutagenic potential of lindane in whole animals and in isolated cells are inconsistent. While lindane was found to be mutagenic in isolated cells in some studies, it was not found to be mutagenic by most assays in animals or bacteria. The results of these studies are summarized below.

a) Chromosome Aberrations in Occupationally-Exposed Humans:
Manufacturing workers exposed to lindane (males), were not observed to have an increased frequency of chromosome aberrations in their lymphocytes (Kiraly et al., 1979). Another unpublished study of lymphocytes from occupationally exposed humans by Desi et al., 1972, has been cited to have similar findings (IPCS, 1991). Details on the study design were not available.

b) Studies in Animals:
Frequency of chromosome aberrations were not increased in herds of pigs with high exposure to insecticides including lindane. The evaluation of exposure was very crude, through the analysis of selected substances in sedimented dust collected in the fattening rooms (Rubes, 1987).

No effect of lindane treatment was observed in mice on the frequencies of micronuclei (Jenssen and Ramel, 1980) or sister chromatid exchanges (SCE) (Guenard et al., 1984, as cited in IPCS, 1991). A single oral dose of a mixture of thimet/pthorate and lindane (1.40 mg/kg) was not mutagenic to mice (Savkovic et al., 1985). Frequency of chromosomal aberrations in the bone marrow cells of Syrian hamsters was unchanged following treatments (i.p.), with a commercial preparation of lindane (10%) (Dzwonkowska and Hubner, 1986). In another study, i.p. injection of 50, 75 or 100 mg/kg of lindane caused a significant increase ($p < 0.05$) in chromosome aberrations and micronuclei in the bone marrow cells of chickens (Bhunya and Jena, 1992); oral administration of the same doses caused only a non-significant increase in chromosome aberrations (Bhunya and Jena, 1992). A significant increase ($p < 0.05$) in single stranded breaks (SSB) was observed in DNA extracts of hepatic nuclei from lindane-treated female rats (per oral dose of 30 mg/ kg) (Hassoun et al., 1993). The significance of SSB in predicting the mutagenic potential of lindane is not clear. Lindane, injected as a 0.001% solution, was unable to induce sex-linked recessive mutations in *Drosophila melanogaster* (Benes and Sram, 1969).

c) Studies in Isolated Cells:
i) Evaluations of Transformation Frequencies:
Lindane treatments at 100 , 50 and 10 μg/ml, increased the cell transformation frequency of BALB/c 3T3 cells significantly and in a dose dependent fashion ($p < 0.05$ at lower two doses, to $p < 0.01$ at the highest dose) (Perocco et al., 1995). This transformation ability of lindane needs further evaluation with other cell lines. In a study that assayed mutation rate as the rate for acquiring resistance to 6-thioguanine, lindane did not significantly increase the mutation rate of Chinese hamster V79 cells (Tsushimoto et al., 1983).

ii) Evaluations of Potential for DNA-Damage:
In cultured human peripheral lymphocytes and in rat thymocytes, a 500 μg/ml dose of lindane caused an increase in unscheduled DNA synthesis (p values not stated) and an inhibition of the cell repair processes following UV radiation, thus increasing the potential for mutations (Rocchi et al., 1980). In another study, unscheduled DNA synthesis was not found to be induced by lindane in cultured human fibroblast cells (Ahmed et al., 1977).

Lindane caused a slight increase in the frequency of chromosome breaks and gaps in Chinese hamster fibroblasts. Increase in polyploidy was not observed with lindane treatments and it was classified in the "suspicious" group among 134 chemicals that were screened for mutagenicity in this study (Ishidate and Odashima, 1977). No DNA damage was detected in another study using a single-cell gel electrophoresis assay of cells from mice that were treated with technical-grade HCH (Sasaki et al., 1997). A formulation of technical HCH containing 6.5% lindane was found to cause a significant increase ($p < 0.05$) in chromosome aberrations and SCE in human peripheral lymphocytes in one study (Rupa et al., 1989). Note that this study did not use pure lindane and the effect could be due to any of the other HCH isomers. In another study, lindane caused a suppression of cell division and induced chromatid breaks in human peripheral blood lymphocytes; details on this study were not available and it was declared inadequate by the WHO (Tzoneva-Maneva et al., 1971, as cited in IARC, 1979).

