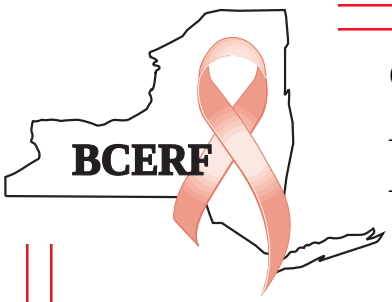

Cornell University
Program on Breast Cancer and Environmental Risk Factors in New York State (BCERF)*

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Critical Evaluation of Simazine's Breast Cancer Risk

by

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Authors' Note: The reader is encouraged to read Appendix B prior to reading this Critical Evaluation. Appendix B includes an explanation of the approach used in writing BCERF Critical Evaluations and an explanation of the BCERF Breast Cancer Risk Classification System.

I. Chemical Information

A. Common Name: Simazine (Meister, 1998)

B. Chemical Name: 6-chloro-*N*,*N*'-diethyl-1,3,5-triazine-2,4-diamine (Meister, 1998)

C. Chemical Formula: $C_7H_{12}ClN_5$ (Montgomery, 1993)

D. CAS Registry Number: 122-34-9 (Meister, 1998)

E. Chemical Structure: (WSSA, 1994)

Simazine

F. Trade Names*: Trade names include: Agrisimazina® (Cequisa); Caliber® 90 (Norvartis); Drexel® Simazine (Drexel Chemical Co.); Gesatop® (Norvartis); Herbanzin® (Herbitecnica Industria de Defensivos S/A); Princep® (Norvartis); Sanazine® (Sanachem Ltd.); Sarvave® (Lainco, S.A.); Simanex® (Makhteshim-Agan); Simapron-50® (Probelte, S.A.); Simatylone® (Chimac-Agriphar S.A.); Simazin® (Sintagro Ltd.); Simazina Atanor® (Atanor S. A.); Simatylone® (Chimac-Agriphar S.A.); Simin® (Industrie Chimiche Caffaro S.p.A.); Sim-Trol® (Sostram Corp.); and Totazina® (Diachem S.p.A.) (Meister, 1998).

G. Trade Names of Mixtures*: Derby®, simazine + metolachlor (Norvartis); Duacit®, simazine + terbutryn (Luxan B.V.); Herbimix®, simazine + atrazine (Herbitecnica Industria de Defensivos); Linusim SA®, simazine + linuron (Sintagro Ltd.); Pathclear®, simazine + diquate + paraquat (ZENECA Agrochemicals); Sartax®, simazine + diuron + metazachlor (CFPI Agro); Simatrol® 55, simazine + atrazine + amitrole (Industrie Chimiche Caffaro S.p.A.); Simazat®, simazine + atrazine (Drexel Chem. Co.); Simazol®, simazine + amitrole (Makhteshim-Agan); Terbutrex Combi®, simazine + terbutryn (Makhteshim-Agan); Terraklene®, simazine + paraquat (Sopra); Topanex®, simazine + diuron + glycophosate (Aragonesas Agro); Trevi 10® simazine + diuron (Calliope S.A.); Tropazin®, simazine + glyphosate (Herbitecnica Industria de Defensivos) (Meister, 1998)

***Note:** Trade names are used herein for convenience and informational purposes only. No endorsement of products is intended and no criticism of unnamed products is implied. Trade names of simazine and mixtures containing simazine listed here are those currently in use in 1998.

H. Major Transformation Products:
Simazine can be photodegraded to produce low levels of the mono-*N*-deethylated metabolite (2-chloro-4-[ethylamino]-6-amino-*s*-triazine); the di-*N*-deethylated metabolite (2-chloro-4,6-bis[amino]-*s*-triazine); and hydroxy simazine (2-hydroxy-4,6-bis[ethylamino]-*s*-triazine). Photodegredation is considered to be a minor route of degredation of simazine in surface soils (WSSA, 1994).

Hydrolysis of simazine can occur in the roots of tolerant plants to produce hydroxy simazine. This occurs through a benzoxazinone-catalyzed hydrolysis (WSSA, 1994; Kearney and Kaufman, 1969). Hydrolysis of simazine has also been reported under controlled conditions in soil treated with simazine (Harris, 1967). The *N*-dealkylation of side chains can also occur in some plants. In some tolerant plants, including corn, a transformation route includes conjugation of simazine with glutathione (chemical structure/formula not specified) (WSSA, 1994). The major metabolite of simazine by aerobic microbial degradation in soil is hydroxy simazine (WSSA, 1994). Under anaerobic conditions, transformation products in soil have included dealkylated forms of simazine, 2-chloro-4-(ethylamino)-6-amino-*s*-triazine and 2-chloro-4,6-bis(amino)-*s*-triazine; and dechlorinated hydroxylated products, 2-hydroxy-4,6-bis(ethylamino)-*s*-triazine, and 2-hydroxy-4-ethylamino-6-amino-*s*-triazine (IARC, 1991).

In vitro studies, using ^{14}C radiolabled simazine and hepatic microsomes isolated from mice and rats, have shown that simazine is metabolized by cytochrome P-450 enzymes primarily via *N*-dealkylation by the loss of the ethyl group to form 2-chloro-4-

(ethylamino)-6-amino *s*-triazine. Very small amounts of the didealkylated product, 2-chloro-4,6-diamino-*s*-triazine, were detected (Adams et al., 1990). Male Charles River rats fed simazine were found to excrete the dealkylated 2-chloro-4-(ethylamino)-6-amino *s*-triazine, as well the di-deethylated form, 2-chloro-4,6-diamino-*s*-triazine, in their urine (Bradway and Moseman, 1982).

