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Critical Evaluation of Dichlorvos' 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 Cancer Risk Classification System, before reading this Critical Evaluation.

I. Chemical Information

A. Common Names: dichlorvos, DDVP, dichlorfos, DDVF (Worthing, 1991).

B. Chemical Name: 2,2-dichloroethenyl dimethylphosphate, 2,2-dichlorovinyl dimethylphosphate (Worthing, 1991; WHO, 1989).

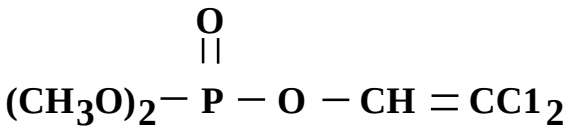
C. Chemical Formula: $C_4H_7Cl_2O_4P$ (WHO, 1989).

D. Formulator' Trade Names: Acivap® (Agro Biochemicals Industries Ltd.); Agro-DDVP® (AGRO-SAN Kimya Sanayi ve Ticaret A.S.); ANAPAV®, ANOPOV®DDVP; Fly Fighter® (AMVAC); Dedevap®, Mafu®, Oku® (Bayer); Cekusan® (Cequisa); Chimac DVP® (Chimac-Agriphar S.A.); Devikol® (Devidayal Pvt. Ltd.); Didivane® (Diachem S.p. A.) Herkol®, Marvex Super®, No-Pest Strips®, Prentox® DDVP, Vapona®, Verdican®, Verdipor®, Verdisol®, Elastrel® (Fermenta Animal Health); Luvon® (Lupin Agrochemicals Ltd.) Vapona® (Micro flo Co.); Midiltipi DDVP® (Midiltipi Agro-Chemicals, Inc); Dadasul® (Sulphur Mills Ltd.); De De Vap® (Vapco) (Meister, 1998).

E. Trade Mixes: Fly-Die® (+ piperonyl butoxide + pyrethrins) (HACO, Inc.); Ransbeck® (+ phosalone) (Rhone-Poulenc); Safrotin® Aerosol (+ propetamphos) (Sandoz Agro, Inc); Piran® (+ dibromochloropropane) (Tamogan Chemicals Ltd.) (Meister, 1998).

F. CAS Registry Number: 62-73-7 (WHO, 1989).

G. Chemical Structure:



H. Major Metabolites: dimethyl phosphoric acid and dichloroacetic acid (WHO, 1989).

II. History of Use and Usage:

A. History of Use and Nomenclature:
Dichlorvos is a synthetic insecticide and belongs to the family of chemically related organophosphate pesticides (OP). Although first synthesized in the late 1940s (IARC, 1991), its commercial manufacture started much later, in 1961. The annual production of dichlorvos was as high as 4.2 million pounds (lbs) in the late 1970s, and fell to 992,000 lbs by 1989. More recent estimates are not available, but are likely to be lower due to the many recent cancellations of its use (ATSDR, 1997). Dichlorvos can be released into the environment as a major degradation product of other OP insecticides, such as trichlorfon, naled, and metrifonate (Hofer, 1981; Murphy et al., 1996; ATSDR, 1997; Pettigrew et al., 1998).

Dichlorvos can kill insects when ingested, or absorbed through the integument or via spiracles (Worthing, 1991). It is effective against a wide range of pests such as mushroom flies, aphids, spider mites, caterpillars, thrips, whiteflies, gypsy moth, spruce budworm, forest tent caterpillars, fruit flies, codling moth, corn borer and boll weevil (Meister, 1998).

B. Usage:
In agriculture, dichlorvos was used to protect stored crops from insect damage. It was used in livestock industry to control external parasites on animals such as fleas and ticks. Dichlorvos was also added in animal feed as an anthelmintic (worming agent) for swine, horses and dogs (ATSDR, 1997). It was used in mushroom houses to control flies and insects (IARC, 1979), and was added directly to the water in fish farms, to control fish parasites (WHO, 1989).

Dichlorvos was used in food processing and packaging rooms, and in food service establishments to control insect pests (USEPA, 1995). In 1974, dichlorvos ranked among the three pesticides that were most frequently applied by pest control operators (ATSDR, 1997). Besides agricultural use, dichlorvos was applied as a fumigant in outdoor and public use areas to control Diptera and mosquitoes, and thus prevent the spread of malaria (ATSDR, 1997). It was used to control flies, mosquitoes, caterpillars, cockroaches and other pests in buildings, aircraft, public transportation vehicles and outdoor areas (ATSDR, 1997; WHO, 1989). Dichlorvos was a popular household insecticide that was applied as an aerosol or liquid spray, or incorporated into resin strips and flea collars for pets. The National Household Pesticide Usage study reported that dichlorvos was the most frequently detected pesticide in 8,254 households surveyed in the U.S. during the mid-1970s (ATSDR, 1997).

The annual agricultural use of dichlorvos was estimated as 248,000 lbs during 1982 (ATSDR, 1997). Estimates done in late 1980s indicate that 60% of dichlorvos used worldwide was for plant protection, 30% was for public hygiene and vector control, and 10% to protect stored crops (WHO, 1989). In the U.S., use in

livestock (beef, dairy, swine sheep, poultry, other animals) industries, tobacco warehouses and greenhouses accounted for approximately 60% of all the dichlorvos used in 1988. In the same year, food processing, sanitation and public health used 25%, and the rest (15%) was used for domestic purposes (against household pests and in pet collars and No-Pest Strips®) (Mueller, 1988). Dichlorvos was among the several organophosphate pesticides (OP) shipped by the Department of Defense for use during the Gulf War (GWVI, 1996).

III. Current Regulatory Status:

A. Regulatory Status:
Based on evidence from animal studies in the late 1980s, the Environmental Protection Agency (EPA) classified dichlorvos as a "probable human carcinogen." On February 24, 1988, EPA initiated a Special Review for pesticide products containing dichlorvos in response to concerns about its carcinogenicity and neurotoxic potential. The Special Review is EPA's formal process to determine if the use of a pesticide poses unreasonable risks to people or the environment. All labels of dichlorvos products were revised to include the signal words "Danger-Poison" and directions for appropriate use. There were concerns about exposure to dichlorvos through food residues, during application, or reentry after application, and through treatment of pets (USEPA, 1995). In 1995, EPA proposed cancellation of many domestic uses such as hanging pest strips, room foggers and pet flea collars. It proposed cancellation of use of dichlorvos on: stored non-perishable raw and processed foods, in warehouses, commercial institutions, industrial areas, food manufacturing and processing facilities, on ornamentals, on turfgrass and in airplanes (ATSDR, 1997). The uses that were proposed to remain were: for insect control in mushroom houses, greenhouses, kennels, garbage dumps, manure piles and animal premises; for automated application to livestock; fumigation of passenger buses; and in insect traps. Treated areas were required to have warning signs posted to restrict re-entry (ATSDR, 1997).

In response to EPA's proposed cancellations, Amvac Chemical Corp., the sole registrant of technical dichlorvos (99.9% active ingredient), voluntarily agreed to delete certain household, food crop, aerial and aircraft applications of dichlorvos (USEPA, 1995). Amvac was allowed to sell its existing stocks with old labels for a one-year period, ending in 1996. Household use of dichlorvos has since been restricted to dog and cat flea collars, impregnated resin strips and total release foggers. Non-total release foggers and aerosols were restricted to be applied by licensed pest control operators only (USEPA, 1995). A new fact sheet has been prepared by the EPA for "bug bombs" or total release foggers to explain and emphasize the label requirements on such devices (USEPA, 1998a). The Food and Drug Administration (FDA) has required that labels of pest-strips carry warnings to discourage their use in restaurants and kitchens (ATSDR, 1997).

B. Clean Water Act Requirements:
Dichlorvos has been designated as a hazardous substance under the Federal Water Pollution Control Act and is regulated by the Clean Water Act Amendment. Discharges of 10 lbs or more of dichlorvos need to be reported to the National Response Center (HSDB, 1997). However, Maximum Contaminant Levels (MCL) or Health Advisories (HA) have not been established (USEPA, 1996). Dichlorvos has not been detected in drinking water in the U.S. (ATSDR, 1997). It is rapidly hydrolyzed in water to dimethyl phosphoric acid and dichloroacetic acid (WHO, 1989).

C. Workplace Regulations:
The Occupational Safety and Health Administration (OSHA) has set the maximum allowable level in workplace air at 1 mg/m^3, based on average exposure of 8 hours / day, 40 hours / week (ATSDR, 1997).

D. Food Tolerances:
The maximum amount of each pesticide that is permitted to occur on the edible portion of raw agricultural commodities and in processed foods, called "tolerance", is set by the EPA. For some pesticides such as dichlorvos, the residues may decline rapidly, and the limits are set for the levels detectable at harvest (IARC, 1991). The tolerances for dichlorvos are: 0.05 parts per million (ppm) in tomatoes; 0.5 ppm in cucumbers; 1 ppm in lettuce; 0.02 ppm in milk; 0.05 ppm in eggs and poultry meat; and 0.02 ppm in goat, sheep and cattle meat (USEPA, 1998b).