Human nasal mucosal cells (NM) were found to be more sensitive to genotoxic effects of lindane than human gastric mucosal cells. Lindane (0.5 μm/ml) caused a significant reduction ($p < 0.01$), in the percentage of intact NM cells from rats and humans. The authors suggest that inhaled lindane may thus be more damaging to humans than the lindane consumed in food (Pool-Zobel et al., 1994). DNA-damage in this study was detected as alterations in the electrophoretic mobility of the DNA liberated after cell-lysis. However, chemicals are known to alter DNA-mobility in an electrophoretic assay without being mutagenic (for example, high salt concentrations). The possibility that increasing lindane concentrations could affect its electrophoretic mobility by changing the effective electric current

to which it is exposed, but not necessarily causing DNA damage, was not addressed in this study.

A meeting abstract reported that lindane caused chromosome breakage in cells, but did not provide details about the assay system (Grant, 1973). Lindane was found to increase the *in vitro* rate of tubulin assembly in porcine brain cells by 30%. Although disrupted rate of tubulin assembly could theoretically cause aneuploidy, such an effect was not assayed for in this study (Albertini et al., 1988).

Studies have looked for a direct interaction of chemicals with DNA as evidence supporting their role in causing DNA-damage. In two studies lindane was found to bind to DNA very weakly (Gopalaswamy and Nair, 1992; Iverson et al., 1984). However, this negative result does not necessarily indicate that lindane is not genotoxic, since a direct macromolecular interaction may not be necessary for a genotoxic effect. This was demonstrated in a comparative study in which the alpha-isomer had a three fold lower DNA-binding ability than lindane, but a greater potency for tumor induction in the liver of mice that were exposed by oral gavage (Sagelsdorff et al., 1983).

d) Studies in Bacteria and Yeast:
Gamma-BHC was unable to revert auxotrophic mutants of *E. coli or Salmonella typhimurium* (Ashwood-Smith, 1972; IPCS, 1991), and was not mutagenic to yeast (Fahrig et al., 1974, as cited by Moutschen-Dahmen et al., 1984). Similar results were reported for a HCH mixture (IARC, 1979; Shirazu et al., 1976). A commercial formulation that included lindane (Lindex, 4.5% lindane), increased the frequency of prototrophic revertants in yeast, but this effect could be due to any of the other HCH isomers.

e) Summary, Mutagenicity:
Lindane exposures were not associated with mutagenic effects in studies of exposed human populations (Kiraly et al., 1979; IPCS, 1991) and most studies in animals (Rubes, 1987; Jenssen and Ramel, 1980; IPCS, 1991). One study has indicated an increase in the number of transformed foci in BALB/c 3T3 cells in response to lindane (Perocco et al., 1995). This result needs verification with other cell lines. Most of the studies have evaluated the potential of lindane to cause DNA damage (Rocchi et al., 1980; Ahmed et al., 1977; Ishidate and Odashima, 1977; Sasaki et al., 1997; Rupa et al., 1989; Pool-Zobel et al., 1994) without adequate evidence to show that it actually does damage DNA in animals. Studies of the mutagenicity of lindane in animals, bacteria and yeast do not support a strong genotoxic effect.

4. Evidence of Tumor Promotion
There are many reports on the tumor promotion ability of technical lindane (Munir et al., 1984). Since technical lindane consists of isomers that have been reported to have a tumor promotion effect, it is essential to evaluate pure lindane separately.

One study found increased tumors in liver and lungs of lindane treated mice, but only in mice of a specific genetic background. Dominant mutations at the agouti locus, A^{vy} can be phenotypically expressed as the "obese yellow" or the "lean pseudoagouti". Obese yellow A^{vy}/a, pseudoagouti A^{vy}/a, and lean black a/a female mice were fed 160 ppm lindane for 6, 12, 18 or 24 months. Tumor incidences in animals treated with 160 ppm lindane for 24 months are presented in Table 1.

The yellow mice had statistically increased incidence of tumors when compared to black mice even in the absence of lindane treatments, indicating an effect of the mutation at the agouti locus. Lindane treatments caused a statistically significant dose-related increase (p values for increases caused by lindane-treatments were not stated) in the number of liver adenomas, combined liver tumors, lung hyperplasia and tumors. While obesity may be a factor for the increased incidence of tumors observed in the yellow mice, the higher incidence of the tumors in the lean pseudoagouti mice suggests a possible role of the A^{vy} mutation in affecting lindane's metabolism or the cell's susceptibility to lindane or its metabolites (Wolff et al., 1987). A different study has reported that yellow mice have increased tissue storage of lindane and its polar metabolites with increasing age and obesity (Chadwick et al., 1985). The tumor promotion effect of lindane needs to be tested in non-genetically altered strains of experimental animals.

