Critical Evaluation of Diazinon’s Breast Cancer Risk

by

Renu Gandhi, Ph.D. and Suzanne M. Snedeker, Ph.D.**

*The institutional home of BCERF is the Institute for Comparative and Environmental Toxicology (ICET) in the Cornell Center for the Environment

** Address correspondence to:
Dr. Suzanne M. Snedeker
110 Rice Hall
Cornell University
Ithaca, NY 14853
Phone (607) 254-2893
Fax: (607) 255-8207
E-mail: sms31@cornell.edu

Supported by grants from:
New York State Dept. of Health
USDA-Regional NYC174423

This report is posted on the BCERF web-page at: <http://www.cfe.cornell.edu/bcerf/>. Permission may be requested to reproduce the final report, without alteration of text or tables, as long as credit is given to the authors, BCERF and Cornell University.
List of Tables and Figures

Figure 1. Chemical Structure of Diazinon............................................................................................ 1
Critical Evaluation of Diazinon’s Breast Cancer Risk

**Authors’ 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.

**Introduction:**
Diazinon was selected to be evaluated based on its high use in the fruit and dairy industries, the two major agricultural industries of New York State (NASS, 1995; Patridge et al., 1991). We also considered its use in and around homes and public places and the high potential for non-occupational exposure. Non-agricultural and urban use of diazinon has been increasing and can account for as much as two-thirds of its total use in the United States (US) (Larson et al., 1995). Diazinon is used to control a wide variety of insect pests in and around homes, offices, fair grounds, zoos and other public places (ATSDR, 1996). The many case-reports in the literature present evidence for the high potential of accidental and non-occupational exposure to this insecticide (Maddy and Edmiston, 1988; WHO, 1998).

The increasing urban use of diazinon has led to its frequent detection as a water contaminant in urban watersheds of New York State (NYS) (Wall et al., 1998). Diazinon is one of the chemicals that is on the Environmental Protection Agency’s (EPA) Drinking Water Contaminant Candidate List (EPA, 1998). There has been no cancer risk classification for diazinon by EPA, the National Toxicology Program (ntp) or the International Agency for Research on Cancer (IARC) (ATSDR, 1996).

**I. Chemical Information**

**A. Common Names:** diazinon, dimpylate (Worthing, 1991).

**B. Chemical Name:** O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate (Worthing, 1991).

**C. Chemical Formula:** C\textsubscript{12}H\textsubscript{21}N\textsubscript{2}O\textsubscript{3}PS (Montgomery, 1993).

**D. Chemical Structure:**

![Chemical Structure of Diazinon](image)

**E. Formulators’ and Basic Producer Trade Names**: Acinon® (Agro Chemicals Industries Ltd.); Diazichem® (Agrochemical Industries Co. Ltd.); Basudin®, D.Z.N.®, Neocidol®, Sarolex® (Novartis); Drexel® Diazinon (Drexel Chemical Co.); Knoxout® (W.A. Cleary Chemical Corp.); Knox Out® 2FM (Elf Atochem North America, Inc.); Diagran® (Agroquimicos Versa, S.A. de C.V.); Diazol® (AGRO-SAN Kimya Sanayi ve Ticaret A.S.); Diazinomobeed® (Arab Pesticides Industries Co. - Mobeed); Vitanon® (Asiatic Agricultural Industries Pte. Ltd.); Hezudin® (Hectas Ticaret T.A.S.); Danol® (Insecticidas Internacionales, C.A.); Biazin® (Koruma Tarim A.S.); Laidan® (Lainco S.A.); Dizalux® (Luxan B.V.); Diazain® Newsinon® (Medmac); Diazolin® (Midiltipi Agro-Chemicals, Inc.); Woprozinol® (B.V. Industrie- & Handelsanderndeming Simonis); Vibasu® (Vietnam Pesticide Co.) Diagron®, Dianon®, DiaTerr-Fos®, Diazajet®, Diazatol®, Diazide®, Dizinon®, Dyzol®, Gardentox®, Kayazinon®, Kayazol®, Nipsan®, Spectracide® (Meister, 1998).

**F. Trade Mixes:** Diafos® (+chlorpyrifos) (Chimac-Agriphar S. A.); Scorpoton® (+ chlorpyrifos) (Helb USA, Inc.; KickStart® (+ carboxin + Lindane) (Helena Chemical Co.); Corsario® (+ cypermethrin) (Insecticidas Internacionales, C.A.); Dizalin® (+ lindane); (Luxan, B.V.); Ethiometon® 4 (+ thiemetom) (Novartis); Captain Diazinon Seed Treater® (+ Captain); Germate Plus® (+ carboxin + lindane), Kernel Guard® (+ Captain + Lindane) (Trace Chemical Inc.); Vibapa® (+ BPMC) (Vietnam Pesticide Co.); Agrox® 2-Way (+ captain), Agrox® D-L Plus (+ captain + lindane) Agrox® Premiere (+ captain + lindane + metalaxyl) (Wilbur-Ellis Co., Seed & Grain Protectant Products) (Meister, 1998).

**G. Discontinued Names:** Adizon® (Atabay Agrochemicals & Veterinary Products, Inc.); Agrox® 3-Way (+ captan + lindane) (Chipman Chemicals); Alfa-tox® (+ methoxychlor) (Ciba-Geigy); D 264® (Drexel Chemical Co.); Bean Seed Protective® (+ captan + streptomycin) (Hopkins Agricultural Chemical Co.); Dymet® (+ methoxychlor) (Sierra Crop Protection Co.); Drawzion® (Wacker-Chemie GmbH); PT® 265 (Whitmire Research Laboratories); Dazzle®, Fezudin® (Zuelling Pte.) (Meister, 1998).

**H. CAS Registry Number:** 333-41-5 (Montgomery, 1993).

**I. Major Metabolites:**
Diazinon treatments of experimental animals have shown that diazinon or its metabolites do not accumulate in body tissues. Excretion is rapid and occurs mainly through the urine. Diethyl phosphate (DEP) and diethylthiophosphate (DETP) were found excreted as end products in the urine of diazinon-treated cows, dogs and rats (FAO/WHO, 1993; Hayes et al., 1980). DEP and
DETP are also the major metabolites found in the urine of diazinon-exposed humans (Hayes et al., 1980; Richter et al., 1992).

In rats, the major metabolites identified were three pyrimidinols, 2-isopropyl-6-methyl-4(1H)-pyrimidinone, 2-(alpha-hydroxyisopropyl)-6-methyl-4(1H)-pyrimidinone and its beta isomer (FAO/WHO, 1993). Diazoxon, a toxic but transient intermediate was found in trace amounts in the urine of diazinon-treated rats. Some other polar metabolites, found in small amounts in the urine have not been identified (FAO/WHO, 1993).

In vitro studies on biotransformation of diazinon by liver microsomes of various species have identified hydroxydiazinon, isohydroxydiazinon, dehydroxydiazinon, their oxons and diazoxon as transient intermediates (FAO/WHO, 1993).

J. Mode of Action:
Diazinon, like other organophosphate pesticides, disrupts nerve transmission in insects by inhibiting the acetylcholinesterase enzyme (ATSDR, 1996).

II. History of Use and Usage

A. History of Use and Nomenclature:
Diazinon is a synthetic insecticide and a member of the family of organophosphate pesticides (OP). It was first registered for use as an insecticide in the US in 1956 (ATSDR, 1996). Ciba-Geigy Corporation produced O,O-dimethyl-O-(2-[1-methylethyl]-4-methyl-6-pyrimidinyl) phosphorothioate under the trademark name Diazinon® until 1994 (ATSDR, 1996). Diazinon has contact insecticidal activity against a wide variety of adult and juvenile forms of flying insects such as flies, fly maggots, mosquitoes, beetles; crawling insects such as cockroaches, bedbugs, lice, ants; and ticks, fleas and spiders (WHO, 1998). Some common agricultural and non-agricultural uses of diazinon have been described below.

1. Agricultural Use and Usage:
Diazinon has been used in agriculture as a nematicide and insecticide against soil insects and pests of fruits, vegetables, tobacco, forage, field crops, rangelands and pasture. It is also used to keep greenhouses and mushroom houses free of flies. It is a non-systemic insecticide. It is most often used on fruit trees, horticulture crops, corn, potatoes, rice, sugarcane, tobacco and in vineyards (Meister, 1998). In NYS, it is used for the production of apples, peaches and pears (NASS, 1995). Diazinon sprays, dips, powders and ear tags may be used to control ticks and fleas on animals and in livestock facilities (ATSDR, 1996).

An estimated 1.2 million lbs of active ingredient (AI) of diazinon was used per year for agricultural purposes in the US during 1990-1993 (Gianessi and Anderson, 1995a). Diazinon ranked as the 24th most used insecticide nationwide. In NYS, an estimated 16 thousand lbs AI of diazinon was used per year for agriculture during the same time period, ranking it as the 21st most used insecticide (Gianessi and Anderson, 1995b). In 1982, it was estimated that 47% of diazinon used in the US was for agricultural purposes. Diazinon used on field crops accounted for 21% of its use, with 12% on alfalfa, 5% on corn, 5% on soybeans, 5% on vegetables, 3% on fruit and nut trees, 2% on wheat, 2% on cotton and 2% on sorghum (ATSDR, 1996).

2. Non-Cropland Use and Usage:
Diazinon was used to protect golf courses from infestations of many soil borne arthropods and nematodes (Frank et al., 1991; Meister, 1998). Its use on golf courses and sod farms was discontinued in 1986 following a concern about its toxicity to birds and aquatic life.