Hydroxy Simazine
(2-hydroxy-4,6-bis[ethylamino]-*s*-triazine)

Mono-dealkylated Simazine, via deethlyation
(2-chloro-4-[ethylamino]-6-amino-*s*-triazine)

Di-dealkylated Simazine
(2-chloro-4,6-bis[amino]-*s*-triazine)

II. History of Use and Usage

A. History of Use and Usage:
Simazine is in the *s*-triazine herbicide family. It was first registered for use in 1957 by Ciba (now Norvartis), and in the mid-1990s Ciba produced 80 to 90 percent of the technical product (USEPA, 1994). Simazine is a selective systemic pre-emergence herbicide used in the United States (U.S.) for control of broadleaf and grassy weeds primarily on corn (40%), citrus crops (22%), alfalfa (11%) and grapes (7%) (Gianessi and Puffer, 1991). It is also used to control weeds on strawberries, peaches, cherries, apples, pears, cranberries, certain nuts, olives, pineapples, asparagus, sugar cane, tea and coffee. Its non-cropland uses include weed control in industrial areas (vacant lots), right-of-ways, established Bermuda grass, in turf grass sod production, golf fairways, and ornamental and tree nursery stock (Meister, 1998). Simazine was used in lakes and ponds to control submerged weeds and algae, and as an algaecide in swimming pools and hot tubs until its registration for many aquatic uses were canceled in the 1980s and mid-1990s (see section III on 'Regulatory Status, EPA') (Tomlin, 1994; USEPA, 1994).

B. Current Usage:
Simazine use on U.S. croplands during 1990-93 was estimated to be 3.98 million pounds (lbs) of active ingredient (AI). It is ranked as the 20th most used herbicide in the U.S. (Gianessi and Anderson, 1995b). In California, 1.1 million lbs was used in 1993, mostly to control weeds on grapes, citrus, fruit and nut crops, and right-of-ways (Bartowiak et al., 1995). Use on New York State (NYS) cropland during 1991-93 was estimated to be 88.9 thousand lbs of AI, making simazine the 9th most used herbicide in NYS (Gianessi and Anderson, 1995a). The U.S. Environmental Protection Agency (EPA) has estimated that 1.9 to 3.3 million lbs of simazine (AI) is used per year for non-crop uses (Gianessi and Puffer, 1991).

III. Current Regulatory Status

A. Regulatory Status, EPA:
Simazine was classified as a Restricted Use Pesticide in 1984 by the EPA because of concern for its potential to contaminate ground water. The restricted use classification was withdrawn in 1985, but the EPA required labeling to include a ground water advisory, and aquatic invertebrate toxicity statements. Simazine is currently classified as a General Use Pesticide.

In 1993, EPA conducted a risk assessment for simazine use as an algaecide in swimming pools, hot tubs and whirlpools. The risk assessment concluded that water treated with simazine algaecides represented an unacceptable cancer and non-cancer health risk to children and adults. Most registrants voluntarily canceled their use of simazine algaecides effective April 15, 1994. Registration for remaining algaecide products which were not voluntarily canceled were canceled through a Notice of Intent of Cancel announced in the Federal Register on July 7, 1994 (USEPA, 1994). In November of 1994, the EPA placed simazine, along with the triazine herbicides atrazine and cyanazine, under Special Review. The EPA initiated the Special Review because the agency

concluded that these three *s*-triazines are possible human carcinogens, and that exposure to these herbicides from consuming treated foods, and / or contaminated water may pose a cancer risk. Other concerns included potential risks to pesticide applicators and mixers exposed to triazine pesticides (USEPA, 1994). It is expected that the EPA may make its preliminary decision regarding the regulatory status of simazine in 1998, with a final decision anticipated sometime in 1999 (BNA, 1997).

B. Drinking Water Standards and Health Advisories:

1. MCL: The EPA has set Maximum Contaminant Level (MCL) for simazine in drinking water at 0.004 mg/ L (USEPA, 1996). The MCL is an enforceable limit for the maximum allowable concentration of a chemical in public drinking water supplies.

2. HA: Health Advisory (HA)* levels for simazine in drinking water are as follows:

10 kg child:

- One-day = 0.07 mg/L
- Ten-day = 0.07 mg/L
- Longer term 0.07 mg/L

70 kg adult:

- Longer term 0.07 mg/L
- Lifetime = 0.002 mg/L

* The HAs are nonenforceable limits of the concentration of the chemical in drinking water that is not expected to cause any adverse non-carcinogenic health effects when consumed for no more that the time period specified, with a margin of safety (USEPA, 1996).