Another study evaluated the cytopathological effects of the combined topical application of 18 mg lindane (purity unspecified) and 9.1 mg of the insecticide diazinon on 80 female albino rats. No macroscopic changes were seen on application of either compound separately, but a synergistic interaction was proposed due to the enhanced cytopathological changes visible in the epidermal layer after combined applications (Dikshith et al., 1974). A study of dd mice that were fed a combination of 250 ppm lindane and 250 ppm polychlorinated biphenyls (PCBs) in diet for 24 weeks did not observe a higher incidence of liver tumors in animals fed a combination of both chemicals when compared to the incidence of liver tumors in animals that were fed each compound separately (Ito et al., 1973a; Ito et al., 1973b).

Stimulation of DNA synthesis may be an indicator of a tumor promotion-event. Lindane did not stimulate DNA synthesis in the liver of rats or mice that were given 1 mM/kg lindane by oral gavage (Busser and Lutz, 1987). Another possible mechanism that is being evaluated for hepatic tumor promotion is the induction of protein kinase C (PKC) activity; lindane was found to be a potent stimulator of the activity of purified PKC *in vitro* (Moser and Smart, 1989).

Other studies have evaluated the promotion ability of lindane by quantitating the induction of γ–glutamyltranspeptidase (GGT) positive liver foci. Lindane increased the number GGT-positive foci in the livers of partially hepatectomized female Sprague-Dawley rats that were initiated with 0.3 mM/kg of diethylnitrosamine (DEN) (Kitchin et al., 1994; Pereira et al., 1982). Another study has reported that 30 mg/kg lindane in diet

Table 1. The Effect of Lindane Treatments on Mice Carrying the A^{vy} Mutation

Tumor Type	Phenotype	Genotype	Control	Treated
Liver adenomas	Y	A^{vy}/a	9%	35%
	P	A^{vy}/a	5%	12%
	B	a/a	6%	3%
Liver carcinomas	Y	A^{vy}/a	13%	17%
	P	A^{vy}/a	2%	5%
	B	a/a	3%	1%
Combined (liver adenomas and carcinomas)	Y	A^{vy}/a	22%	52%
	P	A^{vy}/a	7%	17%
	B	a/a	9%	4%
Lung hyperplasia	Y	A^{vy}/a	15%	72%
	P	A^{vy}/a	10%	76%
	B	a/a	10%	82%
Lung tumors	Y	A^{vy}/a	4%	19%
	P	A^{vy}/a	6%	14%
	B	a/a	2%	3%

Y = yellow
P = pseudoagouti
B = black

The statistical analysis done in this study analyzed the increases in incidence in response to the mutation, but not in response to lindane treatments (Wolff et al., 1987), and have not been included here.

for 15 weeks could significantly enhance ($p < 0.05$) GGT-positive foci and have more potent an effect than the positive control phenobarbitol (Schroter et al., 1987). These studies indicate mechanisms that may lead to the promotion of hepatic tumors in lindane treated animals.

A study reported that liver microsomes of lindane-fed mice and rats, metabolize aflatoxin B_1 (AFB_1) into more mutagenic metabolites (Decloitre and Hamon, 1980). In contrast, in another study, lindane treatments caused a complete inhibition of the incidence of aflatoxin B1 (AFB1) induced liver tumors in female Buffalo rats (Angsubhakorn et al., 1989). The authors of both studies suggest that lindane alters the liver metabolism of AFB1. Besides the conflicting results of the above two studies, a third study reports that lindane does not significantly alter the metabolite profile of benz[*a*]anthracene in rats, suggesting no effect on liver enzymes involved in the metabolism of this carcinogen (Jacob et al., 1985).

5. Signal Transduction and Intercellular Communication

Cells use intercellular communication to regulate their growth. Disruption of intercellular communication is a mechanism that has been suggested to lead to cell transformation. Lindane caused a significant inhibition ($p < 0.001$) of the intercellular communication in rat uterine myocytes (Criswell and Loch-Caruso, 1995). It caused an inhibition in intercellular communication in Chinese hamster V79 cells as well, but at much higher concentrations (15 μg /ml) than the concentration of tumor promoter control 12-*O*-tetradecanoylphobol-13-acetate (TPA) that was required for the inhibition (0.1 μg/ml) (Tsushimoto et al., 1983). Another study reported a significant and dose-responsive ($p < 0.05$) decrease in intracellular communication of mouse hepatocytes at non-cytotoxic concentrations of lindane (Ruch et al., 1987). The inhibition of communication was reversible when lindane was removed (Klaunig et al., 1990). No studies were found that evaluated the ability of lindane to disrupt intercellular communication in mammary cells.