Diazinon is available for use in homes to control flies, fleas, ants, silverfish, spiders, cockroaches and other household insects (WHO, 1998; Meister, 1998). It may be used in liquid, dust and granular forms (WHO, 1998). Diazinon may be used in the form of pest strips or sprays in indoor areas and offices. Besides home and garden uses, diazinon may be used in sprays, dips, or pet collars in veterinary applications (ATSDR, 1996). Diazinon is used to control flies around areas where food or animal waste may collect, such as fair grounds, zoos, animal facilities and garbage collection centers.

Non-agricultural use accounted for 43% of the total amount of diazinon applied in the US in 1982 (5.8 million lbs) (ATSDR, 1996). Home and garden use was estimated to be two to four million lbs AI diazinon annually in 1994 to 1995 (Aspelin, 1997). Another three to four million lbs AI diazinon was used annually by industrial and commercial applicators during this time. By one estimate, non-agricultural and urban use of diazinon can account for as much as two-thirds of its total use in the US ( Larson et al., 1995).

III. Current Regulatory Status

A. Regulatory Status:
Diazinon was determined to be an avian hazard and its use on golf courses and sod farms was cancelled in 1986 (USEPA, 1996a).

B. Clean Water Act Requirements:
Diazinon has been designated as a hazardous substance. Discharges of more than one lb of diazinon are required to be reported under the Clean Water Act (ATSDR, 1996). There has been no maximum contaminant level (MCL) set for its presence in public drinking water supplies. Health advisories (HA) have been set as follows:

Health Advisory:
10 kg child
• One day = 0.02 mg/L
• Ten day = 0.02 mg/L
• Lifetime = 0.0006 mg/L

70 kg adult
• Long term = 0.02 mg/L
• Lifetime = 0.0006 mg/L
HAs are non-enforceable limits of the concentration of the chemical in the drinking water that is not expected to cause any adverse non-carcinogenic health effects when consumed for no more than the time period specified, with a margin of safety (USEPA, 1996b). Health advisories for non-carcinogenic toxicants are derived from the No Observed Adverse Effect Level (NOAEL). The NOAEL of 0.02 mg/kg/day of diazinon was determined in a study of human volunteers who received 0.025 mg/kg/day diazinon for 34 to 36 days and showed no plasma and erythrocyte cholinesterase inhibition or clinical effects (FAO/WHO, 1993). Thus, for a 10 kg child, the health advisories at 0.02 mg/L confer a ten-fold safety factor over the NOAEL.

C. Workplace Regulations:
The Occupational Safety and Health Commission (OSHA) has set the maximum allowable level in workplace air at 0.1 mg/m³ for eight hours per day and 40 hours per workweek (ATSDR, 1996).

D. Food Tolerances:
EPA sets tolerances or the maximum amount of pesticide permitted to occur on the edible portion of raw agricultural commodities and in processed foods. Some of the residue tolerances for diazinon are: 0.1 parts per million (ppm) for potatoes and soybeans; 0.5 ppm for apples; 0.7 ppm for peaches and corn; and 0.75 ppm for grapes, melons and peanuts (USEPA, 1998). OPs are undergoing a risk assessment review under the 1996 Food Quality Protection Act and some of these tolerances may be modified in the future.

IV. Summary of Evidence of Overall Carcinogenicity (Non-Breast Sites)

A. Human Studies:
1. Case Reports:
While there are numerous reports of diazinon poisonings and health effects, none have reported cancer incidences. Acute reversible pancreatitis has been observed following severe cholinergic syndrome in some cases of diazinon poisoning (WHO, 1998). The clinical effects due to acute diazinon exposure found in the case reports were not indicative of carcinogenicity and have not been included here.

2. Population-Based Case-Control Studies:
The only epidemiological studies done on cancer incidences in diazinon-exposed populations have been case-control studies among agricultural workers and one case-control study on childhood brain cancer. In most of these studies the small numbers of subjects did not allow for an evaluation for the effect of diazinon after adjusting for other confounding exposures.

Cases of non-Hodgkin’s lymphoma (NHL) among white men (n = 987) and matched controls from three case-control studies in Iowa/Minnesota, Nebraska and Kansas (n = 2,895) were pooled together to evaluate the risk for NHL in association with use of different agricultural chemicals. The controls were matched for age, marital status, smoking history and state of residence. The risk for NHL for lindane use was reduced from an Odds Ratio (OR) of 1.5 [95% confidence interval (CI) 1.1 to 2.0] to 1.3 (95% CI 0.9 to 1.9) after an adjustment was made for diazinon use (Blair et al., 1998). This result suggests that diazinon may have contributed to the increased risk for NHL observed from lindane use. The OR for risk of NHL in association with diazinon exposures should be evaluated in this pooled population.

A subset of the population described above had been previously reported to have an increased risk for NHL from use of diazinon (Cantor et al., 1992). Cases of NHL (n = 622) among white male farmers from Iowa and Minnesota were compared to 1,245 population-based controls matched for age, vital status and state of residence. The OR for NHL was increased (OR = 1.5; 95% CI 0.9 to 2.5) in the group of 27 cases and 39 controls who had ever handled diazinon. The increased risk for NHL was significant in the group of 14 cases and 12 controls who had used diazinon before 1965, 15 to 18 years before diagnosis (OR = 2.6; 95% CI 1.2 to 5.9) (Cantor et al., 1992). The numbers of cases exposed to diazinon in this subset was small.

In another case-control study of 184 women diagnosed with NHL and 707 controls from agricultural workers in eastern Nebraska, the risk of NHL was significantly increased (OR = 4.5; 95% CI 1.1 to 17.9, p < 0.05) in six cases and five controls who had personally handled OP (Zahm et al., 1993). However, the increase in risk was not statistically significant for the four subjects who reported using diazinon (OR = 4.1; 95% CI 0.4 to 43.2). This study is one of the few that has evaluated occupational exposures to women agricultural workers. However, only a few women had handled diazinon in the study, limiting the statistical power of the study, and the potential for exposure to other pesticides was high.

A study of 578 white men with leukemia and 1,245 population-based controls was conducted in Iowa and Minnesota (Brown et al., 1990). An increased risk for leukemia was observed in association with the use of OP on animals (OR = 1.5; 95% CI 1.0 to 2.1). The small increase in risk for leukemia for mixing, handling or applying diazinon was not statistically significant (OR = 1.2, 95% CI 0.6 to 2.1). The risk for leukemia for less frequent users of diazinon on crops was higher (one to four days per year, OR = 2.1) than the risk for more frequent users (five to nine days per year, OR = 0.5). The results were not conclusive because of the small number of cases that had reported having used diazinon.

A population-based, multi-center case-control study was conducted to determine the association between pesticide exposure and risk of multiple myeloma in 698 cases (men and women) from Detroit (MI), Utah, Washington State and Atlanta (Morris et al., 1986). Cases were identified using the cancer registries serving the four areas. Controls (n = 1,683) were from the same geographical areas, selected at random by random-digit telephone dialing in three states, or by random selection of households from within two counties in Washington. The cases and controls were given a questionnaire with a list of toxic and other chemicals and asked to recall past exposures. For individuals who had died or were too ill, the questionnaire was filled out by a relative. There was a significantly increased risk for multiple myeloma among those who acknowledged being exposed to pesticides (OR = 2.9; CI 1.5 to 5.5). The same percentage of cases and controls (0.3%) recalled being exposed to OP. Thus, the increased risk for multiple
myeloma was not associated with exposure to OP, including diazinon. Only a small number of cases and subjects in this study recalled exposure to OP. Proxy responses were used more often for cases (38%) than controls (1%), creating the potential for recall bias.

The risk for childhood brain cancer was evaluated in a case-control study of children exposed to diazinon through family use of the insecticide in the home, gardens or orchards (Davis et al., 1993). Childhood brain cancer cases (n = 45, all white children, male and female) were identified through the Missouri Cancer Registry. The two groups of controls were cancer-free friends of the cases (n = 85), or other childhood cancers cases (n = 108). Family use of diazinon in the garden or orchards was found to be associated with a significant increase in childhood brain cancer (OR = 4.6; 95% CI 1.2 to 17.9) in comparison with the cancer-free control group. The increase was not significant if the control group of other cancers was used for comparison (OR = 1.4; 95% CI 0.4 to 4.7). The small number of cases (n = 7) and controls (n = 17) that were exposed to diazinon and the lack of data on level or duration of exposure and the potential for recall bias were limiting factors of this study. However, the results point out the need for future studies of cancer incidences in populations exposed to diazinon, especially children, through its use in homes, gardens and orchards.

3. Summary:
All the epidemiological studies described above have evaluated the risk of cancer in populations that were exposed to many different chemicals including diazinon. The small numbers of cases in most of these studies do not allow for an evaluation of the cancer risk that can be attributed to diazinon exposures specifically. The risk for NHL has been observed to be significantly increased in male agricultural workers who had handled diazinon 15 to 18 years before diagnosis in one case-control study (Cantor et al., 1992). In another case-control study, the risk for NHL among four female agricultural workers who reported using diazinon, the increase in risk for NHL was not statistically significant (Zahm et al., 1993). Diazinon exposure was not found to be associated with an increase in the risk for leukemia (Brown et al., 1990) or multiple myeloma (Morris et al., 1986). The frequency of use of pesticides including diazinon was significantly higher in families with cases of childhood brain cancer than a cancer-free control group of families (Davis et al., 1993).

B. Experimental Animal Studies:
Except for a cancer-bioassay conducted by the National Cancer Institute (NCI), all evaluations of the effects of diazinon in experimental animals have been presented in unpublished reports. Some of the unpublished reports were kindly provided to us by Novartis Crop Protection, Inc. (formerly Ciba-Geigy). We have included brief abstracts of other studies as reported by WHO (1998).