A strict interpretation of the Delaney Clause would mandate a zero tolerance of any chemical shown to cause cancer in man or animal, in ready-to-eat or processed food. Dichlorvos was reported to cause cancer in experimental animals in the late 1980s (Chan et al., 1988; Chan et al., 1991). Dichlorvos food tolerances were thus potentially violating the Delaney Clause. In 1991, the Federal Food, Drug and Cosmetic Act (FFDCA) proposed to revoke the food additive regulation that permitted dichlorvos use on packaged or bagged non-perishable food. This revocation was stayed indefinitely after a petition from Amvac, but the use of dichlorvos on packaged foods has been proposed for cancellation (USEPA, 1995).

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

A. Human Studies:
There are many reports on the clinical and non-cancer effects of dichlorvos. These effects are mainly mediated through its inhibition of acetyl cholinesterase enzymes. Dichlorvos has also been

implicated as a causative agent of contact dermatitis in case-control studies (Matsushita et al., 1985). Toxic effects of dichlorvos have been extensively reviewed (IARC, 1991; ATSDR, 1997; Gillett et al., 1972; Tinker, 1972; WHO, 1989) and are not included here to restrict the discussion to carcinogenic effects.

1. Case Reports:
Case-reports of exposure and subsequent diagnosis of cancer are not sufficient evidence to establish a cause and effect relationship. Cancer has a long and variable latency period during which time a person is typically exposed to many potential carcinogens. However, case-reports can serve as useful indicators for an association that needs to be followed in large epidemiological studies.

One report documents that parents of two cases of childhood leukemia recalled using dichlorvos and propoxur (Reeves et al., 1981). This case-report is not adequate evidence in itself for a cancer causing potential of dichlorvos. However, it adds weight to the evidence from case-control studies of leukemia discussed below.

2. Population-Based Case-Control Studies
A few case-control studies have evaluated the risk for cancer in association with exposure to dichlorvos and other pesticides. However, all the studies were based on small numbers of cases, making them inadequate as evidence for a carcinogenic effect of dichlorvos in humans. They do raise some concerns that need to be addressed in future studies.

In one case-control study, 578 leukemia cases among white male farmers in Iowa and Minnesota and 1245 population-based controls were evaluated for exposure to pesticides (Brown et al., 1990). The odds ratio (OR) for leukemia was significantly increased among the 26 farmers who had ever mixed, handled or applied dichlorvos (OR = 2.0; 95% confidence interval (CI) 1.2-3.5). The risk was higher among the 12 farmers who had used dichlorvos at least 20 years before the time of interview (OR = 2.4; 95% CI 1.1-5.4). The risk was elevated among farmers who used dichlorvos on animals frequently (> 10 days per year), based on a small sample of five cases and four controls. This study adjusted ORs for vital status, age, state of residence, tobacco use, family history of lymphopoietic cancer, high-risk occupations and high-risk exposures in a logistic analysis. However, the number of farmers exposed to dichlorvos was small. Since questionnaires were used to assess exposure to dichlorvos, recall bias could not be ruled out. Increased risk for leukemia in this study was also observed in association with other pesticides, and the role of dichlorvos, by itself, could not be evaluated due to the small number of cases diagnosed.

The above population of white male farmers was also evaluated for risk of developing non-Hodgkin's lymphoma (NHL) (Cantor et al., 1992). The risk for NHL was slightly, but not significantly increased in farmers who had ever handled dichlorvos (OR = 1.2; 95% CI 0.7-2.2, based on 20 cases and 38 controls), or among those who had handled it prior to 1965 (OR = 1.8; 95% CI 0.8-3.9, based on 12 cases and 17 controls). The ORs were adjusted for vital status, age, state, cigarette smoking status, family history of lymphopoietic cancer, high-risk occupation and high-risk exposures.

A different population-based case-control study from eastern Nebraska evaluated the risk for NHL among white women agricultural pesticide users. This study included 184 NHL cases and 707 controls matched for race, sex, vital status and age. Among the six cases and five controls who had personally handled OPs, the OR for NHL was significantly increased (OR = 4.5, 95% CI 1.1-17.9) (Zahm and Babbitt, 1993). However, dichlorvos was one of several OPs that the women could have been exposed to, and only one of the women interviewed reported having handled dichlorvos specifically. The very small numbers in both these studies do not allow an evaluation of the effect of dichlorvos on the risk for NHL.

A case-control study has evaluated the incidence of multiple myeloma among 173 white male farmers in Iowa and 650 population-based controls (Brown et al., 1993). The risk for multiple myeloma was found to be increased, but not significantly, among farmers who had ever mixed, handled or applied dichlorvos as an animal insecticide (OR = 2.0; 95% CI 0.8-5.0), based on seven cases and 21 controls. Failure to use protective equipment did not increase the risk further. The ORs were adjusted for vital status and age. Smoking and education were evaluated, but not found to be confounding factors.

Families of 153 cases of childhood cancer cases identified through the Missouri Cancer Registry and 85 cancer-free white children were interviewed for home use of pesticides. Almost 10% of the participating families acknowledged use of dichlorvos containing No-Pest Strips" (Davis et al., 1992). A comparison of 45 brain cancer cases and 85 cancer-free controls indicated a significantly increased risk for childhood brain cancer (OR = 3.7; 95% CI 1.0-13.7) associated with family use of No-Pest Strips". The increased risk for brain cancer was not significant if the comparison was done with a control group of 108 other childhood cancer cases (OR = 2.0; 95% CI 0.6-6.3) (Davis et al., 1993). This study was of limited value because of the small number of subjects, potential for recall bias and the lack of detailed exposure information. It does indicate the need for larger case-control studies on population groups who may have been exposed as children to dichlorvos and subsequent cancer incidences.

3. Cohort Studies:
Mortality rates of a cohort of 2384 manufacturing workers (87% white men, 10% white women, 3% black men, <1% black women)

at a plant in Colorado that produced aldrin, azodrin, Vapona® (dichlorvos) and other pesticides were compared to the general population of Colorado State. The median follow-up period was 29 years for the cohort, with a minimum follow-up of 11 years for any employee. The vital status of the subjects was determined through the National Death Index, the Social Security Administration Death Files, the Colorado Division of Motor Vehicles, a credit bureau and individual tracing. The standardized mortality ratio (SMR) was calculated as the ratio of deaths among cohort members to the expected number of deaths, multiplied by 100. The overall cancer mortality rate of the cohort was similar to that recorded for the general population of Colorado State. The SMR for lymphopoietic cancer in the cohort was slightly, but not significantly increased (SMR = 146; 95% CI 83-237). However, this increase was significant among black males, based on three deaths (SMR = 1318; 95% CI 272-3853, $p < 0.05$). A common exposure pattern or a single causative exposure was considered unlikely for these three workers since they had held different job responsibilities (Amoateng-Adjepong et al., 1995). White women in this cohort had no overall increase in deaths from all causes, but a non-significant increase was observed in deaths from lung cancer (SMR = 256; 95% CI 53-747) (Amoateng-Adjepong et al., 1995). There was a significant increase in rate of hepatobiliary cancer in the group of white, male workers who were hired between 1950-1959, based on 5 deaths (SMR = 427; 95% CI 139-997, $p < 0.05$). These five cases had been hourly workers with a high potential for pesticide exposure. However, the period of employment associated with the increased mortality (1950-1959) predates the start of commercial manufacture of dichlorvos in 1961, making dichlorvos exposures unlikely as the cause. The incidence of hepatobiliary cancers was not significantly increased in the rest of the cohort (Amoateng-Adjepong et al., 1995).

4. Summary:

Male farmers in the Midwest who handled dichlorvos were found to have a significantly increased risk for leukemia in one case-control study (Brown et al., 1990). Risk for NHL or multiple myeloma was not observed to be significantly increased in male farmers who had used dichlorvos (Brown et al., 1993; Cantor et al., 1992). The risk for NHL was significantly increased among a very small group of female farmers who had used OP, but only one woman reported using dichlorvos (Zahm and Babbitt, 1993). Another study reported family use of dichlorvos to be associated with a significantly increased risk for childhood brain cancer (Davis et al., 1993). The small numbers and potential for exposure to other pesticides in the two studies indicating positive associations (Brown et al., 1990; Davis et al., 1993), do not permit an evaluation of whether dichlorvos caused the increase in incidence of leukemia or brain cancer. However, both these studies raise concerns about possible carcinogenic effects from dichlorvos exposure in humans that need to be followed in larger populations-based case-control studies.

B. Animal Experimental Studies:

There have been many studies on the carcinogenic effects of dichlorvos in experimental animals. Some of the studies described below provide evidence for a cancer-causing effect of dichlorvos at contact sites such as the stomach and esophagus. Studies that reported on mammary neoplasms are discussed in Section VB.1.

1. Mice:

In a study conducted by the National Cancer Institute (NCI), groups of 50 male B6C3F1 mice were given 0, 10, 20 mg/kg dichlorvos (99% pure) by gastric intubation, 5 times / week for 103 weeks. Groups of 50 females were similarly treated, but with 0, 20, or 40 mg/kg of dichlorvos. Average survival rates were 70% for control males, 50% for control females, 56% for dichlorvos-treated males and 63% for dichlorvos-treated females. There was a significant increase ($p < 0.01$) in squamous cell papillomas in the forestomach of female mice, but only in the high-dose group (18/50). The incidence of squamous cell papillomas in the low-dose group females (6/49) was not significantly different to that in the controls (5/49). Forestomach carcinomas were found in two females in the high-dose group, but none in the vehicle-treated controls. The combined incidence of papillomas or carcinomas in the stomach of female mice was significantly increased in the high dose group ($p < 0.01$). The incidence of squamous cell papillomas of the forestomach was increased in male mice as well, but not significantly (Chan et al., 1991). The low survival rates may have reduced the effective numbers of mice that survived long enough for tumors to develop. Although the number of surviving animals was small, the histopathological evidence of tumor progression and the dose-dependent increase of lesions in treated female mice suggest a carcinogenic effect of dichlorvos at a primary site of contact, the forestomach.