C. Food Residue Tolerances:

The Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) are responsible for monitoring the levels of simazine residues in domestic and imported foods and animal feeds. Because of new legislation set forth in the 1996 Food Quality and Protection Act, the tolerances for simazine may be reset according to the new guidelines. Until the tolerances are reset, current tolerance levels for simazine residues on raw agricultural commodities and animal feed are: meat or fat portions of meat from animals, 0.02 ppm (includes cattle, goats, horses, poultry and hogs); eggs, 0.2 ppm; milk, 0.2 ppm; nuts, 0.1 to 0.25 ppm; most stone, pom and citrus fruits and berries, 0.25 ppm; and animal fodder and grasses, 0.25 to 15 ppm. The combined tolerances for residues of simazine and its metabolites 2-amino-4-chloro-6-ethylamino-*s*-triazine and 2,4-diamino-6-chloro-*s*-triazine include: 0.2 ppm for bananas and 12 ppm for fish (USEPA, 1998).

IV. Summary of Evidence of Overall Carcinogenicity

A. Human Studies (non-breast sites):

1. Case-Control Studies of Agricultural Workers:

Two case-control studies on the effect of triazine exposure on the incidence of ovarian cancer have been conducted on female farm workers in the Alessandria province of Northern Italy (Donna et al., 1984; Donna et al., 1989).

Authors' Note: We have provided a summary of the study by Donna, et al. (1984) because it has been frequently cited as evidence of a relationship between triazine herbicide exposure and the risk of ovarian cancer. However, the study only mentions that exposure to "herbicides" were assessed by interview in this study, and there is no mention of specific exposure to triazine herbicides anywhere in the paper. In a later study published by the same authors in 1989, it noted that in the first 1984 study interviewed subjects frequently reported exposures to triazine herbicides, and that in the 1970s there was a high use of triazines, especially atrazine, and to a lesser extent, simazine, in the region of Italy where both studies were conducted.

The 1984 study included 60 women with primary mesothelial ovarian tumors and 127 hospital based cancer-controls with no evidence of ovarian cancer that were matched by year of diagnosis, age ($\pm$ 2.5 years) and residence. Exposure was estimated by means of a questionnaire as follows: "definite exposure"- the subject or next of kin described personal use of herbicides; "probable exposure"- subject was a farmer after 1960 when use of herbicides was extensive, or resided in areas with known herbicide usage; or "no exposure"- denied personal use of herbicides. An elevated, but nonsignificant relative risk (RR) of 2.20 (95% Confidence Interval [CI] 0.77-6.32) was observed in the women " probably" exposed to herbicides, while a significantly elevated RR of 4.38 (95% CI 1.90-10.07) was reported in the women with ovarian cancer who were exposed to herbicides (combined "definite" and "probable" exposure groups) as compared to the cancer case-controls (Donna et al., 1984).

A subsequent study using more appropriate population-based controls found that women "definitely" exposed to triazines had a significantly elevated risk (RR = 2.7; 90% CI 1.0-6.0, p = 0.10) for ovarian neoplasms compared to case-controls. Those who were "possibly" exposed to triazines had a lower RR of 1.8 (90% CI 0.9-3.5) (Donna et al., 1989). This study was based on 65 women with diagnosed ovarian neoplasms, and 126 controls (two for each case, matched for age $\pm$ 5 years) who were recruited by random selection from electoral rolls of surrounding towns. Triazine exposure was assessed by means of an interview-administered

questionnaire. Criteria for "definitely exposed" to triazines included "subjects who were involved in the preparation or use of triazine herbicides or who worked in corn cultivation with reported use of herbicides" (Donna et al., 1989). It should be noted that this study used 90% rather than the usual 95% CI in their statistical analysis.

This study has been criticized because of the possibility of selection bias (Minder, 1990). This included interviewer bias, since the interviewer may have been able to distinguish between the cases, and the controls who were recruited much later. Minder also suggested that cases with higher-socioeconomic status may have not sought treatment at the local hospital, but may have sought treatment elsewhere in larger cities, resulting in an incomplete recruitment of ovarian cancer cases. The authors responded to the concerns of Minder, stating that the interviewers were not aware of the disease state of those interviewed, and their response also discussed the completeness of recruitment of ovarian cancer cases (Crosignani et al., 1990).

While other researchers have evaluated relationships between agricultural triazine exposure in Midwestern states, none of these studies specifically reported whether these populations were exposed to simazine. Since cyanazine and atrazine are the predominant triazine herbicides used for weed control in the corn belt of the Midwest, it is probable that the "triazine" exposures in the Midwestern studies were predominantly to atrazine and cyanazine. For this reason, we have not included these triazine studies in the simazine Critical Evaluation, but they are included in the cyanazine and atrazine Critical Evaluations.

There is a paucity of studies on agricultural exposure to simazine and cancer risk in fruit and nut growing states that use simazine for weed control, such as California and Florida. Because of the large migrant population involved in the work force in these states, including women and children, it presents the problem of assessing health risks in an exposed mobile population. Assessment of health risks in migrant farm workers is also difficult because this population does not always have access to or seek out adequate health care, and they may be exposed to multiple pesticides over the course of the year.

2. Cohort Mortality Studies of Manufacturing/Production Workers:

The mortality among males who worked at triazine herbicide manufacturing plants was recently reported by Sathiakumar, et al. (1996). Women were not included in this study because of insufficient numbers of subjects. The study examined the Standardized Mortality Ratios (SMRs = observed number of deaths among cases, multiplied by 100, divided by the number expected based on general population mortality rates) for all cancer deaths and cancer deaths at specific sites. The study was based on reported deaths during a 16 year period from 1960 to 1986 of 2,683 men with definite or probable manufacturing exposure to triazine herbicides, and 2,234 men with possible exposure to triazines. No information was available on the types of triazines manufactured at the plants. Most of the definitely / probably exposed group (83%) began triazine-related work between 1960 through 1979, but only 12% were exposed for more than five years. In the group possibly exposed to triazines, 30% had been exposed to triazines for more than five years. The SMRs were not elevated for overall cancer mortality in the probable / definitely triazine exposed group (SMR = 85, 95% CI 46-142). This may indicate a "healthy worker effect" in this cohort.