1. Mice:
Groups of B6C3F1 mice (50 of each sex) were fed either 100 or 200 ppm diazinon for 103 weeks. Untreated mice (25 of each sex) served as matched controls. Survival rates were at least 84% at week 78. The incidence of hepatic adenomas and carcinomas was significantly increased (p = 0.046) in male mice treated with the low dose of diazinon (number of tumors / number of animals examined = 20/46). A dose-related effect was not observed: 13/48 (27%) male mice fed the high dose of diazinon had these tumors, which was not a significant increase over the incidence in 5/21 (24%) controls. The incidence of liver tumors was not increased in diazinon-treated female mice: 0/47 of low dose group, 3/49 (6%) of the high dose group, and 2/23 (9%) controls had liver tumors (NCI, 1979). The combined incidence of lymphoma and leukemia was observed to be increased, but not significantly, or in a dose-dependent manner in both the diazinon-treated groups, in 11/47 (23%) female mice fed the low dose, 10/49 (20%) fed the high dose of diazinon, compared to 3/23 (13%) controls. The incidences of mammary gland neoplasms observed in this study are given in Section V.B.1. of this report.

In an unpublished report submitted to the United Kingdom Ministry of Agriculture, Fisheries and Food (MAFF) (as reported in WHO Report, 1998), B6C3F1 mice (60 of each sex per dose) were fed 0, 100, 200, 300 (males only) or 400 (females only) mg/kg diazinon (purity unspecified). After 24 months, there were reported to be no treatment-related histopathological lesions. Pathology results, tumor incidences, survival rates and body weight gains were not available to critically evaluate this study.

2. Rats:
Groups of F344 rats (50 of each sex per dose) were fed either 400 or 800 ppm of diazinon for 103 weeks. Matched controls consisted of groups of untreated rats (25 of each sex). The survival rates of all groups were higher than 84% at week 78 (NCI, 1979). There was a significant increase (p = 0.011) in the combined incidence of lymphomas and leukemias in 25/50 (50%) male rats fed the low dose of diazinon, compared to the incidence in 5/25 of controls (20%). The increase in incidence of lymphomas and leukemias, in 12/50 (24%) male rats fed the higher dose of diazinon was small and not significant, indicating a lack of a dose-dependent effect. The combined incidence of lymphoma and leukemia was 6/50 (12%) for both the diazinon-treated groups of female rats, a small but not significant increase over the incidence in controls (2/25 = 8%). The incidence of mammary gland neoplasms is given in Section V.B.2. of this report.

In an unpublished toxicological study conducted by Ciba-Geigy Corporation (Kirchner et al., 1991), Sprague-Dawley rats (30 or 40 of each sex, per dose) were fed 0.1, 1.5, 125 and 250 ppm diazinon (87.7% pure in soy oil). A control group of animals were fed a diet containing the vehicle alone (soy oil). At 12 months, ten animals were killed from each group. Another ten animals were allowed to recover for four weeks and then killed. The remaining animals were maintained on treatments until 99 weeks. Survival rates were variable and were as low as 30 to 35% in some diazinon-treated groups. The authors state that the high moribundity was due to senescence-related symptoms in these rats. None of the histopathological lesions observed at final sacrifice were considered to be treatment-related by the authors. Male rats that had received the highest dose of diazinon had a
significantly increased (p = 0.025) incidence of pancreatic focal islet cell hyperplasia compared to vehicle-treated controls. Pancreatic focal islet cell hyperplasia was observed in 1/20 (5%) vehicle fed controls and in 7/20 (35%), 5/20 (25%), 6/20 (30%) and 7/20 (35%) male rats that had received 0.1, 1.5, 125 and 250 ppm diazinon, respectively. The incidence of this disease in female rats was not available. The increased incidence of pancreatic focal islet cell hyperplasia in male rats was not considered to be treatment-related since there was no dose-related trend, although it should be noted that the incidence was increased in all the diazinon-treated males. (Kirchner et al., 1991).

In another unpublished report submitted to MAFF (as reported in WHO Report, 1998), F344 rats (75 of each sex per dose) were fed 0, 0.1, 0.5, 150 and 300 ppm diazinon (purity not specified). Rats fed the highest dose had increased ulceration of the stomach including hyperplasia of the epithelium (statistical analysis not available). The report stated that no neoplastic lesions were observed in the controls or diazinon-treated rats in the study. No details were available on the incidence of pathological lesions or survival rates.

3. Dogs:
In an unpublished study, Beagle dogs (four of each sex, per dose) were fed 0, 0.1, 0.5, 150 and 300 ppm diazinon (87.7% pure) for 52 weeks. The authors report that histopathological differences between controls and treated groups were unremarkable. Pituitary cysts were found in 1/4 (25%), 0/4 (0%), 1/4 (25%), 2/4 (50%) and 3/4 (75%) females that were fed 0, 0.1, 0.5, 150 and 300 ppm diazinon, respectively. The increase in incidence of pituitary cysts in the females was dose-related and significant for the group fed the high dose of diazinon (p < 0.05), but was not considered as a treatment-related effect by the authors because of the historically high incidence of such cysts in dogs (Rudzki et al., 1991). This study cannot be regarded as a cancer bioassay due to the small sample size and the short duration of only 52 weeks.

In another study on dogs that was also done at Ciba-Geigy (Barnes, 1988), atrophy of pancreatic acini was observed in one male dog that was fed the highest dose (300 ppm of diazinon) for 13 weeks. While this result is not significant by itself, it has been pointed out here since pancreatic effects have been reported in diazinon-treated rats (Kirchner et al., 1991), and in case reports of diazinon poisonings in humans (WHO, 1998).

4. Monkeys:
In an unpublished study conducted for Ciba-Geigy (Cockrell et al., 1966), Rhesus monkeys (three of each sex per dose) were orally treated with a daily dose of 0, 0.1, 1.0 and 10 mg/kg diazinon. The doses were lowered by half after 34 days and treatment was stopped after 106 weeks. Four of the 18 diazinon-treated monkeys did not survive the full term of the experiment. Diazinon-treated animals had reduced weight gains compared to the controls (statistical analysis not available). Histopathological analysis did not reveal any pathology that could be attributed to diazinon treatments. It should be noted that this study, because of the small number of animals used per dose group and the short duration of exposure, can not be regarded as a cancer bio-assay.

5. Summary:
Diazinon-treated male mice had a significantly increased incidence of hepatic carcinomas in one treatment group (NCI, 1979). Incidence of hepatic tumors in diazinon-treated female mice was not affected. Male rats in one diazinon treatment group had a significant increase in the combined incidence of lymphomas and leukemia (NCI, 1979). One study of diazinon-treated male rats observed a significant increase in the incidence of focal islet cell hyperplasia in the pancreas (Kirchner et al., 1991).

C. Current Classification of Carcinogenicity by Other Agencies
1. IARC Classification:
Diazinon has not been classified for carcinogenicity by IARC (ATSDR, 1996).

2. NTP Classification:
Diazinon has not been classified for carcinogenicity by NTP (USDHHS, 1998).

3. EPA Classification:
Diazinon has not been classified for carcinogenicity by EPA (ATSDR, 1996).

V. Critical Evaluation of Breast Carcinogenicity
A. Human Studies:
There have been no case reports of breast cancer among women exposed to diazinon. Case-control studies on health effects in women exposed to this insecticide have not reported breast cancer incidences.

1. Human Tissue Levels:
No reports were found on the presence of diazinon residues in human breast milk.

B. Experimental Animal Studies
1. Mice:
In one study conducted by the NCI, groups of B6C3F1 mice (50 of each sex) were fed either 100 or 200 ppm diazinon for 103 weeks (NCI, 1979). Groups of untreated mice (50 of each sex) served as matched controls. Survival rates were at least 84% at week 78. Mammary gland fibroadenomas were observed in 1/47 (2%) female mice that were fed the low dose and 4/49 (8%) mice that were fed the high dose of diazinon compared to 0/23 (0%) controls. The increased incidence of mammary gland fibroadenomas in diazinon-treated mice was not statistically significant.

2. Rats:
The same study described above also evaluated mammary gland neoplasms in groups of F344 rats (50 of each sex per dose) that were fed either 400 or 800 ppm of diazinon for 103 weeks. Matched controls consisted of untreated rats (25 of each sex). The survival rates were higher than 84% at week 78 for all groups (NCI, 1979). Mammary gland neoplasms in female rats were observed in 7/50 (14%) fed the low dose and 4/50 (8%) fed the
high dose of diazinon, compared to 5/25 (20%) controls. The incidence of mammary gland neoplasms was thus slightly, but not significantly lower in treated rats, indicating that diazinon does not affect the incidence of mammary tumors in rats. Another study of long-term exposure effects in rats did not report any increase in incidence of mammary gland neoplasms (Kirchner et al., 1991).

3. Summary:
Case-control studies of health effects in women exposed to diazinon have not reported on breast cancer incidences. No case reports of breast cancer in association with diazinon exposure were found. No increase in incidence of mammary gland neoplasms was reported in diazinon-treated mice or rats (Kirchner et al., 1991; NCI, 1979).