6. Effects on Hepatic Microsomal Hydroxylases

Interspecies differences in hepatic metabolism have been investigated to explain why mice are more susceptible to hepatic tumors than rats to lindane treatments in cancer bioassays. One study has observed that the hepatic mitochondrial extracts from female rats had significantly elevated ($p < 0.05$) microsomal lipid peroxidation rates following lindane treatment (p.o., 30 mg/kg) (Hassoun et al., 1993). Another study reported that hepatocarcinogen-initiated liver cells from mice (from partial hepatectomies and neoplastic nodules), were more resistant to the cytotoxic effects of lindane than similar cells from rats (Ruch et al., 1985). Higher accumulations of reactive and mutagenic metabolites in mice was suggested as a possible reason for their greater susceptibility to developing liver tumors in response to lindane treatments. However, subcellular liver preparations from mice and rats treated with lindane were both found to be negative for any mutagenic activity in *S. typhimurium* by a different study (Glatt and Oesch, 1987).

7. Immunological Effects:

A compromised immune system may decrease the host defenses against cancer. There is limited evidence that lindane treatments may suppress the immune response to mitogens in animals. In one study, female BALB/c mice (6/dose) were fed 0, 0.012, 0.12 and 1.2 mg /kg/day of lindane for 24 weeks. The lymphoproliferative response to Con A (a mitogenic stimulus) was observed to have a biphasic curve in treated mice; there was a significant stimulation ($p < 0.001$) in the lymphoproliferative response during the first four weeks of treatment and significant suppression ($p < 0.005$) after continued lindane treatment at the two higher dose levels (Meera et al., 1992). Further studies are needed to determine if chronic exposures to lindane cause a suppression of experimental animals' immune-response to mitogens, and if the immuno-suppression leads to an increased susceptibility to cancer.

In another study, male albino mice were fed 0, 10, 30 or 50 ppm lindane. The mice fed 50 ppm lindane had a significantly reduced ($p < 0.05$) primary and secondary antibody titer to sheep red blood cells (SRBC) after 3, 6, 8 or 12 weeks of treatment (Banerjee et al., 1996). Similar results were observed in a study of Charles Foster albino rats (5 rats/sex/dose) given 6.25 and 25 mg lindane by gavage for five weeks. A decreased immune response to the *Salmonella* vaccine was observed in comparison to untreated rats (stated by authors as significant; p value not available) (Dewan et al., 1980). A significant suppression in immune response (stated by authors as significant; p value not available) was also reported in a study of rabbits exposed to capsules containing 1.5, 6 and 12 mg/kg/day of lindane, five times a week for five to six weeks (Desi et al., 1978). These studies indicate a general immune suppression of animals in response to lindane treatments. However, the mitogenic response of these immune-suppressed animals was not tested.

Lindane stimulated human polymorphonuclear neutrophils (PMN) *in vitro*, causing an increase in the production of superoxide anions. While the cidal action of the PMN involves production of superoxide anions, the effect of this strong nonphysiological stimulation by lindane has not been tested in whole animals (Kuhns et al., 1986).

Studies that have evaluated the immune responses in experimental animals suggest immuno- suppression effects caused by lindane treatments. Future animal modeling studies need to determine if lindane exposure can affect the immune response of animals to challenges with transplantable mammary tumor cells in multiple species, including the rat and mouse.

8. Summary of Other Relevant Data on Breast Cancer Risk:

Lindane was not mutagenic in most animal and bacterial systems studied. The potential for DNA damage and cell transformation by lindane observed in *in vitro* systems needs further investigation. Lindane can act as a promoter for lung and liver tumors in mice carrying the A^{vy} mutation at the agouti locus. Lindane was not estrogenic in *in vitro* assays, and its

disruption of estrogen-mediated events and steroidogenesis in animals supports that it has some anti-estrogenic effects. Lindane has been found to disrupt intercellular communication in mammalian cells and suppress the lymphoproliferative response to mitogens in mice. These mechanisms need to be evaluated further for their impact on tumor promotion and breast cancer risk in animals.

VI. Other Information

A. Environmental Fate and Potential for Human Exposure:

Lindane adsorbs to organic matter in the soil, causing its mobility to be very slow in organically rich soils. The half-life of lindane can vary considerably depending on the type of soil and temperature. Different studies under different soil conditions have reported half-life for lindane that ranges between 12 days to 1,146 days (IPCS, 1991).