In this same cohort, the risk of mortality from non-Hodgkin's lymphoma (NHL) was elevated in the triazine-exposed workers, but it was not statistically significant (SMR = 385, 95% CI 79-1,124). This was observed for a small number of cases (three observed, 0.78 expected), and two of these NHL cases had worked for less than one year in triazine-related jobs. There was no other cancer that had an elevated SMR in this cohort. It was noted that the insecticide DDT, other insecticides, and fungicides were produced at the plants from 1952 to 1962, but information on exposure of the workers to these chemicals, or previous employment history of chemical exposures was not provided. The follow-up time may not have been sufficiently long in this cohort to detect an increase in exposure-related cancer mortality, since only 21% of the definite/probable group were followed for more than 20 years (Sathiakumar et al., 1996).

3. Summary, Human Carcinogenicity (non-breast sites):

There is evidence of increased risk of ovarian cancer in women agricultural workers exposed to triazine herbicides in one population-based case control study conducted in Italy (Donna et al., 1989). A nonsignificant elevation in deaths from NHL was observed in men employed and exposed to triazine herbicides that may have included exposure to simazine (Sathiakumar et al., 1996).

B. Experimental Animal Studies (non-breast sites):

Chronic feeding studies have reported the induction of livers tumors in male rats and pituitary tumors in female rats fed simazine for up to two years, while in mice, there have been no reports of an oncogenic effect of simazine. It should be noted that the three long-term studies describing simazine's oncogenic potential in rodents were only available in a summary or abstract form. Complete details of the experimental design, tumor incidence, tumor pathology, and other results were not always available. Available results from the studies evaluating simazine's oncogenic potential in experimental animals are summarized below.

1. Mice:
The "Drinking Water Criteria Document for Simazine" included a summary of an unpublished mouse study that assessed the oncogenicity of simazine (Dynamac, 1990). A summary of this study (Hazelette and Green, 1988) was also cited in the EPA Special Review Document on triazines (USEPA, 1994), and has been included in the EPA Integrated Risk Information System's (IRIS) carcinogenic assessment for simazine (IRIS, 1997). Male and female Crl:CD1 (1CR) BR mice, 60 per sex per dose, were fed diets containing 0, 40, 1000, and 4000 ppm simazine for 95 weeks. There was no evidence of a carcinogenic effect of simazine in this study. However, the adequacy of the experimental design of this study can not be determined due to the limited amount of information available. It was noted in the Dynamac (1990) and IRIS database (1997) summaries stated that body weights were significantly depressed ($p<0.01$) in the mid- and high-dose simazine treatment groups compared to controls.

2. Rats:
An unpublished two-year cancer bioassay evaluating the carcinogenic effect of simazine in Sprague-Dawley (SD) rats was available in summary form in the "Drinking Water Criteria Document for Simazine" (Dynamac, 1990), and in the EPA IRIS database (1997). It has also been summarized and cited as an unpublished Ciba-Giegy sponsored-study (McCormick and Arthur, 1988) in the EPA Special Review Document on simazine (USEPA, 1994). Some of the results of this study have been published by in a study authored by Stevens et al. (1994).

Sprague-Dawley (SD) rats, 50 of each sex per dose, were fed 0, 10, 100, and 1000 ppm simazine in the diet for two years. In contrast to the mouse cancer bioassay, there was a significant increase in the incidence of hepatic carcinomas in the 100 ppm dose group , and for combined liver adenoma / carcinoma in males fed the 1,000 ppm simazine diet (Dynamac, 1990). However, commentary on this study in the EPA Special Review Document on triazines stated that the incidences of the liver tumors fell within the range for historical controls (USEPA, 1994).

Although there were statistically significant dose-related trends for kidney tubule carcinomas, and for combined adenomas/ carcinomas in the male and female-simazine treated rats, the kidney tumors were only detected in the 1000 ppm group. There were no significant differences in the incidence of kidney tumors in the simazine treated animals in a pairwise comparison to concurrent controls (USEPA, 1994). In the same study, there was a significant increase ($p<0.01$) in pituitary carcinomas in the female rats fed diets containing 1000 ppm simazine (6/80; 8%) compared to controls feed 0 ppm simazine (1/90; 1%). There were no treatment related differences in the incidence of pituitary adenomas, though the incidence was high in 0 ppm controls (73/90; 81%), and in all simazine-treated groups of female rats (70%-80% incidence of pituitary adenomas) (Stevens et al., 1994; USEPA, 1994). (Note: A significant increased incidence of mammary fibroadenomas and adenocarcinomas where observed in the high-dose 1000 ppm treatment group of this study; these results are discussed in Part V., Section B.2 of this document).