C. Other Relevant Data on Breast Cancer Risk

1. Evidence of Endocrine Disruption

a. In Vivo Studies:
Male and female progeny of pregnant mice exposed to 0, 0.18, or 9 mg/kg/day diazinon during gestation were observed to have significant (p < 0.05) retardation in sexual development in one study (Spyker and Avery, 1977). It was not possible to determine from this study if this retardation was due to an overall toxic effect from prenatal exposure to diazinon, or whether the effect was specific to the development of sex organs. In another paper by the same author, adrenal gland weights and in vitro steroidogenesis were reported to be unaffected in the progeny of dams that were exposed to the low dose (0.18 mg/kg) of diazinon. However, the hepatic capacity to metabolize corticosterone was significantly reduced (p < 0.01) in the progeny of the low-dose treatment group (Cranmer and Avery, 1978). Progeny of pregnant mice exposed to the higher dose (9 mg/kg/day) did not have a significant effect on plasma levels of corticosterone, adrenal steroidogenesis or hepatic corticosterone hydroxylation. However, a significant reduction in adrenal gland weights (p < 0.05) was observed in the progeny of the high-dose treatment group. The authors state that estradiol can stimulate the liver’s capacity for corticosterone hydroxylation. However, a decrease in hepatic corticosterone hydroxylation in the progeny of exposed mice is not sufficient evidence for an anti-estrogenic effect of diazinon.

In a two generation reproductive study, albino rats (30 of each sex per dose) were fed 0, 10, 100 or 500 ppm technical grade diazinon (unknown purity) (Giknis, 1989). The relative testes and ovary weights were not significantly different in the treated and control groups. Uterine and ovarian weight gains were not significantly affected in two studies (see Section IV.B.3. for study design) that evaluated the toxicity of diazinon in dogs (Barnes, 1988; Rudzki et al., 1991). Monkeys that were orally treated with diazinon over 106 weeks (see Section IV.B.4. for study design) were reported to have normal estrus cycle development (Cockrell et al., 1966).

b. In Vitro Assay for Estrogenicity:
Diazinon was not estrogenic in an in vitro test. Diazinon did not induce cell proliferation of estrogen-responsive human breast cancer cells (MCF-7) in the E-SCREEN assay for estrogenicity (Soto et al., 1995).

c. Male Sex Hormone Levels:
Liver enzymes that metabolize corticosterone regulate the circulating steroid levels in the plasma. Thus, a disruption of corticosterone metabolism could lead to a feedback affect on steroidogenesis of sex hormones. Diazinon (10^-8 M), added to the extract of liver microsomes from male Swiss-Webster mice caused a significant inhibition of the testosterone hydroxylases (Donovan et al., 1978). This effect was also observed in liver microsomes extracted from Sprague-Dawley rats, but only at higher (10^-4 M) concentrations of diazinon. We have presented this study because diazinon was observed to affect hepatic corticosterone reductases in vivo in mice (Cranmer and Avery, 1978). We recommend that the hepatic effects of diazinon and any disruption of steroidogenesis be evaluated further.

d. Summary:
Some studies indicate that diazinon may adversely affect hepatic corticosterone metabolism (Cranmer and Avery, 1978; Donovan et al., 1978). However, studies done in vivo (Barnes, 1988; Cranmer and Avery, 1978; Giknis, 1989; Rudzki et al., 1991; Spyker and Avery, 1977), or in vitro (Soto et al., 1995), show no evidence for an anti-estrogenic or estrogenic effect of diazinon. Diazinon’s effect on hepatic hydroxylation of steroids and whether it leads to any endocrine disruption needs to be studied further.

2. Reproductive and Teratogenic Effects:
Studies on reproductive toxicity can sometimes provide evidence for a disruption in estrogen-dependent events. A two-generation study of albino rats orally treated with 0, 10, 100 or 500 ppm diazinon observed no significant effect on organ weights, precoital interval, gestation duration, and other reproductive parameters when animals were treated with < 100 ppm diazinon. At the 500 ppm dose level of diazinon, gestation was prolonged, accompanied by a decrease in number of pregnancies and fertility indices (Giknis, 1989). Other clinical effects were also observed in the dams treated to this dose level, indicating that the dose was toxic. An earlier study of Sprague-Dawley rats that were fed 0, 15, 50 or 100 mg/kg diazinon during days six through fifteen of gestation, reported no treatment-related effects on number of corpora-lutea, implantations, resorptions or viable fetuses (unpublished report by Fritz et al., 1974, as cited in WHO, 1998). Similar results have been reported from other animal studies in rats and rabbits (unpublished reports, as cited in WHO, 1998). Pregnant Carworth Farms Nelson (CFN) rats were orally treated with 0, 40, 50, 60, or 75 mg/kg diazinon through days seven to 19 of gestation in another study. The highest dose was found to be toxic to the dams. Other doses of diazinon did not affect litter size, fetal body weights, brain weights, the number of resorptions, or corpora lutea (Hoberman et al., 1979).

Studies on reproductive toxicity of diazinon have observed that prenatal exposure to diazinon is toxic to litters, but only at high doses that are also toxic to the dams (Giknis, 1989; Hoberman et al., 1979). The reproductive toxicity observed in diazinon-treated animals does not indicate disruption of estrogen-mediated events.
3. Tests of Mutagenicity and Genotoxicity:
Various screening assays have been developed to identify chemicals that increase the frequency of mutations or chromosome aberrations and thus affect cancer risk. Diazinon was not mutagenic in most systems in which it was tested. Based on its lack of activity in at least six genetic bioassays, it was classified as "probably negative" for mutagenicity and genotoxicity by EPA (Waters et al., 1983). Short term genotoxicity tests done by the NCI in mice and rats have shown no evidence for a carcinogenic potential for diazinon (Shelby and Stasiewicz, 1984).

a. Chromosome Aberrations in Occupationally Exposed Humans:
One study has evaluated chromosomal defects in association with exposure to Basudin® E (formulated diazinon), in 34 manufacturing workers (Kiraly et al., 1979). Chromosome deletions and translocations were increased, but not significantly (p = 0.10) among workers exposed to Basudin® E. The two control groups used in this study had variable rates for such aberrations, further reducing the significance of the diazinon-related increase.

Three other occupational cohorts have been evaluated for frequency of chromosome aberrations in blood lymphocytes, but these populations were exposed to many different pesticides including diazinon. Genotoxicity of urine samples from 22 nonsmoking orchard workers who were occupationally exposed to many pesticides including diazinon, was assayed using Chinese hamster ovary cells (CHO). The clastogenic activity of urine specimens was significantly increased during the spraying period (p < 0.001) for the highly exposed orchard workers but not for the unexposed research station personnel (See et al., 1990). A study of 64 workers (floriculturists) in Italy who were exposed to pesticides including diazinon through their work found significantly elevated sister chromatid exchange rates in the peripheral blood lymphocytes of exposed workers (p < 0.01) (De Ferrari et al., 1991). Another study evaluated the frequency of chromosome aberrations in the lymphocytes of 16 pesticide applicators in Idaho who used insecticides including diazinon. A five-fold increase was observed in the frequency of chromatid breaks in lymphocyte cultures, if the lymphocytes obtained during the spraying season were compared to those obtained off-season (Yoder et al., 1973). All these studies indicate an induction of chromosome aberrations in populations that were occupationally exposed to many different kinds of pesticides. Since the orchard workers, floriculturists and pesticide applicators used various pesticides including OPs, the genotoxicity of diazinon specifically cannot be determined from these studies.

b. Studies in Experimental Animals:
Twelve male Wistar rats were fed diazinon (87% pure) in a 1:1 water-ethanol solution by gavage for 28 weeks, while twelve control rats received only the vehicle. Four control rats were left untreated. Histopathological examinations of the liver did not reveal any preneoplastic lesions (Anthony et al., 1986). Results from unpublished reports of studies done for Ciba-Geigy have been summarized in a recent Environmental Health Criteria Report (WHO, 1998). Diazinon did not induce any nuclear aberrations in the bone marrow of Chinese hamsters or mice in these studies. Diazinon did not induce genetic damage or chromosomal loss in DNA-repair defective Drosophila (Woodruff et al., 1983). Diazinon in aquarium water, at concentrations of 5.4 X 10^{-10} M induced a significant increase (p < 0.01) in the frequency of sister chromatid exchanges (SCE) in mudminnows (Vigfusson et al., 1983). On the basis of these results it appears that diazinon has not been observed to be genotoxic in animal systems except for one study in fish (Vigfusson et al., 1983).

c. Studies in Isolated Cells:
Results from different studies of diazinon’s genotoxic effects in isolated cells have been equivocal. Two studies have observed that diazinon treatments along with the metabolic activating S9 mix can cause a significant increase in SCE rates (p < 0.01) in human lymphoid cells (Sobti et al., 1982), and a 20 to 50% increase in SCE in CHO cells (Matsuoka et al., 1979). Diazinon, without S9 activation, was found to induce mutations in the thymidine kinase gene in mouse L5178Y tk/tk: lymphoma cells (McGregor et al., 1988).

In contrast, SCE was not increased in two other studies of CHO cells treated with 0, 0.05, 0.1, 0.2 and 0.4 µg/ml of 99% pure diazinon (Kuroda et al., 1992), or to concentrations ranging between 0.03 to 1.0 mM of 89% pure diazinon (Nishio and Uyeki, 1981).

