

Cornell University

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

**Critical Evaluation Report # 1
May 1998**

**Critical Evaluation of 2,4-D's
Breast Cancer Risk**

by

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Supported by grants from:

New York State Dept. of Health
USDA-CSREES, Proj. no. 97-34369-4005

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Critical Evaluation of 2,4-D's Breast Cancer Risk

by Renu Gandhi, Serge-Alain Wandji, and Suzanne Snedeker

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 Name: 2,4-D (Worthing, 1983)

B. Chemical Name: 2,4-dichlorophenoxyacetic acid (IUPAC) (Worthing, 1983)

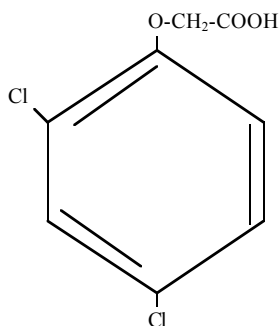
C. Chemical Formula: C₈H₆Cl₂O₃ (Montgomery, 1993)

D. Trade Names: Amoxone[®], Chloroxone[®] Crop Rider[®], Dinoxol[®], Dormone[®], Emulsamine[®], E-3[®], Fernimine[®], Fernoxone[®] (ZENECA Agrochemicals), Savage[®], Salvo[®] Statesman[®] (Platte Chemical Co.), Weed-no-More[®] (Ciba-Giegy Ltd), Aqua-Kleen[®], Lawn-Keep[®], Planotox[®], Desormone[®], Esteron[®], Weedar[®], Weedone[®] (Rhone-Poulenc Ag Co.) Riverdale 2,4-D, Solution[®], Weedestroy[®] (Riverdale Chemical Co.), Navigate[®] (Applied Biochemists), Weed Pro[®] 2,4-D (Cornbelt Chemical Co.), Miracle[®], Weedtrol[®] (Agchem Mfg.), Plantgard[®] (Chevron Chemical Co.) (Meister, 1997).

E. Trade Names of Mixes: Tiller[®] (AgrEvo USA Co.), Duplosan[®], U 46[®] (BASF AG), Chimac Cop Special[®], Chimac Mixte[®], Selectone G[®], Selectyl MD[®], Trimonal[®] (Chimac-Agriphar S.A.), MCPP-2,4-D (W.A.Cleary), Nox-D[®] (Crystal Chemical Inter-America and Duposca), Curtail[®], Lontrel[®] 205, Grazon[®] P+D, Pathway[®], Tordon[®] 101, Scorpion[®] III (DowElanco), Herbanil[®] 368 (Herbitecnica Defensivos Agricolas Ltda.), Durtok[®] 540 (Invequimica S.A.), 2 plus 2[®] (ISK Biosciences Corp.), Brush-master[®], Super Trimec[®], Trimec[®] Bentgrass, Trimec[®] Brush Killer, Trimec[®] Classic, (PBI/Gordon Corp.), Dissolve[®], Triamine[®], Tri-Ester[®], Triplet[®], Veteran[®] 720 (Riverdale Chemicals Co.), Actril[®], Envert[®], Weedone[®], Trio[®] (Rhone-Poulenc Ag Co.), Weedmaster[®] (Sandoz Agro, Inc) (Meister, 1997).

F. CAS Registry Number: 94-75-7 (Meister, 1997)

G. Chemical Structure of 2,4-D: (Meister, 1997)



H. Related Compound: 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Meister, 1997)

II. History of Use and Usage:

A. History of Use:

The synthesis of 2,4-D was first reported in 1941 (IARC, 1986). 2,4-D is structurally similar to indoleacetic acid (IAA), a naturally occurring plant hormone. This similarity allows 2,4-D to mimic the plant hormone and is the basis for the herbicidal action. 2,4-D was originally developed as a plant growth regulator, but its more useful herbicidal properties were discovered in England in 1942 (Lilienfeld and Gallo, 1989). In 1945, Dow Chemical Co. discovered that a 1:1 mixture of 2,4-D and 2,4,5-T was a more effective herbicide than either of the two chemicals alone. The mixture was thereafter referred to as **Agent Orange** (Lilienfeld and Gallo, 1989). 2,4-D was first registered for commercial use in 1948 (Munro et al., 1992). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), was often found as a trace contaminant of 2,4-D preparations before 1975 (IARC, 1986; Johnson et al., 1992). Agent Orange was used in the Vietnam war by the U.S. for defoliation and crop destruction beginning in 1962 and its use increased until 1969, when concerns over its health effects arose. Production and use of 2,4-D decreased markedly in the U.S. after 1969 due to governmental restrictions on its use.

Registrations in the U.S. for 2,4-D and 2,4,5-T in the 1940s included many food crops and the use of these herbicides increased to 17 million kg annually by 1960. Between 1966-1969, over 62 million acres of U.S. agricultural land was sprayed with these herbicides (IARC, 1986). In the late 1980s, an estimated 75% of total usage of 2,4-D has been in control of weeds in wheat and corn fields. Other use include weed control in forests, right-of-ways, rangelands, parks and golf courses, and home lawn and gardens and aquatic weed control (Ibrahim et al., 1991). While the primary use of 2,4-D is as an herbicide, it has minor uses as a growth regulator in prevention of premature dropping of fruit and for the color enhancement of potatoes (Munro et al., 1992).

B. Usage:

1. Agricultural Use:

2,4-D ranked fourth among the most used herbicides in agriculture in the U.S. during 1990-93. During this time, 2,4-D use for agricultural purposes on cropland was 41,938,492 lbs of active ingredient (AI) per year (yr) (Gianessi and Anderson, 1995b). 2,4-D was the seventh most used herbicide in New

York State (NYS), with 141,665 lbs of AI used annually on croplands during 1990-93 (Gianessi and Anderson, 1995a).

2. Non-Cropland Use:

The U.S. Environmental Protection Agency (EPA) estimates that the amount of 2,4-D used nation-wide on non-cropland during 1987 was 12.6 to 28.7 million lbs of AI /yr (Gianessi, 1991).

III. Current Regulatory Status:

A. Regulatory Status:

1. 2,4,5-T:

Because of concerns about carcinogenicity, fetotoxicity, and developmental toxicity, all registrations for 2,4,5-T were canceled by the EPA in 1983 (USEPA, 1996; IARC, 1986).

2. 2,4-D:

A concern was raised regarding the possible carcinogenic role of 2,4-D in a case-control study done in Sweden in the late 1970s (Hardell and Sandstrom, 1979). EPA called for more studies to be done on 2,4-D and announced its intention to publicly review the scientific literature related to 2,4-D (Munro et al., 1992). 2,4-D is currently in "pre-Special Review Status". This term defines "chemicals for which it is public knowledge that they are being considered for formal Special Review" (USEPA, 1996). While more studies are being done in response to EPA recommendation, the Industry Task Force (ITF) on 2,4-D Research Data has agreed to a subset of recommendations from EPA for risk reduction measures in September, 1992. These include a plan to implement a user education program as well as to reduce exposure through modifications to technical and manufacturing use labels (USEPA, 1996). Results of new oncogenicity studies on experimental animals were presented to the peer review committee for EPA in December 1995. A recent report from the Carcinogenicity Peer Review Committee (CPRC) for EPA has classified 2,4-D in group D, "not classifiable as to human carcinogenicity" (USEPA, 1997).

B. Workplace Regulations:

The U.S. Occupational Safety and Health Administration (OSHA) has set the time weighted average (TWA) occupational exposure limit for 2,4-D over an 8 hour workshift at 10 mg/m³ (IARC, 1986).

C. Drinking Water Standards and Health Advisories:

1. Maximum Contaminant Level:

The EPA has set the maximum contaminant level (MCL) for 2,4-D, to be no more than 0.07 mg/L in drinking water. MCL is the maximum permissible level of a contaminant in water which is delivered to any user of a public water system. This is an enforceable limit on the maximum allowable concentration in public water supplies.

2. Health Advisory:

Health Advisories (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, 1996). HA levels for drinking water are as follows (USEPA, 1996):

10 kg child

- One day = 1 mg/L
- Ten day = 0.3 mg/L
- Longer term = 0.1 mg/L

70 kg adult

- Longer term = 0.4 mg/L
- Lifetime = 0.07 mg/L

D. Food Residue Tolerances:

The past food residue tolerances for 2,4-D set by the federal agencies in 1989 included tolerance levels of 0.2 ppm in peaches and potatoes, and 0.5 ppm in grapes and 5 ppm in apples and grapefruit (USDA, 1993).

IV. Summary of Evidence of Overall Carcinogenicity; Non-Breast Sites

A. Human Studies:

Cancer risks from occupational exposures to chlorophenoxy herbicides have been extensively reviewed in an International Agency for Research on Cancer (IARC) monograph (IARC, 1986). IARC concluded that: "There is limited evidence that occupational exposures to chlorophenoxy herbicides are carcinogenic to humans."

Most studies have evaluated the risks due to exposures to multiple herbicides and contaminating dioxins, and not to 2,4-D alone. In this section, we have presented commentary on large scale studies on cancer risks associated with 2,4-D exposure and have summarized other studies assessing cancer risk involving exposure to phenoxyacid herbicides (PH) and other pesticides.

1. Case Reports:

Although case-reports alone are inadequate to infer a causal relationship for the carcinogenicity of chemicals, they provide useful information to justify the need for further epidemiological studies.