Several studies evaluating the oncogenicity of long-term administration of simazine in rodents found no evidence of tumors in animals treated with simazine; however these studies were not appropriate cancer bioassays because of inadequate number of doses and insufficient number of animals per dose (Innes et al., 1969), or an inappropriate use of a subcutaneous route of administration for the test compound (IARC, 1980; Pliss and Zabezhinsky, 1970). Donna, et al. (Donna et al., 1981) detected a significantly higher ($p<0.01$) number of lymphomas in female Swiss mice (3/24) injected with Fogard S, a formulation containing atrazine and simazine, compared to saline injected controls (0/50). However, this study is of limited value for evaluating carcinogenicity of simazine, because of the inappropriate route of administration (intraperitoneal), limited duration of treatment (13 doses over 39 days), inadequate follow-up time (up to 1 year), and loss of animals due to disease (Donna et al., 1981).

3. Summary, Animal Experimental Studies (non-breast sites):
Simazine administration in experimental animal cancer bioassays resulted in a significantly higher incidence of hepatic tumors in male and female rats (McCormick and Arthur, 1988), and pituitary tumors in female rats (Dymamac, 1990; Stevens et al., 1994; USEPA, 1994) fed high-doses of simazine in two-year cancer bioassays. Evidence of carcinogenicity was not observed in long-term feeding studies conducted with simazine treated mice (Hazelette and Green, 1988).

C. Current Carcinogenesis Classifications by Other Agencies:
1. IARC Classification: Group 3, not classifiable as to its carcinogenicity. IARC concluded that there were not adequate date available on the human or experimental animal carcinogenicity of simazine (IARC, 1991). Only studies that are available in the open, peer-reviewed literature are included in IARC evaluations. Hence, the unpublished oncogenesis studies of McCormick and Arthur in rats (1988), and of Hazelette and Green (1988) in mice, were not included in the IARC evaluation.

2. NTP Classification: Not rated (USDHHS, 1998)

3. EPA Classification: The EPA Special Review Document for triazines lists simazine as a Class C Carcinogen (possible carcinogen) (USEPA, 1994).

V. Critical Evaluation of the Evidence for Breast Cancer Risk

A. Human Studies:
No case-control human epidemiology studies were located that evaluated breast cancer incidence or mortality in women exposed to simazine.

B. Experimental Animal Studies:
1. Mice:
There was no evidence of mammary carcinogenicity in mice fed up to 1000 ppm simazine in a 95-week cancer bioassay sponsored by Ciba Geigy (unpublished study, Hazelette and Green, 1988, as cited in USEPA, 1994). The adequacy of this study could not be determined because details on the experimental design of this study were not available.

2. Rats:
Mammary tumors have been observed in a chronic feeding study of simazine in rats (McCormick and Arthur, 1988; Stevens et al., 1994). SD female rats were fed 0, 10, 100, and 1000 ppm simazine in the diet for 104 weeks. The incidence of fibroadenomas, and the incidence of adenocarcinomas of the mammary gland were significantly higher ($p<0.01$) in the only in high-dose 1000 ppm simazine-treated group compared to control animals. The incidence of mammary fibroadenomas was 30% (27/90; number of animals with tumor type/number of animals in dose-group) in the control 0 ppm group, 35% (28/90) in the 10 ppm group, 24% (19/80) in the 100 ppm group, and 51% (41/80) in the 1000 ppm simazine-treated group. The incidence of mammary adenocarcinomas was 18% (16/90) in the 0 ppm control group, 16% (13/80) in the 10 ppm group, 25% (20/80) in the 100 ppm group, and 50% (40/80) in the 1000 ppm simazine-treated group. The incidences of mammary fibroadenomas or adenocarcinomas in the 10 and 100 ppm simazine treated groups were within the range of reported for historical controls (Stevens et al., 1994). There were no significant differences in the incidence of mammary adenomas in the simazine-treated female rats compared to control rats in this study (McCormick and Arthur, 1988 as cited in USEPA, 1994; Stevens et al., 1994).

The mechanism by which simazine induces a higher rate of mammary tumors in female rats fed high doses of simazine has not been established. One of the hypotheses for the induction for mammary tumors in female SD rats is that *s*-triazines may induce changes in the estrous cycle, the hormonally controlled reproductive cycle, which may result in increased serum estrogen levels (Eldridge et al., 1994; Stevens et al., 1994; Wetzel et al., 1994). This hypothesis could not be substantiated by other investigators who found that ovarian hormone levels, including estrogen and progesterone, were depressed in SD female rats treated with the *s*-triazine, atrazine (Cooper et al., 1996). Others have hypothesized that *s*-triazines induce premature reproductive aging in the SD rat (Chapin et al., 1996) and cause mammary tumors to appear earlier in the female SD rat which has a high spontaneous rate of mammary tumor development. Since most of the studies evaluating these hypotheses have been done on atrazine-treated rats, the results of these studies are discussed in the Critical Evaluation on atrazine.

Few studies have evaluated changes in the estrous cycle or early hormonal changes in simazine treated rats. One study reported that rats fed high doses of simazine for two years have changes in serum hormone levels compared to controls (Stevens et al., 1994). Serum estradiol levels were significantly depressed ($p<0.05$) in aging SD rats that had been fed 1000 ppm simazine for 24 months (2 ± 1 ng estradiol /ml) compared to animals receiving 0 ppm simazine (12 ± 6 ng estradiol/ml). Estradiol levels were also lower, but were not statistically significant, in the rats fed the diets containing 10 ppm (8 ± 4 ng estradiol/ml) and 100 ppm (5 ± 2 ng estradiol/ml) simazine compared to controls. Serum progesterone levels were also significantly depressed ($p<0.05$) in SD rats fed 1000 ppm simazine diet (11 ± 9 ng/ml) compared to controls (39 ± 26 ng/ml). Serum prolactin levels were significantly elevated ($p \leq 0.01$) in the 1000 ppm dose group (204 ± 147 ng/ml) compared to 0 ppm controls (29 ± 18 ng/ml) (Stevens et al., 1994).