An earlier NCI study evaluated B6C3F1 mice (50 per dose of each sex) that were given TWA (time-weighted average) doses of 0, 318 and 635 ppm dichlorvos in diet for 78 weeks. Surviving mice were killed 92 to 94 weeks after initiation of treatment. Survival rates were not dose-related. The overall incidence of tumors was increased in male mice that were fed the low-dose of dichlorvos ($p = 0.050$). There was one esophageal squamous cell carcinoma per group of male mice fed the low-dose and female mice fed the high-dose of dichlorvos (NCI, 1977). Three male mice that were fed the low-dose, and one female fed the high-dose of dichlorvos, had focal hyperplasia in the esophagus (NCI, 1977). Although these increases were not statistically significant, their observation was important since spontaneous esophageal tumors are rare in this strain of mice.

In one study, C57B1/6 mice (100 of each sex) were treated with 0 or 0.2 mg dichlorvos (97%) by gavage, either two to three times a week over 50 weeks. Both, dichlorvos-treated males and females had significantly increased incidence of focal hyperplasia of the urinary bladder compared to controls. Details on statistical analysis

and experimental design were not available. The authors reported no significant increase in treatment-related neoplastic lesions [Horn et al., 1987, as translated in (FAO/WHO, 1993)]. The study was inadequate as a cancer bioassay since it used only one treatment dose and did not allow an evaluation of a dose-response effect. Horn et al. conducted a follow-up co-carcinogenicity study, in which similar dichlorvos treatment of mice were accompanied by a weekly subcutaneous dose of 50 µg N-nitrosodiethylamine over 50 weeks (statistical analysis and details on experimental design not available). Dichlorvos was not found to act as a co-carcinogen in this study [Horn et al., 1990, as cited in (FAO/WHO, 1993)]. The short time period (50 weeks) of treatment in both these studies make them invalid as bioassays for cancer.

An unpublished study by Konishi et al., reviewed by (Bremmer et al., 1988), on B6C3F1 mice (50 of each sex) treated with 0, 62 or 124 mg/kg dichlorvos in drinking water for 102 weeks, reports a dose-dependent decrease in all tumors in females. No details on experimental design were available. Since dichlorvos is rapidly hydrolyzed in water, the actual dose received by animals treated using drinking water may have been lower.

2. Rats:

The carcinogenic effect of dichlorvos was evaluated in Fischer F344/N rats (50 per dose, of each sex) treated by gastric intubations with 0, 4 or 8 mg/kg dichlorvos (99% pure), five times per week, over 103 weeks. Survival rates were 62% for controls and 50% for treated animals. A significant increase in incidence of hyperplasia and multiple adenomas of the pancreas ($p < 0.05$) was observed among male rats. Although pancreatic acinar atrophy was significantly increased in the high dose-treated female rats ($p < 0.05$), there was only a small, non-significant increase in pancreatic acinar hyperplasia (Chan et al., 1991). There was a significant and dose-dependent increase in the incidence of mononuclear cell leukemia in dichlorvos-treated male rats ($p < 0.02$). In female rats, the increased incidence of mononuclear cell leukemia was small and not significant.

The low survival rates of both the controls and treated rats in this study indicate that the animals may have been dying of other, non-treatment related causes. Early deaths in animals can lead to an underestimation of slow developing cancers that would become detectable with age had the animals lived longer. One reviewer of the study has pointed out that variable and high rates of spontaneous mononuclear cell leukemia are common in corn oil-dosed male F344 rats, with rates ranging between 4% to 46%. The increase in incidence of mononuclear cell leukemia in treated rats, although significant, does not exceed the range that has been observed for this strain of rats in controls of other bioassays (Bremmer et al., 1988; Mennear, 1994). Details on the incidence of mammary fibroadenomas observed in this study are discussed in Section VB.1.

Other studies described below had either strong limitations, or were invalid as cancer bioassays for the reasons outlined below after each study's design.

Osborne-Mendel rats (50 of each sex) were fed 0, 150 ppm (male) or 326 ppm (female) TWA of dichlorvos over 80 weeks. The surviving animals were killed at 110-111 weeks. Histopathological analysis of the treated animals revealed no treatment-related effects (NCI, 1977). This study was of limited value as a cancer bioassay since it used only one treatment dose.

In a two-year inhalation study, groups of 50 Carworth Farm E rats (CFE) of each sex, were exposed to 0, 0.05, 0.5 and 5 mg/m^3 of dichlorvos. The survival rates in treated and control groups were very low, but not treatment-dependent. Only 11/50 controls and 15/50 males treated with 0.5 mg/m^3 dichlorvos survived the experimental period. Survival of female rats was relatively higher, with 50% of controls and 64% treated rats surviving the experimental period. The incidence of tumors of the thyroid, adrenal and anterior pituitary was higher in treated males (statistical comparison with controls was not available for each organ site). The group of males that were fed the highest dose however, had a significant decrease in incidence of tumors of the adrenal medulla ($p < 0.05$). Tumors in the anterior pituitary were slightly but not significantly increased in the treated females ($p > 0.05$). The poor survival rates of the control rats in this study made a comparison of dose-related effects meaningless (Blair et al., 1976).

In an unpublished study that has been reviewed by EPA, CD rats (40 per dose of each sex) were fed 0, 0.1, 10, 100, and 500 ppm of the insecticide Vapona® for 104 weeks (93% dichlorvos). No oncogenic effects for dichlorvos were reported; however, EPA noted that the maximum tolerated dose (MTD) was not included in this study. Further, the number of animals that were examined after the full 24-month period was small, and no histopathological details were available (Witherup et al., 1967, as cited in USEPA, 1982). Another study conducted by the same authors was also invalid as a cancer bioassay because of the very small number of animals used, the very low survival rates and the use of only one treatment dose (USEPA, 1982; Witherup et al., 1971).

In an unpublished study by Enomoto et al. (as reviewed in WHO, 1989), Fischer rats were treated with 0, 140, or 280 mg/L of dichlorvos in drinking water for 104 weeks. No treatment related effect on tumor incidences was reported. Since dichlorvos breaks down in water rapidly, the effective dose in this study may have been much lower.

3. Dogs:

Beagle dogs (three of each sex) were fed 0, 0.09, 0.32, 3.2, 32 and 256 mg/kg dichlorvos in diet for two years [unpublished study by Jolley et al., 1967, as reviewed by (Bremmer et al., 1988)]. No

increases of any tumors were observed among treated dogs. The number of animals used were inadequate for a cancer bioassay.

Summary, Animal Studies:
A study that was conducted with appropriate experimental design, although limited by its poor survival rates, indicated a significant increase in incidence of stomach tumors in female mice and pancreatic tumors in male rats that were fed dichlorvos (Chan et al., 1991). The increase in tumors in the forestomach in female mice and pancreatic tumors in male rats was dose-dependent, indicating a treatment-related effect. The incidence of esophageal cancers in dichlorvos-treated mice in another study deserves attention since spontaneous cancers at this site are rare. Also, the esophagus is similar to the forestomach, in being a primary site of contact for ingested dichlorvos (NCI, 1977). The increase in incidence of leukemia in male rats (Chan et al., 1991) deserves further attention in light of the evidence, although limited, from human case-control studies discussed previously in Section IVA. Other studies on experimental animals did not indicate dichlorvos treatment-related effects, but were invalid as cancer bioassays due to the small number of animals (Witherup et al., 1971), or inadequate reporting (Blair et al., 1976; Witherup et al., 1971).

C. Current Classification of Carcinogenicity by Other Agencies
1. IARC Classification:
The IARC has classified dichlorvos in Group 2B, *possibly carcinogenic* to humans (IARC, 1991). This classification is based on *inadequate evidence* in humans and *sufficient evidence* in experimental animals for the carcinogenicity of dichlorvos (IARC, 1991).

2. NPT Classification:
On the basis of studies of experimental animals, the NTP has concluded that in rats, there is *some evidence* from males and *equivocal evidence* from females, and in mice, there is *some evidence* from males and *clear evidence* from females for the carcinogenic effect of dichlorvos (ATSDR, 1997).

3. EPA Classification:
The EPA has classified dichlorvos as a Group B2, *probable human carcinogen* (ATSDR, 1997). This classification was based on sufficient evidence for carcinogenicity from studies on experimental animals on dichlorvos.

V. Critical Evaluation on Breast Carcinogenicity

A. Human Studies:
Human Tissue Levels:
Studies of human tissue levels in association with breast cancer were not available.

B. Animal Studies:
1. Mice:
Two long-term carcinogenicity studies in mice that were discussed in Section IVB.1, did not report any increase in the incidence of mammary gland neoplasms in dichlorvos-treated animals (Chan et al., 1991; NCI, 1977).

2. Rats:
In one study, groups of 50 female Fischer F344/N rats were treated with 0, 4 mg/kg and 8 mg/kg dichlorvos (99% pure) by gavage for 103 weeks (Chan et al., 1988; Chan et al., 1991). Survival rates were 62% for controls and 50% for treated animals. The number of mammary gland tumors found in each group are presented in Table 1.