The average dietary intake of diazinon was estimated from reports of its detection in foods in Italy. Cytokinesis-blocked human lymphocytes treated with diazinon at the estimated daily intake level, or 10 and 100 times higher concentrations caused an increase in the frequency of micronucleated cells, but the increase was not dose-related. A mixture of dimethoate, azinophos-methyl and diazinon caused a larger increase in micronucleated cells than diazinon alone. However, the increase in micronucleated cells caused by the mixture was less than the sum of the increases observed after treatments with each of the three chemicals alone (Bianchi-Santamaria et al., 1997). Thus, the increase in effect was not additive or synergistic. Diazinon treatment of human peripheral blood lymphocytes in another study did not cause an increase in chromosome aberrations, but did induce a dose-related trend in abnormally condensed chromosomes (Lopez et al., 1986). The same authors conducted another study in which the lymphocyte cultures were treated with pulses of a non-inhibitory dose of diazinon during different phases (30 µg/ml). A trend for abnormal chromosome condensation was observed in parallel to abnormal chromosome condensation. Older cultures treated with diazinon did not have an increase in chromosome aberrations (Lopez and Carrascal, 1987). These results suggest that actively growing blood lymphocytes exposed to non-toxic doses, may be more susceptible to chromosome damage from diazinon.

d. Mutagenicity Studies in Bacteria and Yeast:
Studies in yeast and bacteria indicate that diazinon is not a strong mutagen. Assays for reversion mutations in bacteria and yeast have been negative (Garrett et al., 1986; Marshall et al., 1976; Nagy et al., 1975; Shirasu et al., 1976; Wild, 1975; Zeiger, 1987). Non-toxic concentrations of diazinon (20 ppm) were not
mutagenic to four tester strains of *Salmonella* in the absence of S9 mix. In the presence of S9, diazinon was found to be mutagenic to only one (TA98) of the four *Salmonella* strains that were tested (Wong et al., 1989).

A modified colorimetric SOS microplate assay was used to compare the genotoxicity of diazinon when mixed with bile salts or in 10% dimethyl sulphonate (DMSO) (Venkat et al., 1995). Diazinon, mixed with bile salts, was less genotoxic than when it was mixed in DMSO. The mutations induced were assayed by the expression of the β-galactosidase gene, which was under the control of *SulA* (SOS DNA repair gene).

**Summary:**

Diazinon was not found to have a strong genotoxic effect in most systems in which it was tested. The three studies that have reported a significant increase in chromosomal aberrations in orchard workers, floriculturists and applicators, evaluated the effects of exposure to many different pesticides, but not to diazinon specifically (De Ferrari et al., 1991; See et al., 1990; Yoder et al., 1973). Diazinon was not found to increase the incidence of preneoplastic lesions in rats (Anthony et al., 1986), or nuclear aberrations in bone marrow of mice and Chinese hamsters (unpublished studies, cited in WHO, 1998). Studies of its genotoxicity have been equivocal in isolated cells, with three out of five assays indicating a genotoxic potential (Kuroda et al., 1992; Lopez et al., 1986; Lopez and Carrascal, 1987; Matsuoka et al., 1979; McGregor et al., 1988; Nishio and Uyeki, 1981). Mutagenicity assay results were mostly negative in bacteria and yeast (Garrett et al., 1986; Marshall et al., 1976; Nagy et al., 1975; Shirasu et al., 1976; Venkat et al., 1995; Wild, 1975; Wong et al., 1989; Zeiger, 1987).

**4. Evidence of Tumor Promotion and Cell Proliferation:**

One study found diazinon to promote lung tumors in mice. A/St bred mice are highly susceptible to pulmonary tumors. Intraperitoneal (i.p.) treatments of these mice with 10 mg/kg diazinon, three times a week for eight weeks, caused a significantly increased incidence of pulmonary tumors in the females (p ≤ 0.05), but not in the males (Maranon et al., 1986). Diazinon’s tumor promotion ability was tested in male rats in another study. Male F344 rats received 200 mg/kg diethylnitrosamine (DEN) i.p., and two weeks later were fed diazinon (500 or 1000 ppm) for eight weeks (Kato et al., 1995). Glutathione S-transferase P (GST-P) positive foci were assayed one week after treatment with diazinon, as preneoplastic indicators of tumor promotion. There was no significant increase in either the number or size of GST-P positive foci in the rat livers of diazinon-treated animals. Other experimental animal studies have reported liver damage in diazinon-treated male rats (Dikshith et al., 1975; Kirchner et al., 1991). However, an evaluation of preneoplastic lesions was not reported for these diazinon-treated rats.

A chemical that increases the rates of cell proliferation may lead to tumor promotion by causing an increased number of tumors of a larger and detectable size. Low doses of diazinon (1 and 10 µM) were found to significantly increase (p ≤ 0.05) the cell proliferation rates of cultured human intestinal and rat intestinal epithelial cells (established cell line) in an *in vitro* assay (Greenman et al., 1997). However, higher concentration of 50 µM diazinon did not induce cell proliferation. In the *in vitro* ESSCREEN assay for estrogenicity, 1nM to 10 µM diazinon concentrations did not induce the proliferation of estrogen responsive human breast MCF-7 cells (Soto et al., 1995).

**5. Immunological Effects:**

An impaired immune system may compromise the ability of the body to fight disease and cancer. One study evaluated the effects of diazinon in pregnant mice that received either 0.18 or 9 mg/kg dose in diet throughout gestation. A significant suppression (p ≤ 0.05) in the immunoglobulin (IgG) concentrations was observed in prenatally exposed male and female pups at 101 days of age. However, at 400 or 800 days of age, the IgG levels of the exposed pups were no longer significantly different than control mice (Barnett et al., 1980). This transient suppression in immune-competence also corresponded with an increased early morbidity observed in litters of diazinon-treated mice.

In another study, the immuno-toxicity of cocaine was evaluated in mice exposed to diazinon (Kump et al., 1996). Treatments of female B6C3F1 mice with 10 or 30 mg/kg diazinon (i.p.) caused no significant difference in antibody response. However, mice that were pre-treated with diazinon and cocaine had a significantly suppressed antibody response to sheep erythrocytes, and the suppression was related to the dose of diazinon (Kump et al., 1996). The authors propose that diazinon inhibits esterases and thus inhibits the metabolism of cocaine through the esterase pathway. This inhibition leads to more cocaine being metabolized through the P-450 enzyme pathway. The metabolites of cocaine formed through the P-450 enzymes are proposed to cause the suppression of the T-cell dependent antibodies response (Jeong et al., 1994). This study does not indicate that diazinon is immuno-toxic by itself, but that it can potentiate the immuno-toxic effects of other toxic chemicals by disrupting the esterase pathway of metabolism.

In a study of fish treated with diazinon, macrophage populations in the kidney and spleen were observed to be significantly increased (p < 0.03), indicating an activation of the immune system (Dutta et al., 1997). Northern Bobwhite eggs were placed in nests without cover in fields that were sprayed with diazinon. Three week old chicks were randomly selected (n = 48) and challenged with a pathogenic strain of bacteria that causes avian cholera. The immunocompetence of the hatchlings was not significantly affected by the diazinon exposure (Dabbert et al., 1996).

Diazinon exposure was found to cause a transient suppression of the developing immune system (Barnett et al., 1980), and potentiated the immuno-toxic effect of cocaine (Kump et al., 1996) in mice. Whether or not diazinon can increase cancer risk in mammals cannot be determined from this evidence. Diazinon’s immuno-toxicity and ability to potentiate the immuno-toxic effects of other chemicals should be evaluated in further studies.
6. Summary of Other Relevant Data on Breast Cancer Risk:
Diazinon was not estrogenic in studies done in vivo or in vitro (Barnes, 1988; Cockrell et al., 1966; Giknis, 1989; Rudzki et al., 1991; Soto et al., 1995). Whether or not diazinon’s effect on hepatic enzymes leads to endocrine disruption needs to be studied further (Cramer and Avery, 1978; Donovan et al., 1978). Diazinon was not found to be genotoxic in most systems in which it was tested. Diazinon promoted pulmonary tumor in mice (Maronpot et al., 1986), but did not promote pre-neoplastic lesions in the livers of rats (Kato et al., 1995). Diazinon induced the cell proliferation rate of intestinal cells (Greenman et al., 1997), but did not induce the proliferation of breast tumor MCF-7 cells (Soto et al., 1995). Prenatal exposure to diazinon was found to disturb the developing immune system in mice (Barnett et al., 1980). The evidence of related mechanisms by which diazinon may affect cancer risk is limited to its lung tumor promotion and transient immuno-toxic effects. Diazinon’s immuno-toxic effects need to be studied further since an impaired immune system may increase the risk for cancer.

VI. Other Information
A. Environmental Fate and Potential for Human Exposure:
1. Occupational Exposure:
Diazinon exposures in agricultural workers have been reported in the population-based case-control studies discussed in Section IV.A. of this report (Blair et al., 1998; Brown et al., 1990; Cantor et al., 1992; Zahn et al., 1993), in orchard workers in Hawaii (Rayner et al., 1972), and case reports (Maddy and Edmiston, 1988). But the levels of exposure to diazinon were not determined in these studies. Occupational exposure to organophosphates including diazinon was also documented by the presence of alkyl phosphate metabolites in the urine of pest control applicators (Hayes et al., 1980; Maizlish et al., 1987; Weisskopf et al., 1988).

Results of studies of occupational exposure suggest that high levels of occupational exposures to diazinon probably occur through the dermal route during spraying operations, and not through inhalation. Air residues of diazinon in storage rooms and offices in commercial pest control buildings were found to be below the allowable limits or threshold limit value (TLV = 100 µg/m³) (Wright and Leidy, 1980). Air samples collected from cabs of pickup trucks used by pest control firms had higher diazinon residue levels when moving (0.58 to 5.15 µg/m³), than when stationary (0.34 to 2.05 µg/m³) (Wright et al., 1982). In another study, the concentration of diazinon in the air of a retail garden store that sold agrochemicals was found to be 3.4 µg/m³ (Wachs et al., 1983). All these studies reported diazinon levels below the TLV (100 µg/m³). One study has evaluated the personnel exposure during applications of diazinon granules using different kinds of equipment, predominantly hand-held spreaders. The highest respiratory exposures occurred during work shifts that involve belly grinder use (a broadcast spreader hung by a strap around the neck). This exposure was observed even for workers who wore respirators during applications. It is possible that high levels of deposition of diazinon on the body and coveralls may have contributed to prolonged respiratory as well as dermal exposures in these applicators (Weisskopf et al., 1988). Since this study used equipment that was more typical of residential homeowner use than large-scale operations, it is also useful in evaluating the potential of non-occupational exposure for homeowners who use this insecticide.