Three cases of soft-tissue sarcomas have been reported among U.S. veterans who were exposed to Agent Orange. One of the veterans with soft tissue sarcoma had been completely soaked in Agent Orange two times (Sarma and Jacobs, 1982). Another case report documented soft tissue sarcoma in a father and son who were both employed at a chlorinated phenol manufacturing plant and may have been exposed to 2,4-D, 2,4,5-T, chlorinated phenols and organic solvents (Johnson et al., 1981). Two other case reports suggest increased incidences of liver cancers among patients in Vietnam hospitals who had been exposed to Agent Orange (Ton That Tung, 1973, reviewed by Sterling and Arundel, 1986; Van, 1984). In another study, four out of the five patients that had cutaneous lesions due to non-Hodgkin's lymphoma (NHL) were reported to have repeatedly sprayed large

areas with chlorophenoxy herbicides (Olsson and Brandt, 1981). Another case report describes acute lymphoblastic leukemia in a patient who had been employed by a lawncare firm for three years during which he was exposed to chlordane, 2,4-D, diazinon and Banvel® D, as well as organic solvents (Infante et al., 1978).

No conclusions can be drawn about cancer risks due to 2,4-D exposures from any of the case reports since the exposures were to many different herbicides and their contaminants, and, in some cases to many explosive and other war chemicals known to be toxic.

2. Occupational Cohort Studies:

a) Manufacturing and Agricultural Workers

There are many cohort studies that have evaluated cancer mortality in association with PH exposures, but only one study included a population exposed to 2,4-D. A study of 878 employees (gender not specified for cohort, control group was male) who had been engaged in the formulation, manufacture, or packaging of 2,4-D and other PH at a Dow Chemical Plant, Midland, MI, reported an overall non-significant increase in deaths from malignant neoplasms. There was a significant excess in the standardized mortality ratio (SMR = ratio of observed to expected deaths multiplied by 100) of lymphatic and haematopoietic cancers (SMR = 3.12, $p < 0.05$); cancers of 'other and unspecified sites' (SMR = 3.88, $p < 0.05$); and non-significant excess in mortality from cancer of the large intestine, Hodgkin's disease (HD), leukemia and aleukemia. No deaths were reported from brain neoplasms (Bond et al., 1988). An investigation into the cause of the observed increase in 'other and unspecified sites' revealed that faulty reporting practices by physicians of the area may have contributed to the observed increase in mortality rates (Bond et al., 1990). The strength of this study was that TWA for exposure to 2,4-D were available.

After four additional years, there were no new deaths from NHL in this cohort, but the cohort continued to have a significant excess in mortality from cancers of 'other and unspecified sites' (SMR = 3.51) (Bloemen et al., 1993). However, only a small numbers of deaths were expected in the four year period with a 74% chance of not seeing a single death from NHL (Blair and Hoar Zahm, 1995). The limitations of the initial study of the cohort and its follow-up were the small number of deaths, the potential for co-exposure to 2,4,5-T and the lack of information on the sites and specific nature of the cancers found to be increased. It is not known if there were any women in the cohort and if they were followed for cancer incidences. This cohort is the only one known to have been exposed primarily to 2,4-D, and it is important that it be followed to assess future cancer deaths.

A study of 69,513 male farmers in Saskatchewan (Canada) reports a trend for increased mortality due to NHL with greater acreage sprayed with herbicides ($p = 0.064$ for the trend) (Wigle et al., 1990). 2,4-D was the major herbicide used in this region. Data on direct exposure to pesticides was not available, but exposure was linked to number of acres sprayed for control of

weeds. The farmers did use a variety of other pesticides and organic solvents that may have also contributed to the observed increased risk.

Other cohorts have been evaluated for the risks due to PH exposures but not to 2,4-D specifically. Incidence of cancer in a Danish cohort of 690 male and 250 female workers assigned to PH manufacturing and packaging operations was compared to the cancer rate in the general population (Lynge, 1985). Allowing for a 10 year-latency period, there was a significant increase in relative risk (RR) for soft tissue sarcoma (RR = 3.67) and rectal cancers (RR = 4.08) among male workers, and cervical cancers (RR = 4.71) among female workers. However, all the increased RRs were based on very small numbers (four cases of each cancer type) and the main PH involved were 2-methyl-4-chlorophenoxyacetic (MCPA) and 2,4,5-T. Another study evaluated the number of deaths from soft tissue sarcoma that were reported for an international cohort of 18,910 PH production workers (including 1,527 women). The increase in number of deaths was statistically significant (SMR = 6.06, $p < 0.05$) after allowing for a 10 to 19 year latency period, based on four deaths. Data on duration of exposure were available for this cohort (Saracci et al., 1991). Although there was exposure to multiple chemicals, these two cohorts are important for future follow-up studies on associations with PH exposures since they include women.

A cohort study of 1,658 male German agricultural workers that had the potential for exposure to a variety of pesticides including 2,4-D reported a two-fold increase in deaths due to bronchial carcinoma ($p < 0.05$). The smoking habits of this cohort were not found to be different from the general male German population. Data on the number of years of employment in agricultural work were available, but no attempt was made to assess exposures to the different pesticides (Barthel, 1981). An abstract from a study of a cohort of 33,669 pesticide applicators in Florida, reported a significant increase (p values not stated) in testicular (SMR 2.49) and prostate (SMR 1.92) cancers among male workers; cervical cancer among women applicators (SMR 3.71); but no cases of soft tissue sarcoma or NHL (Fleming et al., 1997). Besides PH, this cohort also had the potential for exposure to a wide variety of organochlorine and other pesticides. Details of this study are not available.

A small cohort of 618 white male golf course superintendents has been evaluated for cancer-related deaths (Kross et al., 1994). A significant increase (p values not stated) in proportional mortality ratio (PMR = number of deaths in the cohort compared to the expected number of deaths in the general population) was observed for all cancers in general (PMR = 1.36). Significantly elevated PMRs (p values not stated) were also reported for lung cancer (PMR = 1.27), NHL (PMR = 2.11), brain cancer (PMR = 2.42), large intestinal cancer (PMR = 1.78) and prostate cancer (PMR = 2.29). In some golf courses, 2,4-D was the most common herbicide used. Confounding factors such as tobacco use and diet of the cohort were not controlled for in this study. This study raises a concern about the combined exposure of turfcare workers to various

pesticides (herbicides, fungicides, insecticides) and tobacco smoke and suggests a need for more carefully controlled case-control studies of cancer incidences among those involved in turfcare. However, it does not provide causal evidence for the carcinogenicity of 2,4-D.

Other studies have not found a relationship between 2,4-D exposure and increased cancer mortality. A study of a cohort of 20,245 pesticide applicators (99% males, 1% female) in Sweden reported a non-significant increase in risk for Hodgkin's disease (HD) (RR 1.2) and no increased risk for NHL (Wiklund et al., 1987a). This study lacked information on 2,4-D exposure levels. A cohort of 2,239 male PH manufacturing workers in England had a non-significant increase in mortality from NHL (SMR = 2.3), based on only 2 deaths (Coggon et al., 1991). A study of 1,971 male Finnish applicators did not find a statistically significant association between any cancer and exposure to chlorophenoxy herbicides (2:1 mixture of 2,4-D and 2,4,5-T) (Riihimäki et al., 1982; Riihimäki et al., 1983). A ten year follow up of this cohort reported no increased cancer mortalities, and no deaths from NHL or soft tissue sarcoma (Asp et al., 1994). No deaths were seen due to soft tissue sarcoma and NHL in a cohort of 1,222 male forestry workers of Ontario (Canada) exposed to PH (Green, 1991).

b) Summary, Occupational Cohort Studies:

A cohort of 878 employees of a manufacturing plant who had been exposed primarily to 2,4-D had increased incidences of lymphatic and haematopoietic cancers, and cancers of 'other and unspecified' sites (Bond et al., 1988; Bloeman et al., 1990). Further follow-up with better investigation of cancer types should be done on this cohort.

Higher risk of cancer incidences or mortalities that were associated with PH exposures in two or more studies, are soft tissue sarcomas (Lyngge, 1985; Saracci et al., 1991), NHL (Coggon et al., 1991; Kross et al., 1994; Wigle et al., 1990) and cervical cancers (Fleming et al., 1997; Lyngge, 1985). In contrast, other studies of applicators, manufacturing and forestry workers have not observed a significant association between PH exposures and increased cancer mortalities (Wiklund et al., 1987a; Riihimäki et al., 1982; Riihimäki et al., 1983; Asp et al., 1994; Green et al., 1991). All of the cohorts were exposed to many chemicals including 2,4-D, and an association between 2,4-D exposures and cancer types cannot be established based on these studies.

3. Population-Based Case-Control Studies

a) Non-Hodgkin's Lymphoma:

About one half of the case-control studies have observed an increased risk for NHL in association with 2,4-D and PH exposures. A large scale case-control study evaluated 200 cases of NHL (from the cancer registry for the state of Kansas) and 600 controls from the general population, matched for age and vital status (all males). An increased risk for NHL (OR = 2.6, p value not stated) was observed to be associated with farm use of 2,4-D. There was a trend for increased risk for NHL with more frequent use of 2,4-D (≥ 21 days/year, $p < 0.0001$ for trend);

increased years of 2,4-D use (≥ 26 years, $p < 0.0002$ for trend); and time elapsed since first year of use ($p < 0.0002$ for trend) (Hoar et al., 1986). The OR for herbicide use without protective equipment (OR = 2.1) was higher than the OR for use with protective equipment (OR = 1.5).