Table 1. Mammary Gland Lesions Observed in Dichlorvos - Treated Rats

Type of Mammary Gland Lesion	Vehicle Control	4 mg/kg	8 mg/kg
fibroadenoma	9	19*	17*
multiple fibroadenomas	0	6*	3
carcinomas	2	2	0
mammary neoplasms (total)	11	20**	17

*Significantly different from controls, $p < 0.05$
**Significantly different from controls, $p < 0.02$

Significant increases in incidence of mammary fibroadenomas (benign), were observed in both groups of dichlorvos-treated females ($p < 0.05$). The incidence of malignant neoplasms was not increased. When the incidence of all benign and malignant neoplasms was combined, a significant increase was observed in the group fed the low dose, but not the group that was fed the high dose of dichlorvos ($p < 0.02$), indicating a lack of a dose-dependent response (Chan et al., 1991). The low survival rates and the use of only two doses were limiting factors of this study. The strength of this study was that histopathology was done on all animals, including those that did not survive the full term of the experiment. However, it is difficult to predict if the benign mammary gland tumors observed to be significantly increased in the treated animals would have progressed to become malignant lesions with increasing age or longer survival time.

In an inhalation study conducted by Tunstall Laboratories (Shell Research Ltd.), groups of 50 female CFE rats were exposed to 0, 0.05, 0.5 or 5 mg/m^3 atmospheric concentration of dichlorvos for two years (> 97% purity). No treatment-related increases in mammary lesions were observed. The controls had very high rates of spontaneous mammary tumors and very low survival rates (Blair et al., 1976). In the high dose-treated females, there was a statistically significant decrease in the incidence of mammary gland tumors ($p < 0.01$) (Blair et al., 1976). Details on the numbers of

mammary tumors in each group were not reported and the high rates of spontaneous mammary tumors prevent meaningful conclusions from this study.

The above study was reviewed by Reuber (1981), who concluded that "benign and malignant neoplasms, as well as neoplasms of the thyroid, pituitary, adrenal and mammary gland were increased in dichlorvos-treated male rats" (Reuber, 1981). The basis for the conclusion of this review is unclear since: 1) the numbers of mammary tumors were not reported in the original paper (Blair et al., 1976), and 2) Reuber states in his review that he was denied permission to examine the slides (Reuber, 1981).

In a study conducted by NCI (1977), Osborne-Mendel rats (50 per dose, of each sex) were fed a TWA dose of 150 ppm or 256 ppm dichlorvos in corn oil in diet for 80 weeks. Five untreated rats served as matched controls for each of the two treatment groups. Matched control groups from studies of five other chemicals were combined to form a pooled control group of 60 animals of each sex. The surviving animals were killed at 110-111 weeks. Mammary gland neoplasms (benign and malignant) were reported in 12% of the females in the high-dose group, 27% of the females in the low-dose, 20% of the matched controls, and 18% of the pooled controls. The increased incidence of mammary neoplasms in the low-dose group was not statistically significant and was mainly of the benign kind (mammary fibromas and fibroadenomas). Only one mammary carcinoma was detected in the low-dose group, while two mammary carcinomas were observed in pooled controls. In male rats, two animals in the high-dose group and one each, in the pooled controls and the low-dose group had mammary carcinomas. The use of only five rats as matched controls was a limiting factor of this study (NCI, 1977). Reuber re-examined the histological sections from the above NCI study and reported that the dichlorvos-treated male rats had an increased incidence of mammary gland neoplasms. Malignant or benign neoplasms were not significantly increased in either the low-dose or the high-dose groups of male mice when analyzed separately. However, the incidence of malignant mammary gland neoplasms were reported in the review as significantly increased when the entire group of treated male rats (low-dose and high-dose) was pooled and compared to the controls ($p = 0.002$) (Reuber, 1981). A significant increase in carcinomas of endocrine organs (thyroid, adrenal, pituitary and ovary) was reported for the low-dose treated females ($p = 0.000088$). Combining the results of different treatment groups and different organ neoplasms in the review makes the significance of the results unclear, especially since the results of individual endocrine cancers were not statistically significant. It was also not mentioned in this review if rats with neoplasms in multiple organs (metastatic tumors) were counted as one or as multiple tumors.

3. Summary, Critical Evaluation on Breast Carcinogenicity:
No epidemiological studies were available to evaluate the breast carcinogenicity of dichlorvos in women exposed to this insecticide in the past.

A study by Chan et al. (1991) has reported increased (significant in low dose group only) incidence of mammary gland fibroadenomas/adenomas in Fischer rats. Two other studies have reported no significant increase in incidence of mammary gland neoplasms among dichlorvos treated rats (Blair et al., 1976; NCI, 1977), but these studies were limited by either low survival rates, small numbers or high rates of spontaneous mammary gland neoplasms. However, one reviewer of the NCI study (Reuber, 1981) reported that dichlorvos had caused a significant increase in incidence of mammary gland neoplasms in dichlorvos-treated male Osborne-Mendel rats. Two studies in mice did not report increased incidence of mammary gland neoplasms in dichlorvos fed mice (Chan et al., 1991; NCI, 1977). There is a need for another study in rats, using more doses of dichlorvos and better survival rates to verify the results of Chan et al. (1991).

C. Other Relevant Data on Breast Cancer Risk:
1. Evidence of Endocrine Disruption:
a) Effects on Steroidogenesis:
Sprague-Dawley rats (six per group) were treated with 0 or 2 ppm dichlorvos (99% pure) in drinking water for two weeks and evaluated for hormonal disruptions. Dichlorvos was not found to affect the plasma and adrenal corticosterone levels, but did increase the levels of adrenal cholesterol ester and adrenocorticotrophic hormone (Civen et al., 1980). The same author had previously reported a significant inhibition of pregnenolone-stimulated steroidogenesis using 10 μM dichlorvos *in vitro* on isolated adrenal cells ($p < 0.001$) (Civen et al., 1977). These studies indicate that dichlorvos may have the potential to disrupt steroidogenesis. Juvenile male Wistar rats that received either 10 mg/kg of dichlorvos, from day 4 to 23 of life, or 20 mg/kg dichlorvos on day 4 and 5 had a small, but not significant reduction in spermatocytes (Krause et al., 1976). No significant reduction was observed in serum levels of testosterone, luteinizing hormone, or follicle stimulating hormone in adult Wistar rats that were fed 10 mg/kg dichlorvos every second day over two weeks, or 5 mg/kg dichlorvos three times a week for three weeks (Krause, 1977).

b) Delayed Onset of First Estrous Cycle:
Outbred albino rats ($n = 29$), exposed to 2.4 mg/m^3 of dichlorvos in environmental chambers, were observed to have their first estrous cycle significantly later ($p = 0.005$) than unexposed controls ($n = 21$). One half of a No-Pest Strip" was suspended on top of each cage and the rats were exposed to this environment for 8 to 10 hours daily, from birth until the first estrous (Timmons et al.,

1975). The result of this study indicates a delay in an estrogen-dependent event. However, this study did not test for other indicators for estrogenic effects and it is not sufficient to predict an antiestrogenic action for dichlorvos.

2. Reproductive and Teratogenic Effects:
Reproductive toxicity may be suggestive of either endocrine disruption or embryo toxicity. We have included below any studies on reproductive toxicity of dichlorvos that indicate an effect on estrogen-dependent reproductive events.

One study evaluated the reproductive performance of two different mammalian species exposed to dichlorvos by either gavage or inhalation. CF-1 mice (n = 25) were given the MTD of dichlorvos (60 mg/kg, 96% pure) by gavage from day 6 through 15 of gestation. New Zealand Rabbits (n = 12) were similarly treated with a 5 mg/kg dose from day 6 through 18 of gestation. Another group of mice (n = 15) and rabbits (n = 14) were exposed to dichlorvos vapor (4 μg/L for 7 hours per day). No effects on litter sizes or implantation rates were observed in any of the exposed groups in either species (Schwetz et al., 1979).

Similar results were observed in a different study in which Dutch rabbits (n = 20/ group) and CFE rats (n = 15/ group) were exposed to 0, 0.25, 1.25 and 6.25 μg/L of air, for 23 hours daily. There was no effect on the number of pregnancies or the number of live fetuses in rats. Maternal toxicity was observed in rabbits exposed to high doses (Thorpe et al., 1972). In another evaluation of reproductive toxicity, Sherman rats (n = 6) were treated with 15 mg/kg dichlorvos (i.p. on day 11 of gestation). While there was no treatment-related effect on the number of pregnancies and live births, there was a small, not significant increase in malformed fetuses (Kimbrough and Gaines, 1968).

Studies conducted by Shell Chemical Co. found no adverse effect on the reproductive performance of dichlorvos-treated pigs and pregnant sows (Stanton et al., 1979; Young et al., 1979). In a two-generation study in swine, 0, 200, 250, 288, 400 and 500 ppm dichlorvos fed in diet over 37 months had no effect on the number, viability, or reproductive performance of the offspring from the exposed male and female pigs (Collins et al., 1971). Some of these studies reported a small positive effect on litter weight of dichlorvos-treated dams (Foster, 1968; Young et al., 1979). In one study, crossbred sows were fed either basal diet, 500 ppm dichlorvos, 250 ppm copper, or a combination of copper and dichlorvos during gestation and lactation. There was a significant reduction in pre-weaning mortality rate in the group fed dichlorvos and copper (n = 41; p = 0.02) (Thacker, 1991). Two reasons are possible for the increase in litter weight observed in some studies: dichlorvos rids the dam of parasitic infestations, thus allowing for better nourishment of the litter; or it affects the development of the fetus directly. Authors of one study reported similar litter weight gain for groups of dichlorvos-treated sows that were parasite-free at the start of treatment, and groups that were parasite-infected, indicating that dichlorvos was affecting litter weights in ways other than just its anti-parasitic effect in the dam (Young et al., 1979).