2. Potential for Exposure for the General Population:
The general population may be exposed to low levels of diazinon intermittently, through diet or from the air (WHO, 1998). The numerous case reports in the literature also indicate a high potential of accidental poisonings with this insecticide (Adlakha et al., 1988; Balani et al., 1968; Goldman, 1995; Gupta and Patel, 1968; Halle and Sloas, 1987; Hata et al., 1986; Karlsen et al., 1981; Klemmer et al., 1978; Kurt, 1988; Maddy and Edmiston, 1988; Muldoon and Hodgson, 1992; Poklis, 1980; Rao, 1965; Rayner et al., 1972; Reichert et al., 1977; Richter et al., 1992; Shankar, 1978; Sheth et al., 1995; Soliman et al., 1982; Wagner and Orwick, 1994; Wedin et al., 1984). Most high level accidental exposures or poisonings have involved either dermal absorption or ingestion of this insecticide (WHO, 1998). Below, we outline some results of studies of air, food, water and soil levels of diazinon that indicate a potential for exposure to the general population.

a. Air:
Air samples from ten different locations within the US were analyzed for agrochemicals as part of an environmental monitoring study by EPA (Carey and Kutz, 1985). This study reported detectable levels of diazinon in 48% of the air samples collected in 1980 from one location each in South Carolina, Illinois, Alabama, Mississippi, Montana, two different locations in California and in three different locations in Texas. The mean level of diazinon in these outdoor air samples was 2 ng/m³. The maximum level of diazinon detected was 23 ng/m³.

A “Non-Occupational Pesticide Exposure Study” (NOPES) was designed to assess seasonal variations and the total exposure to several pesticides through air, diet, dermal contact and water in 216 homes in two different geographic regions, Jacksonville, Florida and Springfield/Chicopee, Massachusetts (Whitmore et al., 1994). Both the sites have little agricultural pesticide use, but Jacksonville, Florida, with its warmer climate was expected to have higher household insecticide usage. It was estimated that 83% of the population of Jacksonville is exposed to detectable levels of diazinon in indoor air throughout the year. The mean air concentration of diazinon in the homes sampled in Jacksonville ranged between 85.7 ng/m³ in winter, to 420.7 ng/m³ in summer. In Springfield/Chicopee, indoor air contamination was relatively less frequent (10 to 20% detection rate). The mean air concentration (indoor) varied from 2.5 to 48.4 ng/m³ between winter and spring, respectively. These levels were well below the TLV. This study suggests that the potential for non-occupational residential exposure to diazinon from the air may vary depending on the geographic region and household use of the insecticide.

In another study, house dust and air samples were collected and analyzed from nine middle-income households with a child of six months to five years of age in Durham, North Carolina. Residues in houses in which pesticides were used by commercial pest control services or by homeowners were compared to a
control household that reported no pesticide use. Diazinon was detected in the air of only one of the houses. The basement of this house had been professionally treated against insect pests (Lewis et al., 1994). A slow transport of the insecticide, resulted in air residue levels on the first floor in the range of 0.03 to 0.07 µg/m³ diazinon over the 14 day period of monitoring. Movement of diazinon into adjoining rooms was also reported by another study that monitored levels of this insecticide in the air 21 days after its crack and crevice application (Leidy et al., 1982).

Rooms in which pest control strips containing 10% diazinon were used had a steady increase in the level of diazinon in the air, reaching the maximum level (1.34 µg/m³) 15 days after the strips were placed (Jackson and Lewis, 1981). In another study, maximum air levels of insecticide were observed between day 15 and day 30 following the use of diazinon impregnated pest strips in animal facilities (Hinkle et al., 1980). These studies indicate that diazinon residues gradually build up in the air following the placement of pest strips in enclosed areas. However, the maximum air residue levels reached were below the TLV for diazinon.

Airborne residues were monitored after a flat fan spray was used to apply 1% diazinon in three offices. Airborne concentrations of diazinon in the offices peaked at 160 µg/m³ four hours after the spraying. The airborne levels of diazinon remained at the TLV 24 hours after treatment. These results led the authors of this study to recommend that unventilated areas that are sprayed with diazinon be avoided for at least two days following application (Currie et al., 1990).

People who live or work in diazinon-treated areas may be exposed to air residues. The levels of diazinon found in most studies were well within the TLV. As recommended on the label, treated areas should be ventilated. The manufacturer’s guidelines should be followed for re-entry.

b. Food:
The Food and Drug Administration (FDA) conducts studies to determine the level of different pesticide residues that remain in a typical meal or menu items, called “Total Diet Studies.” Total Diet Studies conducted in 1978-1982 have estimated that 11% of US adults are exposed to diazinon through food, but at levels below the acceptable total dietary intakes that have been established by international agencies (Yess et al., 1991). Residue data from Total Diet Studies conducted by the FDA were correlated with food consumption data collected in two large epidemiological studies to estimate the total daily dietary exposure of US adults to diazinon (MacIntosh et al., 1996). Researchers estimated that adults in the US receive 0.5 µg/day diazinon through their diet. Diazinon exposures in this study correlated with the consumption of wheat-based products such as English muffins and pasta. In contrast, no residues above tolerance levels were reported in wheat and wheat products in FDA’s Residue Monitoring Reports from 1997 and 1996 surveys (FDA, 1998a; FDA, 1998b). Diazinon was not detectable (< 0.01 ppm) in grain dust samples from grain elevators in New Orleans (Palmgren and Lee, 1984).

The NOPES study described earlier compared the levels of non-occupational exposures from air and diet in two different geographic regions in the US. In Jacksonville, Florida, estimated daily exposure to diazinon was higher through the air (1,380 ng) than through diet (average of three surveys = 774 ng). One market basket survey done between 1986 to 1987 estimated a daily dietary exposure level (1,140 ng) which was twice the level estimated in 1982 to 1984 (590 ng) or 1987 (593 ng). In Springfield/Chicopee, Massachusetts, daily exposure estimates from diet (586 ng) in 1982 to 1984 were higher than the daily estimated exposure from air (158 ng) (Whitmore et al., 1994). The results of this study indicate that exposure to diazinon for the general population occurs through diet and inhalation and the relative exposure may vary depending on geographic region, food intake and household use of the insecticide.

Diazinon is widely used as an insecticide on fruit trees. In a survey of apples from Ontario, Canada in 1978 to 1986, only 0.3 % of the apples were found to carry detectable residues (0.04 mg/kg) (Frank et al., 1989). Diazinon was found to bioconcentrate in fish (120 fold in carp) that were exposed to 0.012 to 0.021 ppm concentrations in water, with ratios that varied in proportion to the fat content of the fish (Seguchi and Asaka, 1981). However, seven days after the fish returned to clean water, diazinon levels in fish were less than 0.008 ppm, indicating rapid clearance of the insecticide. Thus, diazinon residues in fish may be an indication of a recent pollution event. Milk is not a major route for excretion of diazinon. However, some diazinon was detected in cow’s milk fat (highest level = 0.04 mg/kg) seven days after the lactating animal was ear-tagged with a diazinon-containing product (Spradbery and Tozer, 1996); (see also Section VI.3.b. Diazinon and Lactation).

Foods left in rooms during diazinon treatment, or brought into rooms too soon after treatment may carry detectable levels of diazinon residues. In one study, foods that were left in the room being treated with 1% diazinon as an aerosol for cracks and crevices, had 0.05 ppm as the maximum level of residue (Jackson and Wright, 1975). This level is below the food tolerance levels for agricultural crops (see Section III.D. of this document). Sliced potatoes and television dinners that were placed in rooms 4.5 hours after treatment with diazinon for 30 minutes did not show detectable levels of residues.

The general population may be exposed to small amounts of diazinon intermittently through the food supply. While the residue levels found were below the tolerance levels set for diazinon, OP are undergoing a risk assessment review by EPA, under the 1996 Food Quality Protection Act. The new stringent risk criteria under this act will consider the total exposure to diazinon from different non-occupational sources, such as air, food, water, home and garden use (EPA, 1999).

c. Soil:
A study of diazinon’s degradation in agricultural soils has reported a half life of five days at 20°C, with soil moisture of 60%. The half-life of diazinon was eight days if the soil moisture was 30%, and 118 days in sterile soil, indicating that most of the degradation was microbial (Seyfried, 1994, as cited in WHO, 1998). Diazinon degrades in soil and water through hydrolysis, photolysis and biodegradation (ATSDR, 1996). Diazinon’s degradation in soil is
affected by pH, soil type, organic content, soil moisture and its concentration (WHO, 1998). The ideal conditions for the degradation of diazinon were found to be when it was present at low concentrations, in moist soils with a low pH (WHO, 1998). The major break down product of diazinon through photolysis and hydrolysis is 2-isopropyl-6-methyl-4-hydroxypyrimidine (ATSDR, 1996).

Greenhouse soil that was treated with 15 lbs / acre diazinon was found to have insecticidal activity for 19 weeks after being sprayed, compared to only 14 weeks in the field (Ahmed and Morrison, 1972). In another field study, diazinon was found to be effective in protecting turfgrass from root-feeding insects for 14 days after application (Sears and Chapman, 1979).