Another case-control study evaluated 201 cases of NHL among farmers, identified through the Nebraska Lymphoma Study Group and area hospitals and 725 controls that were matched for age, sex and vital status. An elevated risk for NHL for men was reported among farmers who mixed or applied 2,4-D (OR = 1.5; 95% CI 0.9-2.5; p value not stated). A trend for increased risk for NHL was observed with more frequent handling of 2,4-D (≥ 21 , $p = 0.051$ for trend). Surprisingly, a small, non-significant elevation in risk (OR = 1.7) was found for men who used protective equipment in comparison to workers who did not (OR = 1.2) (Zahm et al., 1990). The data from the above two case-control studies was pooled together and re-evaluated for NHL risk (Weisenburger et al., 1991; Weisenburger, 1990). The risk of NHL was observed to be elevated four-fold in men who were exposed to 2,4-D more than 20 days / yr (Weisenburger et al., 1991; Weisenburger, 1990).

A re-evaluation of 105 NHL cases (all male, from the Department of Oncology, Umea, Sweden) and 335 controls (matched for age, sex and vital status, selected from the National Population Registry) reported a significantly elevated odds ratio (OR = 5.5; p value not stated) for NHL associated with exposure to a commercial mixture of 2,4-D and 2,4,5-T (Hardell et al., 1994). Among four people who had been exposed to 2,4-D alone (3 cases and 1 control), the risk was even higher (OR = 13) (Hardell et al., 1994). This re-evaluation confirmed the previously reports of an increased risk for NHL in this population (Hardell, 1981; Hardell et al., 1981). This study is limited by the small number of cases that were exposed to 2,4-D.

A case-control study of 181 NHL cases (from the regional Cancer Surveillance System) and 196 random cancer-free controls in western Washington found an increased risk for NHL among male farmer workers exposed to organic solvents (OR = 1.74; $p < 0.05$). A logistic regression analysis was used to determine if the increased risk was due to joint exposures with PH. Farmers who reported exposures to PH and organic solvents had a significantly elevated risk (OR = 1.5) over farmers who had exposure to neither agent (Woods and Polissar, 1989). The risks associated with organic solvents and PH alone were OR = 1.12 and OR = 0.85, respectively. Since the information on co-exposures to other chemicals besides the combination of PH and organic solvents was lacking, it cannot be determined if the increased risk observed was due to a specific synergistic relationship, or a general higher use of different farm chemicals.

A two-fold increase in incidence rates of NHL was reported among males living in a rice-growing area of Italy where 2,4-D and 2,4,5-T were used (RR = 2.2; 95% CI 1.4-3.5; p value not stated) (Vineis et al., 1991). This population-based study was

limited by small numbers (30 cases) and the lack of any information about PH exposure.

Other studies have not observed a significant increase in risk for NHL associated with 2,4-D and PH exposure. A population-based case-control study evaluated newly diagnosed cases of NHL (n = 115, all male) in Iowa and Minnesota and 227 controls from the states matched for age and vital status. The OR was slightly, but not significantly increased among farmers who had ever used 2,4-D (OR = 1.2; 95% CI 0.9-1.6) (Cantor et al., 1993; Cantor et al., 1992). Agricultural PH use was associated with a non-significant increase in risk (OR = 1.4, p = 0.26) in a study of 83 NHL cases (all males, New Zealand Cancer Registry) and 168 controls with cancers at other sites (Pearce, 1989; Pearce et al., 1986). The use of controls who had other cancers is inappropriate as it may mask exposure-related associations for chemicals that cause an increase in incidence of different cancers.

In a population-based case-control study, the risk for NHL among women agricultural workers from the eastern Nebraska (119 cases and 471 controls) who had been exposed to PH, was found to be not significantly different from controls (based on a small number of subjects) (Zahm and Babitt, 1993). A nested case-control study of 32 cases of lymphoma (31 males and 1 female) from an international cohort of pesticide applicators and production workers did not reveal a significant increased risk for NHL in association with PH exposure (Kogevinas et al., 1995).

The conflicting results of different studies and the lack of data on 2,4-D exposure history make an assessment of risk for NHL due to 2,4-D difficult. An increased risk with no protective clothing or with increased number of years of exposure is not always seen in the studies indicating a positive association, as would be predicted if 2,4-D was the causative agent (Zahm et al., 1990). Two other considerations need to be made while evaluating the results of the above studies: 2,4-D preparations were often contaminated with dioxins before 1975 (Johnson et al., 1992), and while one study found the use of surrogate or proxy interviews to be satisfactory in assessing exposures (Brown et al., 1991), such a practice has been found to cause an increased recall-bias by another study (Olsen and Bodner, 1996). The positive association found between NHL and joint exposures to PH and organic solvents (Woods and Polissar, 1989) needs further evaluation.

b) Soft-Tissue Sarcoma

Studies conducted on the association of PH exposure and incidence of soft tissue sarcoma have also given inconsistent results. Two out of the 11 studies reported a statistically significant association for soft tissue sarcoma and exposure to PH. These studies are summarized below.

A case-control study in Sweden that evaluated 46 cases and 201 controls found an increase in soft tissue sarcoma incidence associated with PH exposure (RR = 5.3; 95% CI 2.4-11.5, p<0.001) (Hardell and Sandstrom, 1979). A later study interviewed 110 cases and 220 referents and reported a

significantly increased risk for soft tissue sarcoma (RR = 6.8; 95% CI 2.6-17.3, p value not stated) associated with exposure to PH, based on 14 cases and 5 controls (Eriksson et al., 1981). Exposure estimates were from interviews and may have been affected by recall bias.

A small case-control study in Northern Italy reported a higher risk of soft tissue sarcoma among women rice field workers who were exposed to PH (OR = 2.7; 90% CI 0.59-12.37), based on 4 cases and 5 referents (Vineis et al., 1986). This study had limitations, including the small sample size and the lack of information on PH exposure levels. A study of 42 male cases reported a significantly increased risk (RR = 1.7; p = 0.05) of soft tissue sarcoma among farmers, farm managers and gardeners (Balarajan and Acheson, 1984). Although often cited, this study is of limited value since no attempt was made to assess exposures to PH.

In contrast, other studies have found no increase, or a slight, non-significant increase in risk of soft tissue sarcoma associated with PH use. These include a study of 133 white men diagnosed with soft tissue sarcoma and 948 matched controls in Kansas reported no increased risk for soft tissue sarcoma associated with farming (OR = 1.0) (Hoar et al., 1986). A case-control population in New Zealand of 82 male cases of soft tissue sarcoma and 92 male controls who had other cancers had a small, non-significant increase in risk of soft tissue sarcoma associated with PH exposure (OR = 1.3, p < 0.35) (Smith et al., 1983; Smith et al., 1984). Another case-control study of 128 male soft tissue sarcoma cases and 694 cancer free controls in western Washington State reports no association of soft tissue sarcoma with PH exposures (Woods et al., 1987). A study of 354,620 male forestry and agricultural workers did not show an association of soft tissue sarcoma and exposure to PH (Wiklund and Holm, 1986; Wiklund et al., 1987b). No association between soft tissue sarcoma incidences and service in Vietnam was observed by Vietnam veteran's health studies (Greenwald et al., 1984; Kang et al., 1986; Lawrence et al., 1985), but there have been two case reports that were mentioned earlier (Sarma and Jacobs, 1982; Johnson et al., 1981).

c) Vietnam Veterans:

Studies that have evaluated the cancer risks associated with chemical exposures during the Vietnam conflict have been reviewed extensively (IARC, 1987; IARC, 1986; Lathrop, 1988; Sterling and Arundel, 1986; Tindall, 1985; VAO, 1996). Since the Vietnam veterans were exposed to complex herbicide mixtures, as well as to other war chemicals and not to 2,4-D alone, these studies do not further our understanding of the cancer risk specifically due to 2,4-D and have not been included here.

4. Summary of Human Studies on Carcinogenicity at Non-Breast Sites:

In summary, due to inconsistent results of different epidemiological studies, and exposure to multiple chemicals in many studies, no clear association can be made for 2,4-D and increased incidence of cancer in humans. Mortalities due to

lymphopoeitic and cancers of ill-defined sites were increased in a cohort exposed to 2,4-D (Bloemen et al., 1993; Bond et al., 1988). Positive association for NHL has been observed in large case-control studies that have evaluated 2,4-D exposures alone (Hardell et al., 1994; Hoar et al., 1986; Zahm et al., 1990). However, no association for NHL was observed in other case-control studies following 2,4-D exposure (Cantor et al., 1992; Woods et al., 1987) or PH exposures (Asp et al., 1994; Fleming et al., 1997; Pearce et al., 1986).

The evidence for soft tissue sarcoma is even more limited since none of the epidemiological studies have looked at 2,4-D exposures alone. Some studies have indicated an increased risk associated with PH exposure (Eriksson et al., 1981; Hardell and Sandstrom, 1979; Lynge, 1985; Vineis et al., 1986) while others have observed no association (Asp et al., 1994; Fleming et al., 1997; Hoar et al., 1986; Smith et al., 1984; Woods et al., 1987).

Very few studies have involved women. Increased incidence of cervical cancers was reported for a cohort of women exposed to PH in a chemical plant (Lynge, 1985) and a cohort of women pesticide applicators (Fleming et al., 1997). Larger scale studies on women cohorts exposed to 2,4-D are needed to determine if 2,4-D exposure affects cancer incidence or mortality in women.