In summary, evidence from available reproductive toxicology studies indicates that exposure to dichlorvos does not adversely affect the estrogen-dependent events such as implantation rates or litter size in rabbits, mice, rats and pigs.

3. Tests of Mutagenicity:
a) Chromosome Aberrations in Occupationally Exposed Humans:
Cytogenetic analysis of blood lymphocytes of male pesticide applicators indicated significantly elevated sister chromatid exchange (SCE) rates among sprayers (n = 29) who had applied pesticides for more than a year without the use of protective equipment ($p < 0.05$). However, dichlorvos was only one of many pesticides used by these applicators (Crossen et al., 1978).

b) Mutagenicity Studies in Animals:
Many studies in bacteria, yeast, isolated cells and with isolated DNA have reported a mutagenic potential of dichlorvos. However, it has been argued that due to its short half-life, dichlorvos may not have a major genotoxic effect *in vivo* in animals (Wright et al., 1979). The genotoxic potential of dichlorvos has been studied in a variety of ways to assay DNA damage in many different animal systems: mice, rats, hamsters, and fish. Six of the 11 *in vivo* assays discussed below have reported a genotoxic potential for dichlorvos.

Dominant lethal mutations were not observed to be increased in the progeny of male mice treated with a single subtoxic dose of dichlorvos, mated with untreated virgin females (Epstein et al., 1972). A short term assay for unscheduled DNA synthesis (UDS) in the forestomach of mice was initiated following the report by Chan et al., (1991) of increased stomach cancers in dichlorvos-treated mice (see IVB.1). Groups of B6C3F1 mice (five per sex) were given 200 mg/kg of technical dichlorvos (99.8% pure) by gavage, and sacrificed after 2, 4, 8, 10, 12 or 48 hours (Benford et al., 1994). No induction of UDS was observed in response to dichlorvos treatments. However, dichlorvos caused a significant induction ($p < 0.05$) in the focal hyperplasia and replicative DNA synthesis (RDS, an indicator of the number of cells entering S-phase of the cell cycle) in the forestomach of male and female mice. The authors of this study state that the absence of UDS argues against a direct genotoxic potential of dichlorvos (Benford et al., 1994). However, the induced hyperplasia, ignored by the authors, may be indicating a tumor promoting potential of dichlorvos.

In another study, B6C3f1 mice injected intraperitoneally (i.p.) with 5, 15, 25, 35 mg/kg dichlorvos, had no significant induction of SCE in the peripheral blood lymphocytes (Kligerman et al., 1985). Male F344 rats fed 150 ppm and 300 ppm dichlorvos (> 96% pure) for 14 days did not have any proliferative effects in the liver or

kidneys (Cunningham et al., 1994). Cytogenetic analyses of bone marrow and testis of Q strain mice treated to a single dose of 10 mg/kg dichlorvos did not indicate significant treatment-related chromosome damage or clastogenic effects in either tissue (Moutschen-Dahmen et al., 1981). There was no significant increase in dominant lethal mutations which is consistent with the cytogenetic observations.

In contrast, keratinocyte cells isolated from male HRA/Skh mice after a single topical application of 0, 0.05, 0.1, 0.5 or 1.0% dichlorvos had a significant, dose-related increase in incidence of micronuclei ($p < 0.001$) (Tungul et al., 1991). A single topical application of dichlorvos at a dose above 80 mM/kg was found to significantly increase ($p < 0.05$) the percentage of nuclear aberrations in the hair follicles of male CD1 mice (Schop et al., 1990). Oral administration of 1/50, 1/75 and 1/100 of the lethal dose required for 50% kill (LD_{50}) of dichlorvos resulted in a significant increase in the frequency of chromosome aberrations in bone marrow cells of Wistar rats (Nehez et al., 1994). Injection of 3, 6, 15 or 30 mg/kg dichlorvos i.p. to groups of female Syrian hamsters ($n = 6$) was associated with a statistically significant increase in chromosome aberrations at the two highest doses ($p < 0.05$) (Dzwonkowska and Hubner, 1986). One study reported genetic damage, assayed as sperm morphological abnormalities in mice treated with i.p. injections of dichlorvos (Wyrobek and Bruce, 1975). This result was based on sperm morphology alone, without any cytogenetic or genetic assay for chromosome damage.

One study has evaluated the genotoxic effects of 0 or 0.01 ppm dichlorvos (100% pure, diluted to 50% in acetone emulsifier) in fish kept in aquarium water. The rates of chromosomal aberrations in fish exposed to the water for 24, 48, 72 and 96 hours were significantly higher ($p < 0.05$). This study had limitations: the fish used had been collected from many different areas and were not isogeneic (genetically alike), or even farm raised, and may have already been exposed to different chemicals. The analysis of only one fish for each time point in this study prevented an analysis of the background variability among control specimens (Rishi and Grewal, 1995).

c) Mutagenicity Studies in Insects:
Dichlorvos was not found to induce sex-linked recessive lethal mutations in *Drosophila melanogaster* (Sobels and Todd, 1979).

d) Mutagenicity Studies in Bacteria and Yeast:
The mutagenicity of dichlorvos has been extensively studied and reviewed (IARC, 1991; Moutschen-Dahmen et al., 1984; Wild, 1975). In bacteria and yeast, dichlorvos was found to have a mutagenic effect by many studies (Ashwood-Smith, 1972; Benigni et al., 1980; Kada et al., 1980; Nagy et al., 1975; Shirasu et al., 1976; Voogd et al., 1972). One recent study found no evidence for mutagenicity of dichlorvos in bacteria (Hour et al., 1998).

The DNA-methylating ability of dichlorvos in bacteria and with isolated DNA, has been implicated as the mechanism for its mutagenicity by many authors (Braun et al., 1981; Segerback and Ehrenberg, 1981; Wooder and Wright, 1981; Wright et al., 1979). However, the DNA-alkylating ability of dichlorvos *in vivo* has been found to be very weak (FAO/WHO, 1993; Segerback, 1981). Dichlorvos has also been found to be more mutagenic in strains of DNA polymerase-deficient bacteria, indicating that the DNA repair machinery may play a role in preventing some of the dichlorvos damage (Moutschen-Dahmen et al., 1984; Rosenkranz, 1973). In contrast, another study found dichlorvos to be mutagenic in both excision-repair competent, as well as excision-repair deficient strains of *Escherichia coli* (Nagy et al., 1975).

Dichlorvos, at 0.1% volume per volume portion (v/v) in water was observed to increase the mutation rates in four different bacterial strains (Voogd et al., 1972). There was also a dose-dependent effect in *Salmonella typhimurium,* the strain that was tested with multiple doses in this study. In another study, dichlorvos, dispersed in taurocholate micelles, was observed to be more genotoxic in a modified SOS assay than when dissolved in 10% dimethyl sulphonate (DMSO) (Venkat et al., 1995). Dichlorvos was mixed with sodium taurocholate to form water-soluble micelles, to better mimic its physiological state (taurocholate is an end product of cholesterol metabolism found in bile salts). The design of the colorimetric assay had the b-galactosidase gene under the control of the SOS DNA repair gene, *SulA*, which allowed the detection of any mutation that triggered the DNA repair system. Dichlorvos, at concentrations close to 1 mM, induced prophage excision in another assay of mutagenicity (Houk and DeMarini, 1987). Dichlorvos was found to be mutagenic in one of the two strains of *Salmonella* that were tested in one study, providing equivocal evidence on its mutagenic potential (Zeiger, 1987).

The mutagenicity of dichlorvos was recently compared by two different assays: the Ames test, and a newly developed lactam test, designed to detect revertant mutations in the b-lactamase gene (Hour et al., 1998). Dichlorvos was not found to be mutagenic by either of the two assays in this study.

e) Mutagenicity Studies in Isolated Animal Cells:
One study, presented as an abstract, has reported that dichlorvos induced micronuclei in isolated human cells (lymphoblastoid) (Doherty et al., 1996). Other studies in animal cells corroborate this result. Chromosome aberrations and sister chromatid exchanges (SCE) were significantly induced ($p < 0.001$) in a study of isolated Chinese hamster ovary (CHO) cells that were cultured in 1% dichlorvos (> 98% pure) (Tezuka et al., 1980). Dichlorvos (98% pure) added to the culture medium, at 0.03 mM and 0.1 mM concentration, caused a significant induction ($p < 0.01$) in SCE of CHO cells (Nishio and Uyeki, 1981). Another study found

dichlorvos at doses of 40, 200 and 1000 μg/ml to significantly induce SCE ($p < 0.005$ for the trend) in CHO cells (Wang et al., 1988). Dichlorvos was found to induce micronuclei and mutation frequencies in a CHO / hypoxanthine-guanine phosphoribosyl transferase assay (HGPRT assay) with or without S9 activation (Oshiro et al., 1991).