1. Occupational Exposure:

Workers in a technical-grade HCH formulation plant were found to have uniformly high levels of pesticide on their skin (Wolff et al., 1992). Dermal exposure from exposed forearms and contact with contaminated clothing was found to be a major exposure routes during handling of lindane-treated seeds (Fenske et al., 1990). Lindane can be absorbed through the skin at an absorption rate of 9.3% (Feldman and Maibach, 1974). Its absorption from tropical treatments is increased when applied with a fatty-like base or to damaged skin (Solomon et al., 1977; Zesch, 1986). Blood levels of 57 male manufacturing workers in Germany indicated elevated levels of lindane (median = 23 μg/L) among all the exposed workers (Baumann et al., 1980). Blood levels of lindane among 71 agricultural workers were not found to be different than non-exposed controls (Rosell et al., 1993). The mean levels of lindane in the blood of exposed malaria spraymen was not found to be increased, but the total HCH levels were elevated (Gupta et al., 1982).

2. Potential for Exposure for the General Population:

The beta-isomer is the most persistent isomer of technical lindane. Lindane itself is rapidly broken down in warm-blooded animals and disappears within three weeks of the removal of HCH from animal diet (Davidow and Frawley, 1951).

Although the main source of exposure to lindane for the general population is through contaminated food, the amounts of lindane that are estimated to be present in the average diet in the U.S. have been decreasing steadily and are not considered to be a health concern (IPCS, 1991). Lindane is very stable in both freshwater and sea-water (Sieper, 1972). Animal feeding studies have indicated some storage of lindane in the fat tissue (Davidow and Frawley, 1951). These studies together suggest that lindane residues from water may have contaminated fish in the past. A survey of fish caught in U.S. rivers and Great Lakes found the maximum wet weight residue concentration of lindane to have decreased from 0.30 μg/g in collections during 1976 to 1977, to 0.04 μg/g in samples obtained in 1984. However, lindane has been found to occur in more than trace levels at some stations, indicating recent inputs (Schmitt et al., 1990).

Lindane exposure can also occur through dermal absorption in children treated for head lice with Kwell® shampoo. Lindane could be detected in the blood of treated children two to 24 hours after application of Kwell® (Ginsburg and Lowry, 1983). Lindane treatments are now restricted for use against head lice and are prescribed by physicians for patients who have failed to respond to other approved therapies. The manufacturer's instructions warn against effects of overdoses and the use of lindane products along with oil-based treatments (PDR, 1998).

In summary, while there is the potential for exposure to trace amounts of lindane from food, lindane does not persist in the environment or bioaccumulate in animal fat to cause prolonged exposures. The ability of lindane to be absorbed through the skin indicates the need for caution in application of lindane containing prescription products. Based on the average dose rate from air, food and water, and the oncogenic potency factor for lindane, Kastenberg and Yeh have calculated the oncogenic risk for a 15-yr average exposure to be 7.4×10^{-7} (7.4 in ten million) (Kastenberg and Yeh, 1993).

3. Storage and Excretion of Lindane in Humans:

Lindane residues were detected in the fat, breast milk and blood samples of humans in the late 1960s and 1970s. Amounts of lindane that are estimated to be present in the average diet in the U.S. have been decreasing steadily and this is reflected in the decrease in lindane levels that are detectable in human milk and adipose tissue in the U.S.

a) Human Milk and Lactation:

The beta-isomer of HCH is however much more persistent and still detectable in 27% of human milk samples tested from Arkansas women (Jensen, 1991). Studies of human milk samples from most European countries indicate low levels of HCH in general (average around 0.2 ppm in fat), with relatively higher levels in Czechoslovakia, France and Italy. Human milk samples from Asia, particularly from India and China have very high levels (average 6 ppm in fat) of total HCH. The levels in Japan have decreased gradually, but are still higher than European levels (Jensen, 1991). The levels of lindane in the human milk of residents in Zimbabwe were lower than DDT levels, but detectable in 61% of the samples analyzed (Chikuni et al., 1991).

A 1979 study had observed that the lindane levels in breast milk samples from 100 women in Colorado were higher than the levels found in cow's milk (Macy et al., 1979). However, lindane levels of American breast milk samples have been declining since its agricultural use has been virtually eliminated over the past two decades (Sonawane, 1995). The very small amounts of lindane that have been found in breast milk samples in the U.S. are not considered a health concern for infants that are breast-fed (IPCS, 1991).

Studies have analyzed lindane levels before and after lactation to determine if lactation is an effective means of excretion of lindane. A study compared the blood and milk samples from five pregnant women before and after lactation for HCH levels. The blood levels of HCH were not significantly lowered after lactation in this study (Curley and Kimbrough, 1969). Other studies have observed milk levels of lindane to decline after lactation suggesting a reduction in the body burden after excretion of lindane in breast milk (Dillon et al., 1981; Klein et al., 1986).