B. Animal Experimental Studies:

All the cancer bioassays for 2,4-D conducted before 1986 had limitations, and were considered inadequate by EPA (Ibrahim et al., 1991). These cancer bioassays are often cited as evidence for the carcinogenicity of 2,4-D in rodents. We have included them here to point out their limitations.

1. Studies in Mice:

An unpublished oncogenicity study by the Bionetics Research Labs, Inc. that reported a significant increase in reticulum cell sarcomas in female mice was rejected by EPA due to the route of administration used (subcutaneous injection) (Reuber, 1983). In a follow-up study, the maximum tolerated dose (MTD = 100 mg/kg/day) of a commercial preparation of 2,4-D (purity not specified), was administered by stomach tube to the C57BL/6 mice over 18 months. No increase in neoplasms was observed at any site (Innes et al., 1969). Both the studies had limitations since they used small number of animals (18/sex/strain), an insufficient exposure time, and only one dose of 2,4-D.

Due to the limitations of the above studies, EPA recommended that additional cancer bioassays be done. The Industrial Task Force on 2,4-D Research (ITF) responded with a study in which 60 B6C3F1 mice of each sex, were fed 0, 1, 15 or 45 mg/kg/day of 2,4-D in their diet for 2 years. No increase in tumor incidences were observed in any of the groups of 2,4-D (97.5% pure) treated mice (USEPA, 1997). EPA concluded that this study should not be regarded as adequate evidence for no carcinogenic effect of 2,4-D, because the highest dose used was only half of the MTD (Ibrahim et al., 1991).

A new study was initiated, in which female B6C3F1 mice (50/dose) were fed 0, 5, 150 or 300 mg/kg/day, and male mice were fed 0, 5, 62.5 and 125 mg/kg/day of 2,4-D (96.4%) in diet for 2 years. The MTD was reached since reduced body weight gains were observed for males and females that were given the highest dose. A non-significant increase in incidence of primary hepatocellular adenomas was observed in treated female mice, but the highest incidence was in the mice that had received the lowest dose, indicating a lack of a dose response. This study did not report a significant increase in any neoplasms in response to 2,4-D ingestion (Charles et al., 1996a). This study was considered to be a valid animal cancer bioassay by EPA (USEPA, 1997).

2. Studies in Rats:

Weanling Osborne-Mendel rats (25 male and 25 female/dose) were fed 0, 5, 125, 625 or 1,250 ppm of 2,4-D (96.7% purity) in their diet for two years (Hansen et al., 1971). The original report indicated a significant increase in the incidence of malignant tumors ($p < 0.05$) in male rats that were fed the highest dose and the tumors were not restricted to any specific target organ (Hansen et al., 1971). A later study re-analyzed all the histological sections that were available from this study including sections from 6 animals/sex for the group fed the highest dose, the controls, and any tumors that had been sectioned (Reuber, 1983). Based on his re-analysis of the histological sections that were available, Reuber reported an increased incidence of lymphosarcomas in the 2,4-D treated male and female rats (Reuber, 1983). These studies were limited by the small number of animals, the low overall survival rates in both the control and treated groups, especially since only the surviving animals were examined for neoplasms (Reuber, 1983).

The 2,4-D oncogenicity studies conducted before 1986 (Hansen et al., 1971; Hill and Carlisle, 1947; Innes et al., 1969; Reuber, 1983) were all found to be inadequate by EPA as they did not meet the current bioassay guidelines. EPA recommended that additional animal studies be conducted and two more recent studies sponsored by the ITF are summarized below.

In an unpublished study reported to the EPA (USEPA, 1997), Fischer 344 rats (60 rats/sex/dose) were fed 0, 1, 5, 15 and 45 mg /kg /day of 2,4-D (96.4%) for two years. A statistically significantly increase in the incidence of certain brain tumors (astrocytomas) was observed in male rats fed 45 mg/kg/day ($p = 0.0026$), while a non-significant increase was observed for male rats fed 15 mg/kg/day. No brain tumors were observed in the control or treated female rats. However Fischer rats in other bioassays (although not specifically Fischer 344 rats) have been shown to have highly variable frequencies of spontaneous brain tumors, so the significance of the increased incidence of brain tumors in the 2,4-D-treated male rats is unclear (USEPA, 1997). There was a concern that the highest dose of 2,4-D used on rats in this study was not at the MTD as evidenced by the lack of an effect on food consumption.

Due to the concerns about low dose levels, another combined toxicity /carcinogenicity study was initiated in which male and

female Fischer 344 rats (50 of each sex per dose) were fed 2,4-D (96.5%) at 0, 5, 75 or 150 mg/kg/day for 24 months. Ten animals of each sex were sacrificed after 12 months of treatment and evaluated for body weight, organ weight and histopathology. This study did not find evidence of an increase in brain astrocytomas, or any other neoplasms at doses of 2,4-D that were higher than the previous study, indicating that the results of the earlier study may have been due to the variable rates of brain astrocytomas in Fischer rats (USEPA, 1997).

3. Studies in Dogs:

Three beagle dogs/sex were fed with either 0, 10, 50, 100, or 500 ppm of 2,4-D (96.7%) in diet for 2 years (Hansen et al., 1971). No treatment-related neoplasms were observed in this study. This study has been criticized for its short duration by Reuber, who states that canine cancer bioassays should be carried out for six years or longer (Reuber, 1983), and also for the small sample size (Munro et al., 1992).

One veterinary hospital-based case-control study in dogs reported an association between canine lymphoma and 2,4-D use by the owner. Dogs who were exposed to lawns treated with 2,4-D had an increased odds ratio (OR) for canine malignant lymphoma (OR = 1.3; 95% CI 1.04-1.67) (Hayes et al., 1991). The OR was not increased for dogs whose owners did not use 2,4-D (controls), or for dogs who were not allowed into the treated areas. Further, there was a significant trend ($p < 0.02$) for increased risk for dogs with the more frequent use of 2,4-D by the owners. The levels of exposure were assumed to be related to the frequency of 2,4-D application. Although not ideal, this exposure estimate should not invalidate this study, because canine exposures to 2,4-D following lawn-treatments have been documented in other studies (Arnold et al., 1991; Reynolds et al., 1994). The study by Hayes and colleagues has been criticized by Sternberg (Sternberg, 1992) and by a review by the ITF, for the lack of information on exposure levels and the possibility of a recall bias causing the weak positive association observed (Carlo et al., 1992). Further animal studies are needed, since the Hayes study reports some association between 2,4-D exposures and a disease in dogs that resembles a disease also observed in humans, in epidemiological studies. This study is also important since it evaluates a possible association in animals from “real” exposures (more like the kind of exposures that humans are likely to face), unlike cancer bioassays where the animal are fed large doses of > 94% pure 2,4-D.

A one year chronic toxicity study in which 5 dogs/sex/group were fed with 0, 1, 5 or 10 mg/kg/day of 2,4-D (96.7%) did not report any immunotoxic effects but was too short to be regarded as a carcinogenicity study (Charles et al., 1996c).

4. Studies in Sheep:

A study of small-intestinal adenocarcinomas in sheep showed a positive association of the disease with PH use on some farms, although other chemicals were also used and exposures to PH were not documented (Newell et al., 1984).

5. Summary, Animal Studies:

In summary, animal studies on 2,4-D do not indicate a consistent association between exposure and induction of site-specific tumors, making it difficult to establish a causal relationship. Studies in mice have not found 2,4-D to be carcinogenic when fed in diet. Oral administration of 2,4-D was associated with increased incidence of brain astrocytomas in male rats (USEPA, 1997). Other studies in rats have not observed a positive association between 2,4-D exposures and any neoplasms (Charles et al., 1996b). All these animal studies have used the oral route for administration of 2,4-D. Since the greatest exposure to 2,4-D in humans occurs through dermal absorption during application (Munro et al., 1992), the oral administration of 2,4-D to animals does not accurately reflect human exposures. One case-control study has reported an increased incidence of canine lymphoma among 2,4-D exposed dogs (Hayes et al., 1991). This association needs to be evaluated further in a larger study with better exposure assessment.

C. Current Classification of Carcinogenicity by Other Agencies

1. IARC Classification:

IARC has determined that there is limited evidence in humans for carcinogenicity of chlorophenoxy herbicides, including 2,4-D. The evidence for carcinogenicity in animals has been determined to be inadequate. Overall, the IARC has classified 2,4-D along with other chlorophenoxy herbicides in Group 2B “possibly carcinogenic for humans” (IARC, 1987). Note that this classification is based on a monograph published in 1987, before many of the reports suggesting a link between 2,4-D exposures and NHL were available.

2. NTP Classification:

Not classified.

3. EPA Classification:

The Health Effects Division of the Carcinogenicity Peer Review Committee has concluded that 2,4-D should remain classified as a Group D - “Not classifiable as to human carcinogenicity”. The committee concluded that the evidence is inadequate and cannot be interpreted as showing either the presence or absence of a carcinogenic effect (USEPA, 1997).

V. Critical Evaluation of Breast Carcinogenicity

A. Human Studies:

1. Human Tissue Levels:

No reports were found on 2,4-D tissue levels in humans in relation to breast cancer incidences. Traces of the persistent contaminating dioxin, 2,3,7,8-TCDD, are still detectable in the adipose tissues of hospitalized women in South Vietnam, who were heavily exposed to Agent Orange, but not in women in North Vietnam, where there was less war-time exposure (Phuong et al., 1990). Breast cancer incidences were not compared among women in the two regions, but a follow-up study of the health consequences was recommended by the authors (Phuong et al., 1990).