Hepatocytes isolated from phenobarbitol-treated rats were incubated with 500, 1000, 1500 or 2000 μM dichlorvos for 90 minutes (Yamano, 1996). Dichlorvos was found to significantly increase cellular lipid peroxidation and single-strand DNA breaks at doses 500 μM and above ($p < 0.05$). The DNA-damaging effects caused by dichlorvos could not be completely blocked by antioxidants, indicating a lipid peroxidation-independent effect. Dichlorvos treatments of rat tracheal epithelial cells with doses 80 μg/ml or higher, caused a dose-dependent and significant induction in SCE ($p < 0.001$), and a dose-dependent induction in transformation rates (Lin et al., 1988).

4. Evidence of Tumor Promotion:
F344 rats that were fed 8 or 16 mg/kg/day dichlorvos, after receiving a leukemia transplant, developed leukemia earlier than rats that received the transplant alone (Dieter et al., 1990; Dieter et al., 1989). These studies indicate that dichlorvos acts as a tumor promoter for leukemia in rats.

A combination of 20 different pesticides, each at 100 times its acceptable daily intake (ADI) level, was administered to F344 rats that had been injected with a single dose of 200 mg/kg diethylnitrosamine (DEN). Glutathione S-transferase P (GST-P) foci were assayed as pre-neoplastic indicators of a carcinogenic effect. The mixture of pesticides including dichlorvos was found to significantly increase the incidence of GST-P positive foci in the liver of DEN-treated rats ($p < 0.05$) (Ito et al., 1995). Since dichlorvos was just one of the chemicals in the tumor promoting mixture, its role as a tumor promoter cannot be determined from this study.

5. Effects on Hepatic Enzymes:
One of the most potent effects of dichlorvos is the inhibition of cholinesterase enzymes in the liver. It has been reported to be neurotoxic as well. These toxic effects are not the kind that have been linked to an increase in cancer risk and hence, have not been included here.

6. Immunological Effects:
A chemical may indirectly affect the risk for cancer by suppressing the ability of the body's defense system to fight cancer cells. There is some evidence that suggests that exposure to dichlorvos may compromise the immune response of exposed animals.

In one study, C57B1/6 mice were immunized with sheep erythrocytes and treated with a single dose of 120 mg/kg dichlorvos (96% pure) in corn oil (Casale et al., 1983). Two days after treatment with dichlorvos, the treated-mice had a significantly lower immunoglobulin M (IgM) response ($p < 0.05$) to the sheep erythrocytes. The authors suggest that this immunotoxic effect may be in part due to the known toxic effect of dichlorvos on serine esterase enzymes. A later study found dichlorvos (98.5% pure) to significantly suppress ($p < 0.05$) interleukin 2-driven proliferation of mouse T cells (CTLL2) at concentrations ranging from 0.5 to 50 μM (Casale et al., 1993). While these results indicate that dichlorvos has the potential to interfere with immune responses, they do not help predict the effect of dichlorvos on the immune response against cancer cells in these animals. Also, immune response was evaluated to acute exposure only.

In a three-generation immunotoxicity assay, rats were treated with 0, 0.927, 1.27 or 1.85 mg/kg/day dichlorvos by gavage for five days per week for 11 weeks and then allowed to mate. Treatment of females was continued until weaning. Dichlorvos exposures did not cause significant immunosuppression in the first generation. However, in the second generation, rats that were treated with the highest dose of dichlorvos had a significantly decreased plaque-forming response (PFC) to immunization with sheep red blood cells ($p < 0.05$) (Institoris et al., 1995). In the third generation, the decrease in PFC response was not significant. The reason for the different sensitivities of different generations to the immune toxic effect of dichlorvos is not clear. It is difficult to determine if the immunotoxic effect observed in this study could lead to a teratogenic effect.

Male rabbits that were treated orally with technical grade dichlorvos (93% pure) for six weeks had significantly suppressed ($p < 0.01$) humoral-and cell-mediated immune responses at doses higher than 1/20 of the LD_{50} for dichlorvos. The immunosuppression was also significant at 1/40 of the LD_{50} for dichlorvos ($p < 0.05$) (Desi et al., 1980).

Dichlorvos (98% pure) > 3 mg/L, had a significant immunosuppressive effect ($p < 0.05$) on carp lymphocyte proliferation and phagocytosis. It was found to completely suppress any lymphocyte proliferation at concentrations of 24.5 mg/L or higher (Dunier et al., 1991).

7. Summary of Other Relevant Data on Breast Cancer Risk:
Dichlorvos has been reported to have a mutagenic action in bacteria, yeast and isolated cells. However, it has been argued that due to its very short half-life, it may not cause irreparable DNA damage in whole animals. Indeed, the evidence for the genotoxicity of dichlorvos from *in vivo* studies is more equivocal; six studies have observed a genotoxic effect of dichlorvos (Crossen et al., 1978; Dzwonkowska and Hubner, 1986; Nehez et al., 1994; Rishi and Grewal, 1995; Schop et al., 1990; Tungul et al., 1991) and five studies have not (Benford et al., 1994; Cunningham et al., 1994; Kligerman et al., 1985; Moutschen-Dahmen et al., 1981; Sobels and Todd, 1979). Dichlorvos has been reported to cause

immunosuppression (Casale et al., 1983; Casale et al., 1993; Desi et al., 1980; Dunier et al., 1991; Institoris et al., 1995) and tumor promotion in experimental animals (Benford et al., 1994; Dieter et al., 1990; Dieter et al., 1989). Dichlorvos does not adversely affect reproduction in mammals. It has not been tested adequately for estrogenic effects.

VI. Other Information:

A. Environmental Fate and Potential for Human Exposure:
Dichlorvos is rapidly absorbed and degraded in all mammalian species (FAO/WHO, 1993). Dichlorvos metabolism after different routes of administration in man, rat, mouse, swine and hamsters has been reported to be rapid, with similar metabolites in all species (Blair et al., 1975; Hutson et al., 1971a; Hutson and Hoadley, 1972a; 1972b; Hutson et al., 1971b; Page et al., 1971; Page et al., 1972). Dichlorvos degradation follows two main pathways. It is hydrolyzed to dimethylphosphate and dichloroacetaldehyde in the plasma and liver. The vinyl-phosphate bond of dichlorvos is rapidly cleaved as the first step in degradation. A smaller fraction of dichlorvos can be converted to desmethyl-dichlorvos by the glutathione-dependent enzymatic system, which breaks down to methylphosphate and dichloroacetaldehyde. Intermediate metabolites that have been identified in tissues soon after exposure are dimethyl phosphate, dichloroacetaldehyde, dichloroethanol, dichloroacetic acid and desmethyl dichlorvos (Page et al., 1972). There is no evidence of accumulation of dichlorvos or its metabolites in the animal blood or tissues (WHO, 1989). Urine and respired air are major routes for elimination in man and other mammals (Hutson and Hoadley, 1972a).

With recent increased restrictions, the potential for dichlorvos exposure for the general population is relatively low. Although dichlorvos degrades rapidly, studies of populations that are occupationally exposed in the past have reported detectable levels of absorption of this insecticide. The potential for occupational exposure to humans, although low, still exists.

1. Occupational Exposure:
An occupational exposure study of 22 pesticide applicators who used three OPs including dichlorvos reported alkyl phosphate metabolites in 96% of the urine samples collected within 24 hours of a work shift (Hayes et al., 1980). A study of 13 human volunteers who worked as pesticide applicators indicated dermal and inhalation exposures, even among applicators who used protective equipment (Das et al., 1983). At the end of a day's work, average dichlorvos residues were 75.85 μg/ft^2 (0.1 ft^2 = m^2) on the back, 40.9 μg/ft^2 on the chest, 7.02 μg/in^2 (6.5 in^2 = cm^2) on the respirator filter. Urine analysis indicated 0.32 μg to 1.39 μg dichlorvos residues in the total urine sample collected on the day of application, but levels approached zero by the next morning, indicating rapid clearance (Das et al., 1983). These results were also observed in a different study that analyzed OP metabolites in the urine samples of six male pest control operators in Japan. Workers exposed primarily to dichlorvos had a sharp decline in urinary levels of alkyl phosphates within a day of interruption of exposure, indicating rapid clearance (Takamiya, 1994). In another study, Mexican American grape harvesters (25 male and 11 female) were monitored for exposure to OPs, including dichlorvos. Cholinesterase inhibition was observed, especially in female workers post-harvest. However, since there was no evidence of dichlorvos residues on grape leaves, the authors suspect that this group was exposed to OPs other than dichlorvos (Kraus et al., 1981).

Air samples from commercial insecticide storage facilities and vehicles used by applicators were analyzed for dichlorvos residues. Storage facilities had much higher level of dichlorvos in air (0.53 ppb) compared to office rooms (0.04 ppb). Vehicles that were used to store compressed air spraying equipment and the insecticide had a mean air levels of 0.94 ppb dichlorvos (Wright and Leidy, 1980).

All these studies indicate that occupational exposure to dichlorvos was frequent and detectable among pesticide applicators. As mentioned earlier, dichlorvos ranked among the three most frequently applied pesticides by pest control operators in 1974 (ATSDR, 1997). However, there are no epidemiological studies available on the long-term health effects of dichlorvos among pesticide applicators.