A study of fat tissue from 50 children in Germany that had undergone surgery in the early 1980s, indicated that 98% of the children had detectable levels of lindane in their adipose tissue. The authors conclude that the levels were reflective of transfer of lindane through breast milk, but samples of adipose tissue of bottle-fed infants were not available for comparison (Niessen et al., 1984).

b) Adipose Tissue:
Lindane residues were detectable in 88% of the human adipose samples analyzed in Canada in the 1970s (Ames, 1979). A study of adipose tissue samples of 25 persons (19 males and 6 females) from Texas in the mid-1980s found lindane residues in 96% of the samples, with a mean level of 0.20 ppm (Redetzke and Applegate, 1993). No breakdown products were detected. The results in Texas were suggestive of a recent (since there were no breakdown products) dispersed source for lindane, such as food or water.

In a study of levels of organochlorine pesticides in the adipose tissue of 51 human fat samples from a New Zealand hospital, the levels of DDT were found to increase with age, but lindane residues were found to decrease with age (Solly and Shanks, 1974). This result is in agreement with a study done on the blood organochlorine pesticide residues in Virginia residents (Griffith and Blanke, 1975). These studies indicate that lindane does not persist in the human body for extended periods of time.

4. Excretion and Metabolism of Lindane in Animals:
Lindane-fed rats were found to excrete 39% of the insecticide in an unmodified form in urine and feces (Rao et al., 1975). The first step in lindane metabolism leads to a substantial detoxification, but the second step results in the formation of active metabolites (Engst et al., 1976). Di-, tri-, and tetra-chlorophenols have been identified as lindane metabolites, but all possible products have not been identified (Chadwick and Freal, 1972; Grover and Sims, 1965). Lindane and alpha-HCH are both metabolized in part to beta-pentachlorocyclohex-1-ene (beta-PCCH) by microsomal mono-oxygenases. A stable metabolite, beta-PCCH epoxide is acutely toxic to *Salmonella,* making it difficult to test for mutagenicity (Fitzloff and Pan, 1984). Pretreatments with DDT stimulated the metabolism of lindane in rats, but via alternate pathways (Chadwick et al., 1977). One study indicated the presence of HCB as a metabolite, but this result has been debated (Smith, 1991). There is no significant interconversion of HCH isomers during their metabolism (Waliszewski, 1993).

VII. Summary and Recommendation for Breast Carcinogenicity Classification:

There is inadequate evidence to classify lindane as a "human breast carcinogen". We propose that it should be classified in Group 3, *not classifiable as to its breast carcinogenicity to humans* (see Appendix B for BCERF breast carcinogenicity classification scheme). We base this conclusion on the following evidence: Human studies are inadequate to conclude that lindane exposure directly causes breast cancer in women. Case-control studies on the breast carcinogenicity of lindane in humans are inadequate because they were based on very few breast cancer cases (14 total in the two studies) (Wassermann et al., 1976; Djordjevic et al., 1994). The evidence from long-term animal cancer bioassays is not adequate to evaluate lindane's mammary carcinogenicity. Although experimental animals that were treated with lindane have not shown an induction of mammary gland tumors (NCI, 1977; Hanada et al., 1973; Thorpe and Walker, 1973), many of the animal studies were not adequate cancer bioassays because of their short duration (Hanada et al., 1973; Nagasaki et al., 1971; Nagasaki et al., 1972; Ito et al., 1975; Goto et al., 1972; Rivett et al., 1978), small sizes (Fitzhugh et al., 1950; Truhaut, 1954, as cited in IPCS, 1991; Hanada et al., 1973; Thorpe and Walker, 1973) and low survival rates (NCI, 1977). Consequently, we conclude that the evidence for breast carcinogenicity of lindane is inadequate in humans and in animals.

However, there is limited evidence from other relevant data on mechanisms by which lindane may affect breast cancer risk. Lindane can act as a lung and liver tumor promoter in genetically altered mice (Wolff et al., 1987). Lindane has been found to disrupt intercellular communication in mammalian cells (Criswell and Loch-Caruso, 1995; Tsushimoto et al., 1983; Ruch et al., 1987) and suppresses the mitogenic response of the immune system in mice (Meera et al., 1992). Further research is needed to test if lindane can promote breast cancer through these mechanisms.