Widespread spraying of diazinon in open areas was observed to lead to avian toxicity. Diazinon used in sheds that housed ducks was observed to be toxic to young ducklings in an early study done in 1957 (WHO, 1998). Diazinon applications at the label rates (1 kg/hectare) to turfgrass in golf courses, condominium lawns and fairways, were found to cause deaths in Canadian geese in a study conducted in Ontario, Canada (Frank et al., 1991). Its use on golf courses and sod farms was canceled in 1986 in the US after similar reports on its avian toxicity in field studies (USEPA, 1996a; WHO, 1998).

d. Water:
Diazinon does not persist in water for a long time. The half-life of diazinon was estimated to be 70 hours in natural water by one study (Ferrando et al., 1992). The National Water-Quality Assessment Program surveys indicate that herbicides are detected in shallow groundwater much more frequently than insecticides (Kolpin et al., 1998). However, diazinon is among the most frequently detected insecticides. In a nationwide survey of shallow groundwater, diazinon levels at or above the water-quality criteria established for the protection of aquatic life (0.009 µg/L), were observed at 5/1,031 sites (Kolpin et al., 1998).

In a nationwide surface water monitoring program, only 1.2% of the samples collected between 1976-1980 had detectable levels of diazinon, with the maximum residue level of 2.38 µg/L (Carey and Kutz, 1985). A survey of stream and river samples from Ontario, Canada, diazinon residues were detectable in all the samples collected, at levels at or below 0.080 µg/L (Miles, 1976). The variable results from different surveys indicate that diazinon may be present in water transiently, or at specific sites.

In a recent US Geological Survey (USGS) 64 samples of surface water from streams and rivers throughout NYS were analyzed for 47 different pesticides (Phillips et al., 1998). Diazinon was detected in 14% of the samples. Diazinon levels were found to be highest in watersheds draining from urban/residential areas. NYS water-quality criterion for aquatic life (0.07 µg/L) was exceeded at three sites. Two of these were in urban/residential watersheds on Long Island and one was in orchard/vineyard watershed in western NYS. A comparison of these results with an earlier survey of the Hudson and Mohawk River Basin done in 1994 (Wall and Phillips, 1997), indicates that diazinon has been present in the urban watersheds of eastern NYS for at least three years. Another survey was done on surface water samples at three different sites that drain into the Mohawk River. One site was mostly agricultural (Canajoharie Creek), one was urban (Lisha Kill at Niskayuna) and the third was a combination of forested agricultural and urban watershed (Mohawk River at Cohoes). Water samples from Lisha Kill in this survey had 0.55 µg/L diazinon, approaching close to the lifetime HA level set for adults and children (0.6 µg/L) (Wall and Phillips, 1997). In surface water samples collected from 46 different sites (streams and rivers) along the Hudson River Basin between May and August, 1994, diazinon was the most frequently detected pesticide in the urban watersheds (at 6/10 urban sites) (USGS, 1997).

Similar USGS surveys in California have tracked the movement of diazinon residues in watersheds that drain into the Pacific Ocean bays. Following rainfall, diazinon pulses were detected to move from the Sacramento River into the San Francisco Bay (Kuivila, 1993). A survey of pesticide fluxes was conducted on water samples from nine different sites along the Mississippi River basin and its major tributaries (Larson et al., 1995). The total flux of diazinon observed in water samples from the White River basin represented 20% of its agricultural use. This abnormally high flux suggests that a significant non-agricultural or unaccounted use may have been occurring. Also, the peak concentrations of diazinon were observed in this region in late summer, while agricultural applications usually lead to fluxes earlier in the year. The three sites at which diazinon was detected more frequently were near the highest population densities, further supporting that substantial urban use of this insecticide was contributing to the fluxes.

These studies indicate that with increasing urban use, diazinon has become a common water contaminant in urban watersheds. Its agricultural use leads to smaller and more seasonal fluxes in shallow groundwaters around the regions where it is most used.

e. Surfaces:
In a pilot study to assess the risk of exposure to children, handwipe samples from toddlers from eleven homes in California were analyzed for different pesticides. Homes that served as residence for at least one farm-worker were compared to homes that did not have anyone employed on farms. Diazinon was found in the dust of 4/5 farm-worker homes, at levels ranging from 1 to 169 ppm, and 3/6 homes with no farm-workers, at levels of 0.2 to 2.5 ppm (Bradman et al., 1997). Diazinon (total residue amount = 52, 125 and 220 ng) was detected in three handwipe samples, all three from farm-worker homes.

Diazinon residues were detectable in the dust of four of seven houses (average = 740 ng/g) in New Jersey that were surveyed for different insecticides (Roinestad et al., 1993). In one study, surface residues were compared after spraying or fogging applications of technical diazinon (1% oil solution prepared from diazinon and Ultrasene) (Wright and Jackson, 1974). Fogging operations were found to cause relatively more diazinon residues than spraying operations in surface samples collected within one day of application. Widely varying amounts of diazinon residues...
were recovered 0.2 days after spraying operations on collection plates placed on the kitchen counter and cabinets. However, there were no detectable residues (< 0.35 µg) of diazinon on plates placed four days after either fogging or spraying operations, indicating rapid dissipation. In another study, surface contamination levels were found to remain high (peak residue concentration = 38 ng/cm²) 48 hours after application of 1% diazinon spray in offices (Currie et al., 1990). While the levels were not considered high enough to cause significant dermal exposure to the office occupants, it may be prudent for occupants to remove personal belongings such as coffee cups before diazinon pest-control applications.

Summary:
The major routes of exposure for the general population are expected to be through the diet and air residues in treated homes and facilities. While diazinon has been restricted for widespread use in golf courses and sod farms, it is not restricted for use in home lawns and gardens (USEPA, 1996a). An increased presence of diazinon residues in urban watersheds (USGS, 1997) indicates the need to evaluate and restrict these sources of water contamination.

3. Storage and Excretion of Diazinon in Mammals:

a. Storage and Tissue Distribution:
Diazinon is rapidly metabolized and excreted, without significant tissue accumulation in most mammalian species. Since urine is the major route of exposure, the kidneys are expected to be the clearance site for diazinon. Acute renal failure has been observed in association with a case of diazinon poisoning (Abend et al., 1994). Animal studies have observed kidneys to have the highest accumulation of diazinon following i.p. treatment (Tomokuni et al., 1985). An autopsy study of a case of fatal diazinon poisoning revealed traces of diazinon in the blood and 5.1 mg/kg diazinon in the omental tissue (tissue from folds of the stomach and abdominal cavity), but no detectable residues in the liver (Kirkbridge, 1987). The amount or route of diazinon exposure could not be determined in this case. Another autopsy study of a fatal poisoning case has reported the highest concentrations of diazinon in the brain (Heyndrickx et al., 1974).

Cattle that were sprayed with 0.1% diazinon, once a week, had 2.3 ppm diazinon in the fat tissue on the day after the 16th spraying, but the levels reduced to < 0.05 ppm in 14 days. Animals sprayed with 0.05 % diazinon had 0.83 ppm as the highest detectable level of diazinon on the day after the 16th spraying (Claborn et al., 1963).

A study of the coefficient of tissue distribution of diazinon in orally treated Wistar rats (concentration in tissues / concentration in blood) observed relatively higher concentrations of diazinon in the blood than the adipose and surrounding tissues, indicating low tissue absorption and retention (Garcia-Repetto et al., 1995). These studies provide evidence for low tissue accumulation of diazinon.

b. Diazinon and Lactation:
While urine is known to be the major route for diazinon excretion in animals, some studies have evaluated diazinon residues in the milk of treated cattle. Milk from three of five dairy cows dusted with 2% diazinon had detectable levels of residues (0.048 to 0.516 ppm) at 12 hours after treatment (Bourne and Arthur, 1967). Low levels (0.01% of the dose) of unchanged diazinon were detected in the six to 24 hour samples of milk from a lactating cow fed a capsule containing 20 mg/kg of the radioactively labeled insecticide (Robbins et al., 1957). In a study in Australia, cattle were fitted with ear tags (20% active ingredient diazinon) to protect them against buffalo flies. Dairy or butter fat was found to carry 0.01 mg/kg diazinon 58 days after treatment. The maximum residue level in milk fat was found seven days after tagging, at 0.04 mg/kg (Spradbery and Tozer, 1996). The diazinon levels in the fatty tissue of cattle were comparable to the levels detected in butterfat, indicating very little tissue accumulation.

However, in other studies, diazinon fed to lactating cows at 500 ppm of dry matter in silage, or at 2.5 mg/kg did not cause detectable residues to be excreted in the milk (Derbyshire and Murphy, 1962; Lloyd and Matthysse, 1971). Lactating goats exposed to a single dose of 150 mg/kg or 700 mg/kg did not have detectable levels of DETP in the milk (Mount, 1984). These studies indicate that milk is not a major route for excretion of diazinon, although low levels of this insecticide may contaminate milk of lactating animals that are treated with diazinon.

c. Metabolism and Excretion of Diazinon in Mammals:
Absorption through the gastrointestinal tract, metabolism and excretion of diazinon has been observed to be rapid in most mammals, but the yields and rates at which different metabolites are produced may vary in different species (WHO, 1998). Most of the excretion of diazinon metabolites has been observed to be through the urine. Absorption and transplacental transfer of diazinon has been observed in rats (Hoberman et al., 1979).

A human case report indicates that diazinon applied against public lice was rapidly absorbed percutaneously, leading to acute toxicity of the nervous and respiratory systems (Halle and Sloas, 1987). The patient was symptom free by the sixth day, indicating rapid clearance. DEP and DETP have been the major metabolites found in the urine of diazinon-exposed humans (Hayes et al., 1980; Richter et al., 1992). More than half the radioactive diazinon (10 µg /g of whole blood) that was administered i.p. to two female Beagle dogs was recovered as metabolites in the urine within 24 hours (Iverson et al., 1975). DEP and DETP have been found as end products in the urine of diazinon-treated dogs and cows (FAO/WHO, 1993).