2. Human Breast Milk Levels:

2,4-D does not persist or accumulate in the breast tissue or breast fat. No studies were found that have reported its presence in human milk.

3. Human Epidemiological Studies:

There are no case reports for breast cancer that implicated exposure to 2,4-D. The few studies presented below have evaluated the breast cancer incidences among cohorts that were exposed to PH, including 2,4-D. Exposure to PH, including 2,4-D in these studies is not associated with an increase in incidence of, or mortality from breast cancer.

Among the very few cohorts of women that have been evaluated for PH exposures and cancer risk was a cohort of 1,069 female workers employed at a PH manufacturing plant in Denmark. A study of the employment records and the national cancer register reported 13 cases of breast cancer and a RR of 0.9 for all women who had worked in two PH plants. MCPA was the main product of the plant but 2,4-D and 2,4,5-T were also produced, and the probability for exposure to TCDD was low. The RR of breast cancer was low (RR = 0.53) among women assigned to the manufacturing and packaging of PH, and was based on only two breast cancer cases (Lyng, 1985).

Only one death from breast cancer was reported after a 17-year follow-up of an international cohort (IARC) of 1,527 women herbicide applicators exposed to chlorophenoxy herbicides and chlorophenols; a lower mortality rate than expected from breast cancer. There was a non-significant increase in deaths from breast cancer among males in this cohort (SMR = 3.5; 95% CI 42-1246; p values not stated), based on only two cases (Saracci et al., 1991). A follow-up study of a subset of this cohort comprising of 701 women occupationally exposed to chlorophenoxy herbicides reported 7 incidents of breast cancer and a standardized incidence ratio (SIR) of 0.9 (95% CI 0.4-1.9) for breast cancer. The incidence of breast cancer was not increased among women who were probably exposed to TCDD (9 cases; SIR = 0.86); 8 of the cases of breast cancer were diagnosed within 9 years since first exposure (Kogevinas et al., 1993).

A study of a cohort of 50,682 female farmers (from the Swedish, nation-wide, population-based, Cancer-Environment Register) with exposures to pesticides and PH (mainly MCPA and dichloroprop), did not report an increased incidence of breast cancer (SIR = 0.83; 95% CI 0.78-0.88). The SIR did not change between four different time periods of follow-up spanning a total of 16 years (Wiklund and Dich, 1994).

Among 4,582 female Vietnam veteran, the RR for mortality due to breast cancer was found to be non-significantly elevated (RR 1.23; 95% CI 0.62-2.47) when compared to 5,324 non-Vietnam veteran women, based on 17 breast cancer cases (Thomas et al., 1991). A follow up study observed no increase in risk of mortality due to breast cancer (RR 1.03; 95% CI 0.6-1.78) based on 26 cases among the Vietnam veterans (Dalager et al., 1995). Confounding factors that were not controlled for included

smoking, reproductive history, alcohol use and occupational exposure to pesticides since the war.

The studies that have been conducted thus far have several limitations. All the studies were based on small cohort sizes in which the exposure was not limited to 2,4-D and levels of exposure to 2,4-D and other chemicals were not determined. None of the studies controlled for other risk factors for breast cancer, such as advancing age, early menarche, late menopause, older age at first birth, alcohol, diet and family history of breast cancer. Since breast cancer has a high survival rate, epidemiological studies should evaluate breast cancer incidences, rather than mortality, for a more accurate depiction of possible effects due to 2,4-D exposures.

In summary, although the few studies done on the exposure of women to PH and chlorophenoxy herbicides including 2,4-D and breast cancer incidence or mortality do not indicate a positive association, these studies are inadequate to evaluate the effect of 2,4-D exposure on breast cancer risk. Large-scale studies that are controlled for breast cancer risk factors are needed on breast cancer incidences in women who were primarily exposed to 2,4-D. If a large enough cohort with exposures primarily to 2,4-D is not available, better data on level and duration of 2,4-D exposures is needed for the evaluation of a causal relationship.

B. Animal Studies:

The only animal study found to have reported an increase in mammary neoplasms associated with exposures to 2,4-D had many limitations and was declared invalid by EPA. The results of this study have not been considered in our evaluation of the breast carcinogenic potential of 2,4-D. The design and the limitations of this study are presented below.

Groups of 25 Osborne-Mendel rats of each sex were fed 0, 5, 125, 625 or 1,250 ppm 2,4-D (96.7%) in diet for two years in a study conducted by FDA (Hansen, 1971; unpublished report as cited by Reuber, 1983). The combined incidence of benign (fibroadenomas and adenomas) and malignant mammary tumors (adenosarcomas) increased from 45% in control rats (fed no 2,4-D), to 86% ($p = 0.0064$) in rats fed 25 ppm, and 63% ($p = 0.0080$) in rats fed 625 ppm, but was lower for rats fed 5 ppm (40%) and rats fed 125 ppm (35%) (Hansen et al., 1971; as cited by Reuber, 1983). Tissue sections from six animals per group were analyzed histologically at the end of 2 years. A significant dose-related trend was reported for the increase in mammary neoplasms ($p = 0.03$), but a later re-evaluation of the histological sections found that the dose-related trend for mammary carcinomas (not including benign tumors) was not statistically significant ($p = 0.070$) (Reuber, 1983). The increase in the incidence of malignant mammary tumors reported in the original report was modest, from 18% in the controls, to 28% in female rats that were fed the highest dose of 1250 ppm; no statistical evaluation was available (Hansen et al., 1971).

The limitations of this study include the small numbers of animals ($n = 25$ females / dose), the high rate of sporadic mammary lesions in the control group and the low survival

rates (Reuber, 1983). The unavailability of the original report and the inconsistency between the original report and its later review make this study difficult to interpret. Other animal studies on rats have not reported an increase in mammary tumors with long term exposure to 2,4-D (Charles et al., 1996; USEPA, 1997b). Therefore, there is inadequate evidence to show that 2,4-D is a mammary carcinogen in experimental animals.

Summary, Critical Evaluation on Breast Carcinogenicity:

Human epidemiological studies of women, who were exposed to PH during its manufacture, as farmers or as Vietnam veterans (Lynge, 1985) and women herbicide applicators who were exposed to chlorophenoxy herbicides (Saracci et al., 1991) have not reported a significant association between exposure to PH and risk for breast cancer. These studies are inadequate to form a conclusion on the breast cancer risk from 2,4-D exposure. The studies were limited by the small sample sizes, not controlled for breast cancer risk factors, and the women were exposed to many chemicals in addition to 2,4-D. Large-scale studies, well controlled for confounding factors that affect breast cancer risk (such as age, reproductive history, diet and smoking alcohol consumption), are needed on women who were exposed primarily to 2,4-D. In the absence of large groups of women available who were exposed primarily to 2,4-D, the studies should evaluate women for whom the 2,4-D exposure data (levels, duration) is available.

One study in animals has indicated an induction in mammary neoplasms in rats that were fed 2,4-D over a long period of time (Hansen et al., 1971). This study had severe limitations and was not considered a valid bioassay by EPA. In addition to an inadequate number of animals per dose and a low survival rate, the results of this study were also questioned after a re-evaluation of the histological sections (Reuber, 1983). Other carefully controlled studies in animals have not reported an increase in mammary tumors in response to long-term exposure to 2,4-D (Charles et al., 1996a; USEPA, 1997).

C. Other Relevant Data on Breast Cancer Risk:

1. Oncogene Activation:

A study that analyzed the DNA from 28 canine lymphoma cases (20 with known exposure to 2,4-D, 8 with no known exposure), reported that exposure to 2,4-D was not associated with the activation of C-N-*ras*, an oncogene-activating mutation that has been detected in human lymphoma specimens (Edwards et al., 1993).

2. Tests of Mutagenicity:

The mutagenicity of 2,4-D has been studied in humans who were occupationally exposed to this pesticide, mammalian cells, whole mammals, insects, bacteria and yeast. The results are mostly negative or at best equivocal in every system studied.

a) Humans:

While some cytogenetic studies of the blood of individuals occupationally exposed to 2,4-D reported no increase in chromosome breaks and exchanges (Crossen et al., 1978;

Hogstedt et al., 1980; Linnainmaa, 1983; Mustonen et al., 1986), others have reported an increase in chromosomal aberrations in agricultural workers associated with the increased seasonal herbicide use (Yoder et al., 1973). The study showing the positive association had limitations. The results were highly variable within controls, there was little statistical analysis, and 2,4-D was only one of the many pesticides used.

b) Mammalian Cells:

Purified 2,4-D free acid (99%) did not cause chromosomal aberrations in human lymphocytes (Mustonen et al., 1986), or DNA damage in human fibroblasts (Clausen et al., 1990). However, a commercial formulation containing diethylamine salt of 2,4-D was reported as causing chromosome breaks (Mustonen et al., 1986). Another study reported that U 46 D Fluid (a commercial preparation of 2,4-D and its dimethylammonium salt) causes single-strand DNA breaks (Clausen et al., 1990). An increased rate of sister chromatid exchanges (SCE) was observed in cultured human lymphocytes treated with a commercial sample of 2,4-D (free of dioxin contaminants), but the increase was significant ($p < 0.05$) at only the lowest dose tested, and no dose-response effect was observed (Turkula and Jalal, 1985). Unscheduled DNA synthesis was induced in cultured human fibroblasts by 2,4-D Fluid (2,4-D) (Ahmed et al., 1977). The combined cytotoxic effect of U46 D Fluid and a common agricultural residue, copper chloride (CuCl_2), was evaluated on fibroblast cells. A synergistic effect on induction of unscheduled DNA synthesis and DNA repair was observed for cells that were pre-treated with CuCl_2 and then treated with U46 D Fluid (Jacobi and Witte, 1991). No chromosomal effects were reported for 2,4-D (purity not specified) treated bovine kidney cells (Bongso and Basrur, 1973) or cultured murine fibroblasts (Kolberg et al., 1970).