2. Potential of Exposure for the General Population:
a) Food and Water:
Dichlorvos residues on food are readily destroyed by washing and cooking (IARC, 1991). Due to the short half-life of dichlorvos, the potential for human exposure from food and water is relatively low. No dichlorvos residues were found in Total-Diet Studies carried out by the FDA during 1964 to 1979 (Johnson et al., 1981; WHO, 1989). Total-Diet Studies done in the United Kingdom in 1966-67 did not report dichlorvos residues either (Abbott et al., 1970). Analysis of food by the United States Department of Agriculture (USDA) after dichlorvos vapor treatments of food warehouse chambers indicated levels of dichlorvos less than or equal to 0.1 ppm in flour and in packaged foods (Gillenwater et al., 1971; Harein et al., 1971). In Canada, only one out of 262 bovine and porcine samples analyzed between 1973 and 1981 was contaminated with dichlorvos residues (IARC, 1991). A study in Japan on effects of food processing, reports that 40% to 80% of all dichlorvos residues on soybeans can be removed with one or two washes, respectively, and 95% of all dichlorvos is destroyed by cooking for 2.5 minutes (Miyahara and Saito, 1994).

b) Air:
Air residues of dichlorvos have been reported as the major route of exposure to humans in some studies. The Non-Occupational Pesticide Exposure Study (NOPES) sponsored by the EPA was

designed to monitor seasonal fluctuations and relative pesticide exposure to populations in Jacksonville, Florida (high agricultural pesticide use) and Springfield/Chicopee, Massachusetts (low, but not negligible pesticide use). Air samples were collected in spring, summer and winter, between 1986 to 1988. Levels in Jacksonville were higher as expected, and those within the city were highest during the summer. Dichlorvos was one of the top seven pesticides found in high concentrations in indoor air in Jacksonville. It was estimated that 33% of the studied population in Jacksonville had detectable levels of dichlorvos in indoor air during summer, compared to 2% of the population in Springfield/Chicopee. The mean exposure to dichlorvos from air for residents of Jacksonville was estimated at 1,248 ng/day, compared to 66 ng/day estimated for residents of Springfield/Chicopee, Massachusetts (Whitmore et al., 1994). There were no reports indicating dietary exposure to dichlorvos, based on the data obtained from questionnaires on dietary intakes, market basket surveys conducted by the USDA, and the total diet studies conducted by the FDA between 1982 and 1987. The authors of this study concluded that air was the major route of exposure to humans in both cities (Whitmore et al., 1994).

In another study, air samples from seven homes in New Jersey were analyzed for dichlorvos. Six households did not have detectable residues, but one household had a mean concentration of 254.7 ng/m^3 dichlorvos, with a peak level of 354.7 ng/m^3 immediately after application. The residues reduced to non-detectable levels eight weeks after treatment (Roinestad et al., 1993).

A study of deposits on dichlorvos-treated domestic structures found 219 μg/ft^2 dichlorvos deposited on the first day. There was some persistence of the insecticide even after the fifth day with detectable levels of 50.9 μg/ft^2 (Das et al., 1983). Another study analyzed the dissipation of dichlorvos after use of home foggers deployed in accordance with the manufacturer's directions. Air levels of dichlorvos fell to 1 mg/m^3 within 60 minutes. The authors point out that while this level may be acceptable for a TWA exposure of eight hours at the workplace, it may not be the safe level for prolonged non-occupational exposure for someone confined at home. According to this study, safe re-entry levels of dichlorvos for prolonged exposure (considered as 0.06 μg/cm^2) are not reached until 10 hours or more after application (Goh et al., 1987).

Perimeter outdoor applications of dichlorvos around domestic dwellings were reported (presented only in an abstract) to cause low residues of this insecticide in indoor air, but details of this study were not available (Leidy and Stout, 1996). Shell Development Company conducted a study of air and food residues in 15 homes in Modesto, California, following the installation of Vapona® insecticide strips containing dichlorvos. This study reported low residue averages of 0.06 μg/L in air after one day of installation (Collins and DeVries, 1973). A total of 174 meals were collected from these treated homes and analyzed for residues. Either one or two strips were used in the kitchen or dining areas to treat these homes. Twenty four of the meals were found to contain 0.02 ppm, seven had 0.03 ppm, and five had 0.04 ppm of Vapona® (dichlorvos) residues (Collins and DeVries, 1973). These results indicate that low levels of food contamination can result from dichlorvos residues in indoor air.

One study has estimated the "worst case dose absorbed" from dermal and oral exposure combined following indoor application (presented only as an abstract). Exposure after 24 hours was estimated to reach levels of dichlorvos that are known to produce cholinesterase inhibition (Berteau and Mengle, 1986). Details of the study design were not available.

c) Soil:

The half-life of dichlorvos has been estimated to be 16 days in both sandy and silty clay soils (Sattar, 1990). A study that examined the rates of dissipation of trichlorfon insecticide applied to turfgrass reported rapid conversion of trichlorfon to dichlorvos, followed by a decline in dislodgeable residues of dichlorvos to non-detectable levels within five days (Murphy et al., 1996). Safe re-entry time was estimated following lawn treatments with dichlorvos in another study to range between 7 to 12 hours (Goh et al., 1986).

3. Storage and Excretion of Dichlorvos in Humans:

The clinical pharmacology of dichlorvos has been tested directly in human volunteers in many studies (Blair et al., 1975; Hutson and Hoadley, 1972a; Slomka and Hine, 1981). The effect of this insecticide on blood enzymes was tested again in human volunteers who were given small doses of dichlorvos recently, drawing criticism from media and environmental groups (Cushman, 1998).

Dichlorvos is rapidly broken down in human blood, with a half-life of 7 to 11 minutes (Blair et al., 1975). A human male volunteer was monitored after receiving an oral dose of [^{14}C]-dichlorvos. About 27% of the radioactivity was excreted within the first 8 hours in the CO_2 exhaled from the lungs (Hutson and Hoadley, 1972a). Only 7.6% of the total dose was found to be excreted in the urine in the 24 hours after ingestion. After 4 days, measurable amounts of radioactivity could still be found in the urine, but by the ninth day, the levels had dropped to 0.002% of the dosed radioactivity.

A comparative analysis of excretion patterns in humans, hamsters, rats and mice after oral ingestion suggests very similar metabolic fate for dichlorvos in these species (Hutson and Hoadley, 1972a). A recent study evaluated dichlorvos levels in blood of Alzheimer's patients who were receiving metrifonate therapy for inhibition of acetylcholinesterase. Metrifonate was non-enzymatically converted to dichlorvos and rapidly degraded and cleared from the body of these patients, with an estimated half-life of 1.90 to 3.12 hours (Pettigrew et al., 1998).

a) Lactation and Breast Milk:
Breast milk of women in a hospital maternity ward that used Vapona® strips did not inhibit cholinesterase enzymes *in vitro.* An analysis of the enzymes in the blood of breast-fed infants of these exposed mothers also indicated a lack of transfer of dichlorvos through lactation (Cavagna et al., 1970). However, one dichlorvos contaminated (0.1 ppm) breast milk sample was reported by a study conducted in Taiwan [Yeh et al., as cited in (Sonawane, 1995)].

b) Adipose Tissues:
Studies of tissue distribution of dichlorvos in animals indicates rapid clearance and no significant storage of this pesticide in body tissues (Blair et al., 1975; Hutson et al., 1971a; 1971b; Hutson and Hoadley, 1972a; 1972b). One study estimated the half-life of dichlorvos in adipose tissue of animals to be 10.7 days (Garcia-Repetto et al., 1995). An analysis of bovine carcasses sold in Ontario, Canada (1969 to 1981) reported negligible amount (usually 0.1 mg/kg) of dichlorvos in the fat tissue (Frank et al., 1983).

c) Tissue Distribution:
An autopsy done one day after a fatal ingestion of dichlorvos in xylene revealed congestion in lung and kidneys. Disregarding the high levels in heart and spleen which could be due to diffusion from the stomach, the insecticide was most concentrated in the lung and kidneys, with relatively low concentrations in the blood, liver and brain tissues (Shimuzu et al., 1996). Dichlorvos was found mostly in the kidneys of male rats and mice after inhalation or intravenous injection (Blair et al., 1975). Half-life times analyzed *in vitro* ranged from 31 minutes in blood of male rats to 1.7 minutes in rabbit blood (Blair et al., 1975). Studies on different mammalian species have not revealed significant accumulation of dichlorvos or its metabolites in animal tissues (Blair et al., 1975; Hutson et al., 1971a; 1971b; Hutson and Hoadley, 1972a; Page et al., 1972). Dichlorvos was not detected in the brain of rats treated by gavage. Dichlorvos is not expected to cross the blood-brain barrier (Garcia-Repetto et al., 1995).

VII. Summary and Recommendation for Breast Cancer Risk Classification:

A. Breast Cancer Risk:
We propose that dichlorvos be classified in Group 3, *not classifiable as to its breast carcinogenicity in humans* (please see Appendix B for an explanation of the BCERF Breast Cancer Risk Classification Scheme). While there is sufficient evidence for its overall carcinogenicity, there is a lack of evidence of breast carcinogenicity in humans, limited evidence of mammary carcinogenicity in experimental animals and limited mechanistic evidence of its potential to affect breast cancer risk. The evidence is summarized below:

- Human studies: There were no studies available to evaluate the breast carcinogenic effect of dichlorvos in humans.