Whether these hormonal changes contributed to the higher incidence of mammary tumors in the SD rats fed the higher doses of simazine is not known. There is some evidence for a role of prolactin in the growth of mammary tumors in rodents. Early studies by Pearson et al. (1969) demonstrated that injections of prolactin supported the growth of DMBA-induced mammary tumors in ovariectomized / adrenalectomized female rats, and later studies by Koseki et al. (1987) found that prolactin administration increased the levels of estrogen receptor and progesterone receptor in the normal mammary gland, but not in transplanted mammary tumors. The role of prolactin in human breast cancer is less clear, since studies in higher level primates have not shown a role for prolactin in mammary gland development, nor has treatment of women with breast cancer with prolactin-lowering drugs been effective (Kleinberg, 1987).

C. Other Relevant Information on Breast Cancer Risk
1. Evidence of Estrogenicity:
Based on studies reported by several laboratories, simazine is not estrogenic in either *in vitro* or *in vivo* assays. In the E-Screen assay for estrogenicity, simazine did not stimulate cell proliferation in an estrogen-dependent MCF-7 human breast tumor cell line (Soto et al., 1995). A lack of a proliferative response in simazine treated estrogen-dependent MCF-7 breast tumor cells, and PL3 yeast cells was also observed by Connor et al. (1996). No

estrogenic response was demonstrated in simazine-treated HeLa cells transfected with a Gal4-human estrogen receptor chimeric construct and a luciferase reporter gene (Balaguer et al., 1996). Tran, et al. (1996) have reported that simazine was not estrogenic when tested using yeast transfected with the human estrogen receptor (hER) and an estrogen-sensitive reporter. Competition binding assays demonstrated that simazine displaced radiolabeled estradiol from the recombinant hER, suggesting that this herbicide may have some anti-estrogenic activity (Tran et al., 1996).

Several *in vivo* tests have confirmed the lack of simazine's estrogenicity in female SD rats. Ovariectomized rats gavaged with 100 or 300 mg/kg bwt simazine for three days failed to show a significant increase in estrogen-dependent uterine weight gain compared to animals treated with vehicle (Tennant et al., 1994). A lack of an estrogenic uterotropic response in immature uteri was also observed in simazine-treated rats by Connor, et al. (1996). Tennant et al,, (1994), in another experiment found that the administration of 50, 100, or 300 mg simazine /kg bwt for two days significantly depressed ($p<0.05$) estradiol-stimulated tritiated thymidine incorporation into uterine DNA of ovariectomized female SD rats. Lower doses of 1 or 10 mg simazine per kg bwt did not suppress estrogen-stimulated thymidine uptake (Tennant et al., 1994). These tests would suggest that simazine is not estrogenic. The suppression of estradiol-stimulated thymidine incorporation in the uteri of ovariectomized rats suggests that simazine may have some weak anti-estrogenic activity.

2. Reproductive Toxicity:

Mallard ducks exposed to two or 20 ppm simazine prior to the onset of egg laying, and throughout the egg production cycle, displayed no signs of reproductive impairment (Fink, 1975). The reproductive and developmental toxicity of pesticide/fertilizer mixture that was based on levels of pesticides found in California groundwater was assessed in SD rats, and Swiss CD-1 mice (Heindel et al., 1994). This "California" pesticide mixture included two other *s*-triazines, atrazine and cyanazine, among its components. Pesticide mixtures were administered in the drinking water at 1X (0.061 mg simazine /kg bwt/day), 10X and 100X the median concentration reported in California groundwater. These concentrations were well below the Maximum Tolerated Dose (MTD), since these levels were designed to assess health effects of concentrations of pesticides that may actually occur in groundwater. The National Toxicology's Reproductive Assessment by Continuous Breeding protocol was used to evaluate reproductive endpoints, and included measurements of fertility, reproductive performance in the F_0 and F_1 generations, and measures of spermatogenesis. There were no adverse effects reported at any dose of the California pesticide mixture on any reproductive endpoints. There were no treatment-related effects on development. No deaths or dose-related clinical signs of maternal toxicity were reported in treated animals. There were no external, craniofacial, skeletal, viseral or other morphological malformations in pups born to dams treated with drinking water containing simazine during gestation (Heindel et al., 1994).

3. Formation of Co-Carcinogens:

Janzoski, et al (1980) have hypothesized that simazine, which contains a secondary amine group, could react with nitrous oxide in the atmosphere to form nitrosamines. Nitrosomines are carcinogens associated with an increase risk of stomach cancer. Subsequent studies with *in vitro* systems indicated that simazine is capable of undergoing nitrosation by nitrous oxide (Janzowski et al., 1980). However, the extent to which simazine reacts with nitrous oxide to form nitrosomine compounds, and whether such compounds contaminate water supplies or are present in soil has not been determined. Whether nitrosomines can act as co-carcinogens and influence the incidence of non-stomach cancers, such as breast cancer, has not been determined.