In summary, *in vitro* studies indicate that 2,4-D free acid does not cause chromosome aberrations, but there is limited evidence that commercial formulations containing 2,4-D and its amine salts have some DNA-damaging potential. Since details on the formulations used were not available, it is not clear from these studies if the 2,4-D salts caused the DNA-damage. Due to the variable accuracy and sensitivity of the different *in vitro* assays to detect DNA damage, such assays should be used for screening only, and the results verified in other systems.

c) Other Mammals:

In rats, a significant increase ($p < 0.05$) in chromosomal damage (breaks) was reported in the bone marrow cells of animals that were injected with 35 or 70 mg/kg of 2,4-D (purity not specified) intraperitoneally (i.p.) (Adhikari and Grover, 1988), but this route of administration does not accurately parallel human exposures. Oral administration of 100 mg/kg 2,4-D (99% purity) to male Wistar rats did not increase the frequency of SCE in their lymphocytes (Mustonen et al., 1989). Dermal application of 2,4-D (purity not specified) in mice did not increase the frequency of micronuclei in the bone marrow cells of treated animals, but tested positive in a genotoxic assay for increased follicular nuclear aberrations (Schop et al., 1990).

No induction in dominant lethal mutations was observed following a single injection (i.p.) of 125 mg/kg of 2,4-D (commercial formulations) in male mice (Epstein et al., 1972). In another study, bone marrow erythrocytes of mice injected with 100 mg/kg of 2,4-D (purity not specified, < 1ppm dioxin content) did not show a detectable increase in micronuclei when compared to untreated controls. However, gas chromatographic analysis of cell and plasma fractions indicated a very low penetrance of 2,4-D into the cells (Jenssen and Renberg, 1976). The lack of permeability of 2,4-D seen in this study emphasizes the need to evaluate the mutagenicity of 2,4-D under conditions in which it can better penetrate the cell. A recent study has indicated that lipophilic agents can increase the cell permeability to 2,4-D (Witte et al., 1995). Experimental conditions influencing the penetration of 2,4-D into the cell should be evaluated.

d) Insects:

When tested on *Drosophila*, 10,000 ppm 2,4-D fed to larvae was found to induce sex-linked recessive lethal mutations (SLRL) (IARC, 1987; Kale et al., 1995). In adult *Drosophila*, 10,000 ppm 2,4-D (99%) was found to have no effect on SLRL mutation rate by oral administration or by injection (USEPA, 1997; Zimmering et al., 1985).

e) Yeast and Bacteria:

The mutagenicity of pure 2,4-D was negative in the Ames test for mutagens in *Salmonella typhimurium* and *Escherichia coli* (IARC, 1987; Mortelmans et al., 1984; Nagy et al., 1975; USEPA, 1997; Zetterberg et al., 1977). However, one study on *his* mutants of *S. typhimurium* strain TA97a indicated a slight mutagenic effect of 2,4-D (purity not specified), as seen by the increased frequency of revertants (Kappas, 1988).

Two studies have found that experimental conditions such as pH can influence the effects of 2,4-D. In yeast, a dose-dependent increase in the frequency of mitotic gene conversion and recombination was observed in *Saccharomyces cerevisiae*, in response to a sodium salt of 2,4-D but only at low pH conditions (pH 4.5) (Zetterberg et al., 1977). A pH dependence for cell penetration and mutagenicity in yeast was suggested by this study (Zetterberg et al., 1977). In another study, 2,4-D (purity not specified) caused an increase in the rate of mitotic segregation in the fungus *Aspergillus nidulans*, only when it was administered along with the metabolic activating medium S9; no increase was observed in the absence of the S9 activating medium (Kappas, 1988).

f) Summary, Evidence for Mutagenicity:

The evidence for mutagenicity of 2,4-D is equivocal. There are conflicting results in every system that has been used to test its mutagenic potential. Lipophilic agents (Witte et al., 1995), CuCl₂ (Jacobi and Witte, 1991), and pH (Zetterberg et al., 1977), may be affecting the penetration of 2,4-D and the outcome of genotoxic assays. The influence of these factors on 2,4-D penetration of cells and its genotoxic effect, needs further evaluation.

3. Evidence of Tumor Promotion:

CBA X C57/BL hybrid mice (100/group, sex not specified) were painted on the skin with one drop of a 0.5% solution of the carcinogen 3-methylcholanthrene (3-MCA) for three weeks, followed by skin application of a 10% solution of the amine salt of 2,4-D to one group. Mice that received 3-MCA alone or the 10% solution of 2,4-D amine had no papillomas, but 18% of the mice that were applied with both 3-MCA and 2,4-D amine had skin papillomas suggesting a weak skin tumor promotion effect by 2,4-D amine (Arkhipov and Kozlova, 1974, translated by Reuber, 1983).

In an "initiation-selection-promotion" study, 2,4-D (analytical grade) was assayed for its ability to promote liver carcinogenesis in male Wistar rats that were treated with diethylnitrosamine (DEN), 2-acetylaminofluorene (2-AAF) and carbon tetrachloride (CCl₄) (Abdellatif et al., 1990). Groups of 7 to 12 animals were injected with 200 mg/kg DEN (for initiation of tumors), fed 0.03% 2-AAF for two weeks during which time a single 2 ml/kg dose of CCl₄ was given by gavage (for selection) followed by a diet containing 0.05% 2,4-D for 23 weeks (to test for promotion). Although a 2-fold increase in peroxisomal β -oxidation of fatty acids was noticed in 2,4-D treated animals, no incidence of liver tumors was reported in this study.

In another study, male CD-1 mice (25/group) were exposed to a commercial formulation of 2,4-D (including 2,4-D amine) in drinking water for 15 weeks, followed by a single administration of urethan (1.5 mg/g, i.p.). Mice that had been treated with 2,4-D had a moderate increase in number, but not size of urethan-induced pulmonary adenomas, and no dose-response relationship was observed (Blakley et al., 1992). In a similar study female CD-1 mice (15/group) that were exposed to Tordon[®] 202C (a mixture of 2,4-D and picloram) in drinking water were given one i.p. 1.5 mg/g dose of urethan. The number, but not the size of pulmonary adenomas was significantly increased (p = 0.002) in animals that were previously treated with Tordon[®] 202c (Adams et al., 1991). Authors suggest immunological impairment or metabolic factors as possible mechanisms by which 2,4-D may be promoting these lung adenomas.

4. Signal Transduction and Intercellular Communication:

Disruption of intercellular communication is one of the suggested mechanisms leading to cell transformation. 2,4-D (99% purity) was found to significantly inhibit (p < 0.05) intercellular communication in Chinese hamster cells *in vitro* at non-cytotoxic doses. Some additive effect of mixtures of 2,4,5-T and 2,4-D were observed, although the effects were weaker than the positive control 12-tetradecanoylphorbol-13-acetate (TPA) (Rubinstein et al., 1984). 2,4-D should be tested for its ability to disrupt intercellular communication in breast epithelial cells, as a possible mechanism to affect breast cancer risk.

5. Effects on Hepatic Microsomal Hydroxylases:

Agents that act as peroxisome proliferators may induce genotoxic effects due to increased production of oxidative enzymes. Increased hepatocellular carcinomas have been

observed in association with peroxisome proliferation (Warren et al., 1982). 2,4-D has been shown to induce hepatic fatty acid β -oxidation and peroxisomal proliferation in rodents (Hietanen et al., 1985; Kawashima et al., 1984; Mustonen et al., 1989; Vainio et al., 1983) and a hepatic peroxisome proliferation effect in rat Leydig cells (Liu et al., 1996). Daily gastric intubations of 50, 100 and 200 mg/kg of 2,4-D (analytical grade) for three days induced hepatic enzymes such as the P450 IVA1 monooxygenase in male Wistar albino rats (Bacher and Gibson, 1988).

2,4-D may act as a tumor promoter by inducing peroxisome proliferation and oxidative enzymes in the liver. It has not been tested in animals for its tumor promotion effect in the mammary gland.

6. Immunological Effects:

A compromised immune system may affect host defenses against cancer. There is some evidence that suggests that exposure to 2,4-D may compromise the immune response of exposed humans.

Commercial formulations of 2,4-D and MCPA were found to exert short-term immunosuppressive effects in a group of 10 male farmers that were evaluated before and after exposure. A significant reduction was found one to 12 days following exposure ($p < 0.05$) in the circulating helper (CD4), suppressor T cells (CD8), CD8 dim, CD8 cells expressing surface antigen HLA-DR (CD8-DR), cytotoxic T lymphocytes (CTL), and natural killer cells (NK). The mitogenic responses of lymphocytes (to concanavalin A and phytohaemagglutinin) one to twelve days after exposure were significantly lower ($p < 0.01$) than the mitogenic responses before exposure. After 50 to 70 days, other immunological values were comparable to controls, but the mitogenic proliferative responses were still significantly decreased ($p < 0.05$) (Faustini et al., 1996). The CTL and NK cells are involved in the cytolysis of virally infected cells and in cell mediated immunity to tumors; these immune changes could have health implications, and possibly affect cancer risk (Faustini et al., 1996).