There is other evidence that indicates that lindane may not affect breast cancer risk. Lindane does not appear to be estrogenic in both *in vivo* (Cooper et al., 1989; Chadwick et al., 1988; Naishtein and Leibovich, 1969; Uphouse, 1987; Uphouse and Williams, 1989; Laws et al., 1994; Adamec et al., 1974; Lindenau et al., 1994; Singh et al., 1993) and *in vitro* assays (Soto et al., 1995; Ratnasabapathy et al., 1996; Ratnasabapathy et al., 1997). There is evidence that lindane can disrupt hormonal pathways. These disruptions of estrous cycles and a suppression of estrogen-mediated release of pituitary hormones in rodents (Cooper et al., 1989; Chadwick et al., 1988), a suppression of ovulation (Adamec et al., 1974; Chakravarty et al., 1986; Lindenau et al., 1994; Singh et al., 1993) and implantation during early pregnancy (Sircar and Lahiri, 1989). Lindane treatments caused delays in vaginal opening (Cooper et al., 1989). These studies support the conclusion that lindane is not estrogenic and may have some anti-estrogenic action. While

these anti-estrogenic effects are not desirable, there is no evidence that they would pose an increased risk for breast cancer. Lindane ranks among the ten least used pesticides in the U.S. during the early 1990s. It is a RUP. The potential for exposure for the general population to this chemical is thus relatively low.

VIII. Identification of Research Gaps, and Other Recommendations

- Large epidemiological studies are needed on cancer incidences in populations previously exposed to lindane, especially women who may have been exposed occupationally.
- Studies need to evaluate breast cancer incidences in populations that were exposed as children to head lice treatments containing lindane.
- More animal bioassays that meet current guidelines are needed to confirm the association of lindane with hepatic carcinomas in mice and thyroid tumors in female rats. The incidence of mammary tumors should be evaluated in these studies.
- A new estrogen receptor (beta-ER) has been identified and cloned recently (Kuiper et al., 1996). It should be determined if lindane interacts with this estrogen receptor.
- While some mechanisms for tumor promotion by lindane have been suggested, the tumor promoting response to lindane has not been adequately addressed in animals. Lindane needs to be tested for tumor promotion with known mammary carcinogens.

IX. Summary of New Human Studies Currently Being Conducted

We have pointed out the need for more epidemiological studies on cancer rates and endocrine disruption-effects from exposure to lindane. Several ongoing / proposed studies on lindane's effects were found in the CRISP Database (see Appendix A) and are outlined below.

Studies of Occupational Cancer--Pesticides:
(Alavanja, M., Blair, A., NCI, NIH, extracted from the CRISP Database, FY 97, and Personal Communication)
A large scale "Agricultural Health Study" proposes to look at the relationship between agricultural exposures and cancer risk. Enrollment in this study includes 90,000 men and women farmers, pesticide applicators and farmer's wives from Iowa and North Carolina. Besides conducting interviews to determine pesticide use, it will also seek information on lifestyle factors, medical and family history of disease and diet.

Cellular Mechanisms of Endocrine Disruptors:
(Adler, SR, Washington University, St. Louis, extracted from the CRISP Data base, FY 98)
One study that was recently funded by the National Institutes of Health (NIH) () will evaluate health effects from lindane exposure in women. The study proposes to examine a panel of chemicals, including lindane, to determine their estrogenic potential and their effect on women's health and the health of future offspring. The chemicals will be evaluated for their potential to bind the ER and affect gene regulation. These effects will be confirmed in ER "knock-out" mice.

Lindane Modification of Uterine Muscle:
(Loch-Caruso, R., University of Michigan, Ann Arbor, extracted from the CRISP Database, FY 97)
The hypothesis of this proposal is that lindane inhibition of gap junctional communication relaxes uterine muscle and interferes with parturition. The author proposes to study lindane's effects on parturition in rats, and conduct *in vitro* experiments to evaluate the mechanisms by which lindane affects uterine contractility and gap junctional communication. Identification of the mechanism by which lindane affects gap junctional communication and uterine contractility would lead to an understanding of its toxic effects on the reproductive system and other tissues.