Animal studies indicate that the major steps in the metabolic pathway include the hydrolytic and oxidative cleavage of the ester bond leading to the formation of pyrimidinyl derivatives (WHO, 1998). In rats, the major metabolites were 3 pyrimidinols, 2-isopropyl-6-methyl-4(1H)-pyrimidinone, 2-(alpha-hydroxyisopropyl)-6-methyl-4(1H)-pyrimidinone and its beta isomer (FAO/WHO, 1993). Diazoxon, a toxic but transient intermediate was found in trace amounts in the urine. The chemical structures of some of the polar metabolites have not yet been identified. In a study of male Wistar rats and ddy mice injected
with 20 or 100 mg/kg diazinon, peak blood concentrations were observed one to two hours after treatment (700 ng/ml and 80 ng/ml, respectively), followed by a rapid decrease within 24 hours to 50 ng/ml. No oxidative metabolites were detected in the blood. The accumulation was highest in the kidneys (Tomokuni et al., 1985).

In vitro studies on biotransformation of diazinon by liver microsomes of various species have identified hydroxydiazinon, isohydroxydiazinon, dehydroxydiazinon, their oxons and diazoxon as transient intermediates. The yield of these metabolites varied between different species (FAO/WHO, 1993).

VII. Summary and Recommendations for Classification

A. Breast Cancer Risk:

We propose that diazinon 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). This is based on the following:

- **Human studies:** There have been no published studies on breast cancer incidences in women who may have been exposed to diazinon in the past.

- **Animal studies:** No increase in incidence of mammary gland neoplasms was reported in diazinon fed mice or rats (Kirchner et al., 1991; NCI, 1979).

- **Related mechanisms:** There is little evidence on diazinon’s potential to affect breast cancer risk through other mechanisms. Diazinon was not estrogenic in animal studies or in vitro (Barnes, 1988; Cockrell et al., 1966; Giknis, 1989; Rudzki et al., 1991; Soto et al., 1995). Diazinon was not found to be genotoxic in most systems in which it was tested. Diazinon was found to cause pulmonary tumor promotion in female mice (Maronpot et al., 1986). It did not promote GST-P positive foci in rats (Kato et al., 1995). Diazinon induced the cell proliferation rates of intestinal cells (Greenman et al., 1997), but not of human breast cancer epithelial, MCF-7 cells (Soto et al., 1995). Diazinon was found to disturb the developing immune system in mice in one study (Barnett et al., 1980). Further studies are needed to determine whether diazinon impairs the immune system’s ability to fight cancer.

While the evidence above does not show that diazinon increases breast cancer risk, it should be noted that this conclusion is based on the limited scientific evidence that is currently available. There is evidence of non-cancer related clinical effects (neurotoxicity and pancreatitis) from diazinon exposure (WHO, 1998). We recommend that diazinon be used with caution, following all the recommended label guidelines to reduce unnecessary exposure.

VIII. Identification of Research Gaps, and Other Recommendations

- Diazinon has been used widely in agricultural and non-agricultural settings. However, no studies were found on breast cancer incidence rates in women with past exposure to diazinon. Since there is currently no evidence to suggest that diazinon may increase breast cancer risk, we have not recommended an epidemiological study of breast cancer rates in exposed populations. However, animals that were treated with sprays or dips containing diazinon, or have worn flea collars over a long time should be followed for incidences of cancer of the lymphoid tissue and other cancers.

- Case reports of diazinon poisonings and studies in rats indicate that mammalian pancreas may be a target organ for diazinon’s toxicity. Further animal studies are needed on diazinon’s effects on mammalian pancreas.

- Diazinon’s effect on hepatic corticosterone hydroxylases and whether it leads to endocrine disruption needs further study.

- Populations that have been exposed to diazinon should be monitored for their immune responsiveness.

IX. Summary of New Human Studies Currently Being Conducted

**Studies of Occupational Cancer—Pesticides.**

Alavanja, M., Blair, A., Zahn, S., NCI (extracted from the CancerNet at NCI and Personal Communication)

The “Agricultural Health Study” is evaluating 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.

**Strategy to Identify Non-Additive Response to Chemicals.**

Vogel, J.S., University of California, Livermore (extracted from the CRISP Database)

Mice will be exposed to different multiple combinations of OP at environmentally realistic doses to evaluate if there is a non-additive effect to multiple chemicals of this class at low doses.

**Occupational Injury in Hispanic Farmworker Families.**

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 OP 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.
Exposure to Diazinon and Other OP among mixer/loader/applicators applying dormant oil/OP Sprays to Almond Orchards.

R. I. Krieger, University of California, Riverside (extracted from a meeting abstract)

Urine analysis will be used to survey the exposure of OP mixers, loaders and applicators to diazinon and other OP, to measure the extent of absorption and the protection offered by different clothings. Worker exposure will be surveyed in different indoor and outdoor settings in which diazinon is typically used.

Role of Diazinon in Gulf War Illnesses.

A Presidential Committee on Gulf War Veteran’s Illnesses (extracted from the web site http://www.gwvi.gov/ch4.html)

Diazinon 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 diazinon. 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).
X. Bibliography


Wright, C. G., and Leidy, R. B. (1980). Air samples in vehicles and buildings turn up only very low levels of organic phosphate insecticides. Pest Control, 22-26, 68.


XI. Appendix A. Common Abbreviations, Acronyms and Symbols

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACGIH</td>
<td>American Conference of Governmental Industrial Hygienists</td>
</tr>
<tr>
<td>ADI</td>
<td>acceptable daily intake</td>
</tr>
<tr>
<td>AI</td>
<td>active ingredient</td>
</tr>
<tr>
<td>ATSDR</td>
<td>Agency for Toxic Substances and Disease Registry</td>
</tr>
<tr>
<td>BCERF</td>
<td>Program on Breast Cancer and Environmental Risk Factors in New York State, based in Cornell’s Center for the Environment, Institute for Comparative and Environmental Toxicology</td>
</tr>
<tr>
<td>CAS</td>
<td>Chemical Abstract Service</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control and Prevention</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CI</td>
<td>chlorine</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>Co</td>
<td>company</td>
</tr>
<tr>
<td>CRISP</td>
<td>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)</td>
</tr>
<tr>
<td>DEN</td>
<td>diethylnitrosamine</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulphonate</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>E-SCREEN</td>
<td>screening assay for estrogenicity that measures proliferative response in estrogen-dependent breast tumor cells</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GST-P</td>
<td>Glutathione S-transferase P</td>
</tr>
<tr>
<td>HA</td>
<td>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 noncancerous health effects when consumed for no more than the time period specified, with a margin of safety</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer, headquartered in Lyon, France</td>
</tr>
<tr>
<td>ICET</td>
<td>Institute for Comparative and Environmental Toxicology</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>i.p.</td>
<td>interperitoneal</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>L</td>
<td>liter</td>
</tr>
<tr>
<td>lbs</td>
<td>pounds</td>
</tr>
<tr>
<td>m</td>
<td>meter</td>
</tr>
<tr>
<td>MAFF</td>
<td>United Kingdom Ministry of Agriculture, Fisheries and Food</td>
</tr>
</tbody>
</table>

MCF-7: Michigan Cancer Foundation; cells derived from human breast tumor
MCS: multiple chemical sensitivity
MCL: Maximum Contaminant Level; enforceable limit set by EPA which sets the maximum level of a contaminate in a public drinking water supply
µg: microgram
mg: milligram
MTD: maximum tolerated dose
n: number of subjects/animals in the group
NCI: National Cancer Institute
NHL: non-Hodgkin’s lymphoma
NIH: National Institutes of Health
NOAEL: no observable adverse effect level
NOPES: Non-Occupational Pesticide Exposure Study
NTIS: National Technical Information Service; repository for federal agency technical reports
NTP: National Toxicology Program
US: United States
USDA: United States Department of Agriculture
USEPA: United States Environmental Protection Agency
USGS: United States Geological Survey
WHO: World Health Organization

Symbols:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>alpha</td>
</tr>
<tr>
<td>β</td>
<td>beta</td>
</tr>
<tr>
<td>γ</td>
<td>gamma</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µM</td>
<td>micromolar</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>&lt;</td>
<td>less than</td>
</tr>
<tr>
<td>&gt;</td>
<td>greater than</td>
</tr>
<tr>
<td>%</td>
<td>percent</td>
</tr>
<tr>
<td>p</td>
<td>p value</td>
</tr>
<tr>
<td>±</td>
<td>plus or minus</td>
</tr>
<tr>
<td>=</td>
<td>equal to</td>
</tr>
<tr>
<td>®</td>
<td>registered trademark</td>
</tr>
</tbody>
</table>
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 (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 US 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-revised 10/98 sms

I. Chemical Information
   A. Common Name
   B. Chemical Name(s)
   C. Chemical Formula(s)
   D. CAS # (Chemical Abstract Service Number)
   E. Chemical Structure
   F. Trade Name(s)
   G. Trade Names of Mixtures
   H. Major Metabolite(s)/Breakdown Products

II. History of Use, Usage
   A. History of Usage and Uses
   B. Current Usage (when applicable)

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

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

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

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

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

VIII. Identification of Research Gaps, and Other Recommendations

IX. Brief Summaries of New Human Studies Currently Being Conducted

X. Bibliography

XI. Appendix A. Common Abbreviations, Acronyms and Symbols

XII. Appendix B. Critical Evaluations of Breast Cancer Risk
BCERF Breast Cancer Risk Classification Scheme: (adapted from the IARC Preamble by S.M. Snedeker; revised 12/97, 10/98 sms)

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 strong 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 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 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, or (b) 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.