Female offspring of CD-1 mice that were exposed *in utero* to *n*-butylester of 2,4-D, had a non-significant reduction in lymphocyte proliferative response to mitogens (Blakely and Blakely, 1986). However, adult female BDF1 mice that were fed *n*-butylester of 2,4-D had a slightly increased lymphocyte proliferative response to mitogens following exposures (Blakely, 1986).

We conclude that there is very limited evidence from one study in humans that 2,4-D may compromise the immune system (Faustini et al., 1996). This study had limitations. It studied a small number of farmers who were exposed to 2,4-D and many other chemicals. However, the observation of reduced mitogenic response of lymphocytes in exposed farmers (Faustini et al., 1996) needs to be evaluated in a larger study and over longer periods of time. Epidemiological studies on cancer incidences in farmers, applicators or manufacturing workers exposed to 2,4-D

should include an evaluation of the immune system. The animal studies conducted so far are inadequate to determine if 2,4-D exposures can damage the immune system. More studies are needed to determine if adult and *in utero* exposures to 2,4-D can compromise the immune response. If immune-suppression is observed, the experimental animals should be challenged with mammary tumors cells to evaluate if the immune-suppression affects breast cancer risk.

7. Evidence of Endocrine Disruption:

a) Leydig Cells

In one study, isolated rat Leydig cells that were treated with 2,4-D were found to release significantly more estradiol into the medium. The response to 2,4-D was similar to the response to other chemical peroxisome proliferators that have been implicated in the formation of Leydig cell tumors *in vivo* (Liu et al., 1996). However, 2,4-D reduced the non-stimulated release of testosterone, an effect that was unlike most peroxisome proliferators and tumor promoters. This study suggests that 2,4-D can affect the steroidogenic function of Leydig cells *in vitro*. It does not provide adequate evidence to suggest that 2,4-D may act as a Leydig tumor promoter or for endocrine disruption *in vivo*.

b) Estrous Cycle:

The estrous cycle is the reproductive cycle in rodents that is regulated by ovarian and gonadotropic hormones. In a study that has been presented only as an abstract, injection of 2,4-D at doses of 1 mg/kg/day and 12 mg/kg/day into female rats over a period of two months was reported to cause a prolonged diestrus phase and changes in the duration of estrus and metaestrus (Vin et al., 1990). This study does not provide adequate evidence to indicate that 2,4-D causes endocrine disruption or affects the reproductive cycles in rats since details of analysis were not presented. No other studies were found to have analyzed 2,4-D for endocrine disruption *in vivo*.

c) Estrogenicity:

2,4-D 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). E-SCREEN test is an *in vitro* screen for estrogenicity and does not provide conclusive negative evidence on the *in vivo* estrogenic potential of 2,4-D.

d) Effects on Growth Factors :

A published abstract reports that 2,4-D stimulates the production of transforming growth factor-beta (TGF- β) from MCF-7 human breast tumor cells (Lorick et al., 1995). TGF- β is known to inhibit the growth of normal human breast epithelial cells and estrogen receptor (ER) negative breast cancer cells, but not ER positive breast cancer cells. The complete role of TGF- β in mammary gland development and tumorigenesis is not well understood (Koli and Arteaga, 1996). Therefore, we cannot conclude whether the 2,4-D mediated induction of TGF- β in MCF-7 cells reflects a potential for *in vivo* effects on mammary gland growth and thus breast cancer risk.

e) Summary, Endocrine Disruption:

While 2,4-D is not estrogenic, as determined by an *in vitro* screen for xenoestrogens (Soto et al., 1995), it can modify steroidogenesis in rat Leydig cells (Liu et al., 1996) and there is very limited evidence from a published abstract that it may disrupt the length of reproductive cycles in rats (Vin et al., 1990). The cause for the delay in estrous cycle progression in animals by 2,4-D needs further evaluation.

8. Reproductive Effects:

Reproductive toxicity may be suggestive of either endocrine disruption or embryo toxic effects.

Reproductive effects were studied in (20 female and 10 male/dose) Osborne-Mendel rats that were fed 0, 100, 500 and 1500 ppm 2,4-D over three successive generations. No deleterious effects were observed on litter size (for 6 litters produced), but the survival time and weight of the weanlings was decreased (Hansen et al., 1971). These effects of 2,4-D do not indicate reproductive toxicity in response to endocrine disruption.

Animal studies on reproductive effects done so far do not support the role of 2,4-D as an endocrine disruptor.

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

The evidence for the mutagenicity of 2,4-D is equivocal in most systems studied. 2,4-D was found to inhibit cell-cell communication in some mammalian cells (Rubinstein et al., 1984). There is limited evidence that 2,4-D may compromise the human immune system (Faustini et al., 1996). There is were two studies that have reported that 2,4-D acted as a lung tumor promoter (Adams et al., 1991; Blakley et al., 1992) and a weak skin tumor promoter (Reuber, 1983). Several studies have shown peroxisome proliferation and an increase in oxidative enzymes in the liver in response to 2,4-D, which may lead to genotoxic effect through the production of oxygen free radicals and a tumor promotion effect (Hietanen et al., 1985; Kawashima et al., 1984; Liu et al., 1996; Mustonen et al., 1989; Vainio et al., 1983). However, 2,4-D was not found to promote hepatic tumors in rats (Abdellatif et al., 1990) 2,4-D was not estrogenic in the *in vitro* E-SCREEN assay, (Soto et al., 1995). Although one published abstract reported that 2,4-D delayed the estrus cycle in female rats (Vin et al., 1990), a reproductive toxic effect has not been shown for 2,4-D (Hansen et al., 1971).

VI. Other Relevant Information

A. Environmental Fate and Potential for Human Exposure:

1. Environmental Fate:

2,4-D does not persist in soil because of its rapid degradation. The average persistence of its phytotoxic effect is detected for one to four weeks in warm, moist soil. (WHO, 1989). Dislodgeable residues of 2,4-D show a rapid decline from 8% in the first hour following application to turf, to 1% after 24 hours (Harris and Solomon, 1992).

2. Occupational Exposure:

The populations at most risk for occupational exposures today are those who work in manufacturing plants, or as herbicide applicators, forestry, agricultural, and turfcare workers. There is a report on workers in container recycling operations getting exposed to 2,4-D (Guidotti et al., 1994). The highest urinary concentrations of 2,4-D (0.3 to 8 mg/L) have been reported from ground-spraying operations in forestry work (IARC, 1986). A mean urinary concentration of 1.37 mg/L was measured in workers involved in the production and manufacture of 2,4-D (IARC, 1986).

3. Sources of Exposure for the General Population:

Since 2,4-D does not persist in the environment or bioaccumulate in animal fat, diet is not a major source of 2,4-D exposure for the general population (WHO, 1984). Homeowners who apply 2,4-D to their yards without protection may be exposed. People who launder 2,4-D-contaminated clothing may also be exposed to this chemical. In a study that attempted to estimate the exposure of bystanders, volunteers were monitored for exposure after controlled activities on recently sprayed turf (Harris and Solomon, 1992). The levels of 2,4-D in urine samples from most volunteers indicated that they had had very low exposures. The highest exposure to 2,4-D was found in a barefooted volunteer in shorts, who removed his shirt for 30 minutes during the exposure period (Harris and Solomon, 1992).

4. Absorption and Excretion:

During occupational exposure and application of 2,4-D, dermal absorption is a major route of entry into the body. A study of the urine concentrations of 2,4-D of occupationally exposed sprayers indicated peak concentrations within the first three days (Knopp and Glass, 1991). Absorption of 2,4-D by the human body was rapid following oral administration and more than 80% of the dose was excreted unchanged in the urine of five male volunteers who ingested the chemical (Sauerhoff et al., 1977). No notable metabolic transformation of 2,4-D has been detected (Lilienfeld and Gallo, 1989), and it is cleared from the human body within 2 to 4 days (Munro et al., 1992).

Dermal absorption of 2,4-D in rats is dependent on the site of application. 2,4-D has been shown to pass the placental barrier in mice (Ulm and Springer, 1994), pigs and rats (Munro et al., 1992).

VII. Summary and Recommendation for Breast Carcinogenicity Classification

The evidence for 2,4-D causing breast cancer in humans and animals is inadequate. There is very limited evidence of possible mechanisms by which 2,4-D may affect breast cancer risk. Until these mechanisms are studied further, 2,4-D should be classified in Group 3, as *not classifiable for its breast carcinogenicity to humans* (see Appendix B). Our conclusions are based on the following. In humans, the few studies

published on women cohorts exposed to 2,4-D occupationally have not demonstrated an increase in breast cancer incidence or mortality. However, it should be noted that the available studies are not of sufficient quality, number, or size to assess 2,4-D's potential to be a breast carcinogen. In addition, these studies did not adequately control for other breast cancer risk factors and the cohorts were exposed to multiple pesticides and/or war chemicals in addition to exposure to 2,4-D. In experimental animals, studies have not demonstrated an induction of mammary gland neoplasms with 2,4-D administration, with the exception of one study that has been deemed invalid because of inaccurate histological evaluation of tumors (Hansen et al., 1971). There is very limited evidence that 2,4-D may have the potential to affect breast cancer risk by other mechanisms.