- Animal studies: There is *limited evidence* on mammary gland carcinogenicity of dichlorvos from studies in experimental animals. Most studies done in experimental animals are invalid as cancer bioassays by contemporary standards. One study that used sufficient exposure time and large number of animals (Chan et al., 1991), reported an increase in incidence of mammary gland fibroadenomas/adenomas (benign) in one of the two groups of female Fischer F344 rats that were fed dichlorvos. These results need to be confirmed in a cancer bioassay in rats that uses at least three treatment doses of dichlorvos and has better survival rates. Such a study would allow an evaluation of whether dichlorvos causes a dose-dependent increase in the incidence of benign mammary tumors in rats, and if malignant tumors develop because of higher survival rates of exposed animals.

- Related mechanisms: There is *limited evidence* for an effect of dichlorvos on cancer risk through other related mechanisms. Dichlorvos has been found by most investigators to be mutagenic in single-cell organisms and in isolated cells (IARC, 1991). Evidence for its genotoxic potential *in vivo* is equivocal, which may be due to its very short half-life in animal blood (Blair et al., 1975). There is limited evidence that dichlorvos may act as a tumor promoter in rats, causing an increase in incidence of leukemia (Dieter et al., 1990; Dieter et al., 1989), and in mice, by causing increased hyperplasia in the stomach (Benford et al., 1994). There is evidence that dichlorvos causes immunosuppression in mammals (Casale et al., 1983; Casale et al., 1993; Desi et al., 1980; Dunier et al., 1991; Institoris et al., 1995). It is not known however, if the dichlorvos-caused immunosuppression in animals affects cancer risk. While dichlorvos could be affecting risk for all cancer through these mechanisms, these have not been tested directly for their effect on breast cancer risk.

Dichlorvos use has now been restricted. It is rapidly degraded in air, soil and water. It does not persist in the environment or in body tissues, and is not found as a contaminant in drinking water or food. Thus, the potential of the general population for exposure to dichlorvos is low. However, there is still a risk for occupational exposure to dichlorvos for those involved in the manufacture of dichlorvos and other OPs; applicators that use foggers; and veterinarians and people who work with livestock. Dichlorvos was a very popular insecticide in the past and studies have shown that pesticide applicators were at high risk for exposure to this insecticide. As a non-specific carcinogen dichlorvos could also be affecting breast cancer risk. However, currently there is no evidence to support or refute this conclusion due to the lack of available studies on cancer incidences among women pesticide applicators

or those who had a high risk of exposure to dichlorvos. Hence, we recommend further studies below on populations exposed to dichlorvos through their work.

VIII. Identification of Research Gaps, and Other Recommendations

There were no studies available to evaluate the incidence of breast cancer among women who were exposed to this insecticide through its manufacture or its application in agriculture and other industries. Women pesticide applicators exposed to dichlorvos in the past need to be followed for the incidence of breast cancer.

- The increase in benign tumors in the mammary gland of rats treated with dichlorvos (Chan et al., 1991) needs verification. Another cancer bioassay in rats, using at least three dose levels and with high survival rates, is needed to confirm if dichlorvos causes mammary gland neoplasms. These dichlorvos-treated rats should also be evaluated for incidence of leukemia, pancreatic tumors and tumors of the forestomach.
- Small case-control studies have indicated a higher risk for leukemia among farmers who used dichlorvos most frequently. Larger case-control studies are needed for incidence of leukemia among men and women who used this insecticide for dairy, poultry or other livestock.
- One small case-control study has observed an increased incidence of childhood brain cancers in association with dichlorvos exposure. However, the very small numbers and lack of details on exposure in this study do not permit a meaningful conclusion regarding the carcinogenicity of dichlorvos. Larger case-controls studies of populations exposed to dichlorvos as children are needed to evaluate the risk of childhood brain cancer in association with home use of dichlorvos.
- There is some evidence that dichlorvos may promote the incidence of leukemia in rats treated with leukemic spleen cell transplants. Similar studies should be conducted with transplanted mammary tumor cells to determine if dichlorvos can promote the incidence of mammary tumors.
- Studies in experimental animals indicate that dichlorvos causes immune suppression. Experimental animals should be tested with mammary tumor cells to determine if more tumors are established in dichlorvos-treated animals as a result of their immune suppression.
- The estrogenicity of dichlorvos needs to be studied in *in vitro* assays.

IX. Summary of New Human Studies Currently Being Conducted

Studies of Occupational Cancer—Pesticides: (Alavanja, M., Blair, A., Zahm, S., extracted from the CancerNet at NCI and Personal Communication)

The "Agricultural Health Study" proposes to look at the relationship between exposures to agricultural chemicals, including pesticides, 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.

Study of Agricultural Exposures and Cancer Risk (Ward, M., Heineman, E., Zahm, S. and Blair, A., Personal Communication)

Also ongoing at the NCI, are case-control studies of brain and stomach cancer in Nebraska, designed to evaluate the risk of cancer from agricultural exposures, including pesticides.

Organophosphate Immunoactivity (Albrecht, R.M., University of Wisconsin, Madison extracted from the CRISP Database).

An ongoing study seeks to identify alterations in immune cells that are caused by organophosphate chemicals. Immune responses, gene expression and cell differentiation of monocyte/macrophage precursor cells will be followed in the presence and absence of triphenyl phosphate. Murine and human primary cell cultures and cell lines will be tested.

Occupational Injury in Hispanic Farmworker Families (Principal Investigator: McCurdy, S.A., University of California, Davis, extracted from the CRISP Database).

Migrant and seasonal workers in California will be evaluated for occupational injury in association with organophosphate exposure, piece-work versus hourly pay, language appropriate safety training, and the role of multiple employment. The cohort is expected to consist of 500 farmworker families who live in six Migrant Housing Centers close to Davis, California.

Role of Dichlorvos in Gulf War Illnesses (A Presidential Committee on Gulf War Veteran's Illnesses, extracted from http://www.gwvi.gov/ch4.html)

Dichlorvos is one of the OP documented as being shipped for use during the Gulf War. A Presidential Committee on Gulf War

Veteran's Illnesses has reported on several risk factors in veterans of this war, including exposure to OPs including dichlorvos. This committee was terminated in November, 1997. However, the Center for Disease Control and Prevention (CDC), the National Institutes of Health (NIH) and the Agency for Toxic Substances and Disease Registry (ATSDR) have co-sponsored a conference with the aim of developing a research plan to investigate any relationship between chemical exposures and illnesses among Gulf war veterans (Dr. T.D. Spittler, Personal Communication).

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XI. Appendix A. Common Abbreviations, Acronyms and Symbols

ADI	acceptable daily intake
AFB_1	aflatoxin B_1
BCERF	Program on Breast Cancer and Environmental Risk Factors in New York State, based the Cornell's Center for the Environment, Institute of Comparative and Environmental Toxicology
CAS	Chemical Abstract Service
CFE rats	Carworth Farm E strain rats
CfE	Cornell University's Center for the Environment
CHO	Chinese hamster ovary
CI	Confidence Interval
Cl	chlorine
CRISP	Computer Retrieval of Information on Scientific Projects; database of scientific intra and extra mural projects supported by the Dept. of Health and Human Services (i.e., NIH, EPA, USDA)
DDVP	dichlorovinyl dimethylphosphate
DEN	diethylnitrosamine
DMSO	dimethyl sulphonate
DNA	deoxyribonucleic acid
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
FFDCA	Federal Food, Drug and Cosmetic Act
ft	foot
GST-P	glutathione-S-transferase positive
h	hour
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
HGPRT	hypoxanthine-guanine phosphoribosyl transferase assay
IARC	International Agency for Research on Cancer, headquartered in Lyon, France
ICET	Institute for Comparative and Environmental Toxicology
IgM	immunoglobulin M
in	inch
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
lb	pound
LD_{50}	lethal dose for 50% kill
m	meter
µg	microgram
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
MTD	maximum tolerated dose
n	number of subjects/animals in the group
NCI	National Cancer Institute

ng	nanogram
NHL	non-Hodgkin's lymphoma
NIOSH	National Institute of Occupational Safety and Health
NIEHS	National Institute of Environmental Health and Safety
NIH	National Institutes of Health
NTIS	National Technical Information Service; repository for federal agency technical reports
NTP	National Toxicology Program
NY	New York
NYS	New York State
OP	organophosphate pesticide
OR	Odds Ratio
OSHA	Occupational Safety and Health Administration
PFC	plaque forming cells
ppb	parts per billion
ppm	parts per million
RDS	replicative DNA synthesis
RR	Relative Risk
SCE	sister chromatid exchange
SMR	standardized mortality ratio, the ratio of deaths among a cohort, to the expected number of deaths, multiplied by 100
SSB	single stranded breaks
TWA	Time-weighted average
UDS	unscheduled DNA synthesis
U.S.	United States
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

Symbols:

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

XII Appendix B. Critical Evaluations of Breast Cancer Risk

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

The Process

Starting Point - Existing Critical Evaluations on Evidence of Carcinogenicity
- IARC Monographs (**I**nternational **A**gency for **R**esearch on **C**ancer)
- NTP ARC (**N**ational **T**oxicology **P**rogram, **A**nnual **R**eport on **C**arcinogens)
- 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 are critically evaluated for evidence of exposure to agent and breast cancer risk based on "strength of evidence" approach, according to a modification of IARC criteria as listed in the IARC Preamble. (See below for a more detailed explanation of the BCERF Breast Cancer Risk Classification scheme)

The **emphasis of the document** is the 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-carcino genicity, 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 Cancer Risk

BCERF Breast Cancer Risk Classification Scheme & Brief Definitions of Sufficient, Limited and Inadequate Evidence:
(adapted for breast carcinogenicity 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.

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.