4. Mutagenicity:

While the genotoxic properties of simazine have not been studied as widely other *s*-triazines, the weight of evidence suggests simazine is not genotoxic. The Special Review Document on triazines (USEPA, 1994) cites an unpublished mutagenicity study on simazine using the *Salmonella* assay, which was negative (Lasinski et al., 1987). Other studies have evaluated the genotoxic potential of simazine in multiple test systems. The mutagenicity of simazine was tested in bacteria (*S. typhimurium*) and yeast (*S. cervevisiae*) assays with liver microsome activation, plant activation and no activation (Plewa et al., 1984). Simazine tested negative in all *S. typhimurium* genotoxic assays, and only tested positive in a plant activated (maize 1S fraction) *S. cervevisiae* assay. Simazine does not appear to be genotoxic in mammalian *in vitro* systems. Simazine tested negative in lymphocyte sister-chromatid exchange tests, and in alkaline elution assay with rat-hepatocytes, v79 cells, and human lymphocytes (Dunkelberg et al., 1994). Simazine was also found to be non-genotoxic in the chromosome aberration test in human fibroblasts, and did not increase sister chromatid exchange frequency in Chinese Hamster Ovary (CHO) cells (Dunkelberg et al., 1994). Simazine did not demonstrate whole cell clastogenicity in CHO cells exposed two clastogens, adriamycin and araj-C (Biradar and Raybrun, 1995b). The same authors also did not find evidence of chromosomal breakage in CHO cells exposed to simazine at levels similar to those found in public water supplies (Biradar and Rayburn, 1995a). In contrast, simazine tested as Princep 80W (80% simazine) was shown to increase the rate of dominant lethals in *Drosophila melanogaster*. Simazine increased the risk of x-linked lethals, but did not affect the loss of the Y chromosome or the sex chromosome disfunction (Murnik and Nash, 1977).

VI. Other Relevant Information

A. Environmental Fate:

1. Soil:

The half-life of simazine in soil is variable, and has been reported to be in the range of as little as 12 days to as long as 2 years. The persistency of simazine is dependent on soil type, organic matter content, pH, temperature, soil microbes and moisture content. Studies that have evaluated the half-life of simazine under different soil conditions are summarized below.

Chen et al. (1983) reported that the half-life of simazine was influenced by climatic conditions in Taiwan. The half-life for simazine was 18 days in the summer months in when it is hot and wet, compared to 24 days in the cooler, drier winter season. These authors also presented a table which summarized the half-lives of simazine in soils from Canada, Central Europe and Taiwan. Soils from Saskatchewan and Alberta, Canada had the longest half-life ranging from 88 to 101 days. Shorter half-lives were reported for central European countries (Germany, Italy, Holland) that ranged from 54 to 30 days, while Taiwan had the shortest half-life for simazine in soil, in the range of 14 to 18 days (Chen et al., 1983).

The organic carbon content has also been found to influence the half-life of simazine in soil in field studies. In acidic soil (pH 5.4-5.5) from New Zealand, the half-life of simazine was 25 days when the organic carbon level was 4.6%, and the half-life increased to 32 days in soil with organic carbon levels of 9.4%. The authors also provided some information that suggested that higher application rates of simazine increased the persistency of this herbicide in soils (Rahman and Holland, 1985). One of the shortest half-lives published was reported in soil obtained from a Spanish citrus orchard. The half-life of simazine was only 12 days in sandy clay with a pH of 7.4 to 7.5 (Redondo, 1997).

Several reference texts and reviews have also reported half-lives for simazine. Ahrens (WSSA, 1994) has reported that the half-life of simazine in sandy loam under aerobic conditions was 91 days, while the half-life was 70 to 77 days under anaerobic conditions. In an EPA summary, simazine's half-life under controlled aerobic conditions was 110 days, while under anaerobic conditions, the half-life was approximately two years (USEPA, 1994). A recent review of the transport of pesticides in field soils reported that the average half-life of simazine from ten field experiments was 60 days, but information was not provided on the field conditions of these studies (Flury, 1996). The "Drinking Water Criteria Document for Simazine" estimated the half-life of simazine to be between four and six months in soil, and 50 to 70 days in water (Dynamac, 1990).

Many studies have evaluated the persistence of simazine in agricultural soil used for crop weed control. However, simazine was also applied at high rates to control weeds in irrigation ditches. Smith et al. (1975) determined the persistence of simazine applied at 22.4 kg/ha in irrigation ditches in September of 1970. Soil samples from ditch sides, bottoms, and water samples from the ditch and water basin, were taken twice a year over the next three years. After initial application in September, 1970, simazine residues were 12.5 ppm in the ditch bottom and 4.7 ppm in the ditch side, based on a core soil sample taken at 0-15 cm. Samples taken in the upper 7.5 cm had consistently higher levels of simazine residues than samples taken from greater depths. Simazine was persistent in the upper layers of the ditch soil, with the levels only slightly decreasing over three years. Simazine residues in the 0 to 7.5 cm layer from the ditch side were 5.5 ppm in June of 1971 compared to 3.5 ppm in September of 1973. Simazine residues in deeper layers were of less magnitude, usually less than 1 ppm in samples taken at a depth of 7.5 to 90 cm in the ditch bottom or sides during the three year sampling period. Simazine was still detected in water samples taken from the ditch (42 ± 35 ppb) and basin (5 ± 5 ppb) after the sixth irrigation in June of 1973. Simazine residues were not detected in water samples taken after the tenth irrigation in September of 1973. This study indicates that simazine residues can persist in irrigation ditches after application, and are detected in the upper levels of soil from irrigation ditches, and from basin water samples 2 1/2 to 3 years after application.