There is inadequate evidence that 2,4-D may act in animals as a weak tumor promoter for skin and for lung tumors (Adams et al., 1991; Blakley et al., 1992; Reuber, 1983). 2,4-D was found to inhibit intracellular communication in some mammalian cells (Rubinstein et al., 1984) and induce hepatic enzymes, effects that could contribute to tumor promotion (Bacher and Gibson, 1988; Hietanen et al., 1985; Kawashima et al., 1984; Vainio et al., 1983). 2,4-D was determined to be non-estrogenic in the E-SCREEN for xenoestrogens (Soto et al., 1995), but chronic administration of 2,4-D was reported in one abstract to cause a disruption in the length of the estrus cycle in female rats (Vin et al., 1990). Further assays of the estrogenicity of 2,4-D are required. There is some, but limited, evidence that recent exposures to chemicals including 2,4-D may cause immune-suppression in humans (Faustini et al., 1996).

Comment:

2,4-D is one of the few herbicides with large scale non-agricultural use for weed control in homes and gardens and recreational areas. Unlike many other herbicides where exposures are limited to those who mix and apply pesticides on croplands, the potential for exposures to 2,4-D is more widescale. Turfcare workers and homeowners, if they do not take proper precautions when applying 2,4-D products, and to a limited extent people who access treated areas too soon after application of 2,4-D, can also be exposed to 2,4-D. While there is no demonstrated evidence that 2,4-D is a human breast carcinogen, as with any chemical, caution should be exerted in its use, storage, and disposal.

VIII. Identification of Research Gaps, and Other Recommendations

- Large-scale case-control studies are needed on the breast cancer incidences in large populations of women who were exposed primarily to 2,4-D. If the population to be studied has been exposed to other chemicals, better data on level and duration of 2,4-D exposures is needed for an evaluation of a causal relationship.
- Lipophilic environmental contaminants (other pesticides and their metabolites) may enhance the penetration of 2,4-D into tissues and the cell. Animal assays for genotoxicity

should evaluate lipophilic agents that may accompany 2,4-D exposures due to combined use, for their ability to enhance any effects caused by 2,4-D.

- Animal studies are needed to evaluate 2,4-D's potential to be a co-carcinogen and or a tumor promoter of known mammary gland carcinogens such as 7, 12-dimethylbenz[*a*]anthracene (DMBA) and *N*-nitroso-*N*-methylurea (NMU).

Further animal studies are needed to determine if 2,4-D can compromise the immune system. If an immune suppression effect is observed, further studies would be warranted to determine if the immune suppression affects the body's defense against cancer. For an evaluation of breast cancer risk specifically, studies would need to determine if animals exposed to 2,4-D *in utero*, or as adults, and subsequently exposed to transplantable mammary tumors cells, develop a higher incidence of mammary tumors. Studies following populations exposed to 2,4-D should include an assessment of immune function, as well as cancer incidence, to determine if 2,4-D may affect cancer risk by compromising immune function.

IX. Summary of New Human Studies Currently Being Conducted

We have pointed out the need for more epidemiological studies on cancer rates and immune-effects in populations that were exposed to 2,4-D. Several ongoing / proposed studies on 2,4-D effects were found in the CRISP Database (see Appendix A) and are outlined below.

Herbicide Exposure Effect on Natural Killer Cells:

(PI: Ballas, Z.K., University of Iowa, extracted from the CRISP Database FY 96)

A study will evaluate the effects of herbicide exposures among agricultural workers, particularly on natural killer cells. The hypothesis of this project is that 2,4-D increases the incidence of immunologically-related cancers by inhibiting NK cells activity.

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.

MCPA-Probenecid Interaction with 2,4-D in Dogs:

(Gerken, D.F., Ohio State University, extracted from the CRISP Database, FY 95)

2,4-D is generally used in combination with other chlorophenoxy herbicides. A study will evaluate any synergistic effects between 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 2,4-D in dogs. These two herbicides have similar

absorption, distribution and excretion patterns, as well as the same clinical toxicity symptoms.

Environmental Risk Factors for Lymphomas and Sarcomas:

(Tolbert, P.E., Emory University, Atlanta, GA, extracted from the CRISP Database, FY 97)

The investigators propose to investigate patterns of risk in subtypes and groupings of subtypes of non-Hodgkin's

lymphoma, a group of etiologically distinct diseases, that have been so far studied as one. Environmental and occupational exposures that have previously been reported to be associated with all non-Hodgkin lymphomas combined (e.g., herbicides, leather, meat, solvents, formaldehyde, asbestos, radiation, EMF), will be reassessed for subtypes of NHL. Pooled analysis combining data from previous, smaller case control studies will be done. A similar approach is planned for studying sarcoma, a second group of cancers included in the Selected Cancers Study.

X. Bibliography

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XI. Appendix A. Common Abbreviations, Acronyms and Symbols

| | | | |
|------------------|--|----------------|---|
| 2-AAF | 2-acetylaminofluorine | IRIS | Integrated Risk Information System; Database maintained by EPA available through the National Library of Medicine MEDLARS system |
| 2,4-D | 2,4-dichlorophenoxyacetic acid | | |
| 2,4,5-T | 2,4,5-trichlorophenoxyacetic acid | | |
| 3-MCA | 3-methylcholanthrene | | |
| ADI | Allowable Daily Intake, set by the World Health Organization | ITF | Industrial Task Force on 2,4-D Research |
| AI | active ingredient | kg | kilogram |
| ATSDR | Agency for Toxic Substances Disease Registry | L | liter |
| | | LI | Long Island, New York |
| BCERF | Program on Breast Cancer and Environmental Risk Factors in New York State, based in the Cornell's Center for the Environment, Institute of Comparative and Environmental Toxicology | µg | microgram |
| | | m ³ | cubic meter |
| bwt | body weight | mg | milligram |
| C | carbon | MCF-7 | Michigan Cancer Foundation; cells derived from human breast tumor |
| CAS | Chemical Abstract Service | | |
| CCl ₄ | carbon tetrachloride | MCL | Maximum Contaminant Level; enforceable limit set by EPA which sets the maximum level of a contaminate in a public drinking water supply |
| CfE | Cornell University's Center for the Environment | MCPA | 2-methyl-4-chlorophenoxyacetic |
| CI | Confidence Interval | MTD | maximum tolerated dose |
| Cl | chlorine | n | number of subjects/animals in the group |
| CPRC | Carcinogenicity Peer Review Committee for EPA | NA | Not available |
| | | NHL | non-Hodgkin's lymphoma |
| 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) | NIOSH | National Institute of Occupational Safety and Health |
| | | NCI | National Cancer Institute |
| CTL | cytotoxic T lymphocytes | NIEHS | National Institute of Environmental Health and Safety |
| DEN | diethylnitrosamine; a carcinogen in the liver | NIH | National Institutes of Health |
| DMBA | 7,12-dimethylbenz[<i>a</i>]anthracene; known mammary carcinogen | NK | natural killer cells |
| DNA | deoxyribonucleic acid | NMU | <i>N</i> -nitroso- <i>N</i> -methyurea; mammary carcinogen |
| EPA | U.S. Environmental Protection Agency | NRC | National Research Council |
| ER | estrogen receptor | NS | Not statistically significant |
| E-SCREEN | screening assay for estrogenicity that measures proliferative response in estrogen-dependent MCF-7 breast tumor cells | NTIS | National Technical Information Service; repository for federal agency technical reports |
| FDA | Food and Drug Administration | NTP | National Toxicology Program |
| HA | 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 | NY | New York |
| | | NYS | New York State |
| HD | Hodgkin's disease | OR | Odds Ratio |
| IAA | indoleacetic acid | OSHA | Occupational Safety and Health Administration |
| IARC | International Agency for Research on Cancer, headquartered in Lyon, France | PH | phenoxyacid herbicides |
| ICET | Institute for Comparative and Environmental Toxicology | PMR | Proportional Mortality Ratio; PMR = number of deaths in the cohort compared to the expected number of deaths in the general population |
| i.p. | interperitoneal | ppm | parts per million |
| | | ppb | parts per billion |
| | | RR | Relative Risk |
| | | SCE | sister chromatid exchanges |
| | | SIR | Standardized Incidence Ratio; SIR= the ratio of "observed" to expected incidences |
| | | SMR | Standardized Mortality Ratio; SMR= the ratio of "observed" to "expected" deaths |

| | |
|--------------|---|
| TCDD | 2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin |
| TGF- β | transforming growth factor-beta |
| TPA | 12-tetradecanoylphorbol-13-acetate |
| TSH | thyroid stimulating hormone |
| TWA | Time-weighted average |
| U.S. | United States |
| USDA | United States Department of Agriculture |
| USEPA | United States Environmental Protection Agency |
| WHO | World Health Organization |
| Yr | year |

Symbols:

| | |
|---------------|----------------------|
| α | alpha |
| β | beta |
| γ | gamma |
| μg | microgram |
| < | less than |
| > | greater than |
| % | percent |
| p | p value |
| \pm | 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 (International Agency for Research on Cancer)

NTP ARC (National Toxicology Program, Annual Report on Carcinogens)

ATSDR (Agency for Toxic Substances and Disease Registry)

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 or 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.