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XI. Appendix A. Common Abbreviations, Acronyms and Symbols

AFB_1	aflatoxin B_1
BCERF	Program on Breast Cancer and Environmental Risk Factors in New York State, based the Cornell Center for the Environment, Institute of Comparative and Environmental Toxicology
beta-PCCH	beta-pentachlorocyclohex-1-ene
BHC	benzene hexachloride
bwt	body weight
CAS	Chemical Abstract Service
CfE	Cornell 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)
DEN	diethylnitrosamine; a carcinogen in the liver
DMBA	7,12-dimethylbenz[*a*]anthracene; known mammary carcinogen
DNA	deoxyribonucleic acid
EB	estradiol benzoate
EPA	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
FSH	follicle stimulating hormone
GGT	γ−glutamyltranspeptidase
HA	The health advisories are non-enforceable 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
HCB	hexachlorobenzene
HCH	Hexachlorocyclohexane
IARC	International Agency for Research on Cancer, headquartered in Lyon, France
ICET	Institute for Comparative and Environmental Toxicology
i.p.	interperitoneal
IRIS	Integrated Risk Information System; Database maintained by EPA available through the National Library of Medicine MEDLARS system.
kg	kilogram
L	liter
LH	luteinizing hormone
LI	Long Island, New York
μg	microgram
m^3	cubic meter
mg	milligram
MCF-7	Michigan Cancer Foundation; cells derived from human breast tumor
MCL	Maximum Contaminant Level; enforceable limit set by EPA which sets the maximum level of a contaminate in a public drinking water supply
μM	micromolar
mM	millimolar
n	number of subjects/animals in the group
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
NM	nasal mucosal cells
NMU	*N*-nitroso-*N*-methyurea; mammary carcinogen
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
PCBs	polychlorinated biphenyls
PDR	Physician's Desk Reference
PKC	protein kinase C
PMN	polymorphonuclear neutrophils
PMR	Proportional Mortality Ratio; PMR = number of deaths in the cohort compared to the expected number of deaths in the general population
p.o.	per oral
ppm	parts per million
ppb	parts per billion
ppt	parts per trillion
RR	Relative Risk
RfD	Reference Dose
RPAR	Rebuttable Presumption Against Registration
RUP	Restricted Use Pesticide
SCE	sister chromatid exchange
SRBC	sheep red blood cells
SSB	single stranded breaks
SD	Standard Deviation
SMR	Standardized Mortality Ratio; SMR= the ratio of "observed" to "expected" deaths
TPA	12-O-tetradecanoylphobol-13-acetate
TSH	thyroid stimulating hormone
U.S.	United States
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
UV	ultraviolet light
WHO	World Health Organization

Symbols:

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

XII Appendix B. Critical Evaluations of Breast Cancer Carcinogenicity

This includes an overview of the Critical Evaluations and explanation of the BCERF Breast Carcinogenicity 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)
ATSDR (**A**gency for **T**oxic **S**ubstances and **D**isease **R**egistry)

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--EPA's IRIS database source of oncogenicity and regulatory status information
- Grey literature--Studies submitted to EPA that are not published:
  - -Industry generated oncogenicity studies
  - -Some abstracts (short summaries) are on line (IRIS database)
  - -Request reports from industry
  - -Request reports from EPA through Freedom of Information Act

The critical evaluation will include 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 will be provided in synopsis form with some critical commentary, along with the current overall carcinogenicity classification by international (IARC) and U.S. Federal Agencies (NTP, EPA).

Human epidemiological studies, animal studies, and other relevant studies on possible mechanisms of carcinogenesis will be 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 below for a more detailed explanation of the BCERF Carcinogenicity classification scheme)

The **emphasis of the document** will be on critical evaluation of the evidence for breast cancer carcinogenicity, classification of the agent's breast cancer risk, identification of research gaps, and recommendations for future studies. A section will also be 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. CAS # (Chemical Abstract Subject Number)
- F. Chemical Structure

II. History of Use
- 1. Date of first registration
- 2. Uses
- 3. Past Usage / If available, current usage levels in US and NYS

III. Current Regulatory Status
- A. Current Regulatory Status, EPA
- B. Other sections as applicable

IV. Summary on 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. EPA (Environmental Protection Agency)

V. Critical Evaluation of the Scientific Evidence for Breast Carcinogenicity
- A. Humans Studies will include:
  - 1. Case-Studies
  - 2. Human Epidemiological Cohort Studies
  - 3. Human Epidemiological Case-Control Studies
- B. Experimental Animal Studies
- C. Other Relevant Information, including mechanisms by which exposure may affect breast cancer risk (examples: co-carcinogenicity, estrogenicity, endocrine disruptor, mutagenicity, tumor promotion, 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)
- B. When available will summarize information on detection /accumulation in human tissues / and validation of biomarkers

VII. Summary, Conclusions, Recommendation for 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 Carcinogenicity

BCERF Breast Carcinogenicity Classification Scheme
(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.

Brief Definitions of Sufficient, Limited, and Inadequate Evidence:
(adapted for breast carcinogenicity 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.