2. Surface water:

While simazine has been detected in surface waters from Midwestern states, Florida, and several Northeastern states, nearly all of the maximum levels reported are below the MCL for simazine.

The EPA (1994) has compiled summaries of surface water monitoring studies for simazine and other *s*-triazines in the Midwestern corn belt. These summaries were largely based on studies conducted in the mid-1980s to the early 1990s. While the triazine herbicides atrazine, cyanazine, and simazine were frequently detected in the surface waters of the corn belt, simazine was detected less often, and at lower concentrations. For instance, the median concentration of the maximum levels of simazine detected ranged from 0.07 to 0.78 µg per L, compared to 0.83 to 22 µg per L for atrazine, and 0.45 to 4.4 µg per L for cyanazine. This may be due to the lower amounts of simazine, compared to the other triazines, used in controlling weeds on corn fields in the Midwest (USEPA, 1994).

The 1994 EPA report noted that there was little information available on surface waters which drained from areas where simazine was used on non-corn crops, such as orchards or nut trees (USEPA, 1994). One recent study has been published on residues of simazine, and other pesticides, in the canals of southern

Florida (Miles and Pfeuffer, 1997). In this region, the primary use of simazine is in citrus orchards. There were 744 pesticide detections, 74 of these detections were simazine. The highest concentration reported was 2.5 µg per L which is below the MCL of 4 µg per L (mean and median values were not available) (Miles and Pfeuffer, 1997).

There is some information available on simazine levels in surface waters of the Northeastern U.S. The U.S. Geological Survey determined the concentration of pesticides from 46 sites on 42 streams and rivers that fed into the Hudson River Basin of western New York State. Simazine was detected in 28% of the sampled sites, with median concentrations of 0.13 µg per L, and a maximum concentration of 0.55 µg per L (Wall and Phillips, 1997). None of the samples exceeded the MCL for simazine. In water samples obtained from several Vermont streams during 1992 and 1993, 11.8% of the samples (n=17) from the Hungerford Brook had detectable levels of simazine. The mean concentration of simazine was 0.15 µg per L with a maximum concentration of 0.2 µg per L (Gruessner and Watzin, 1995).

3. Groundwater:
Simazine was one of the most commonly detected pesticides in well water in the EPA National Pesticide Survey. The survey estimated that 0.2% of all rural domestic wells (25,000 wells) and 1.1 % of community supply wells (1,080 in number) would have detectable levels of (at least 0.38 µg/L) of simazine in the U.S. (USEPA, 1990). Simazine was the most frequently reported pesticide in the 1995 California Well Water survey (Bartowiak et al., 1995). In California, current uses of simazine include weed control on grapes, citrus, other fruits, nuts and on right-of-ways. Of the 2,202 wells sampled, 142 wells had verified detections of simazine. Levels ranged from 0.05 to 1.0 µg per L; none of the detections exceeded the MCL of 4.0 µg of simazine per L. These levels are lower than those reported in California wells in early 1983 (0.5 to 3.5 µg per L) by the California Dept. of Food and Agriculture as cited by Ritter (1990).

The U.S. Geological Survey, as a part of the National Water-Quality Assessment Program (NAQUA), determined the levels of pesticides in shallow ground water during 1993-95 in 20 major hydrologic basins in the U.S with different land uses (Kolpin et al., 1998). While very low levels of triazine residues were widely detected, samples rarely exceeded the MCL set by the EPA for drinking water. The three pesticide residues detected most frequently (percentage of sites with positive detections) were atrazine (38.2%), deethylatrazine (34.2%) and simazine (18%). However, there were no simazine samples which exceeded the MCL of 4.0 µg/L. The maximum concentration detected for simazine in groundwater was 1.30 µg/L. The land use settings that reported detections of simazine in at least 10% of the sampled sites included: corn and alfalfa (28.0 %), corn (26.2 %), wheat (43.0%), wheat and small grains (11 %), orchard/vineyard (31.7 %), and urban settings (10 %). The only detections that are inconsistent with simazine's uses is the detection in groundwater where wheat crops were grown. Simazine is not usually used as a herbicide on wheat crops, though it is used on alfalfa. The authors suggested that the detections of simazine in groundwater associated with wheat growing sites may be due to the permeable soils and intensive irrigation associated with the wheat crop areas sampled.

B. Dietary Cancer Risk:
Contamination of water supplies with simazine and its breakdown products is the most often cited route of exposure to this herbicide. However, in the EPA's Special Review Document on triazines (USEPA, 1994), concern was expressed regarding dietary cancer risk of simazine and simazine breakdown products. As has been stated previously, the EPA has set tolerance levels for the maximum levels of simazine and its breakdown products in raw agricultural products and animal feed (USEPA,1998). In the Special Review Document on triazines, calculations of cancer risk posed by ingestion of residues of simazine or its breakdown products in food were calculated (see Table 2 in USEPA, 1994). These upper bound cancer risk estimates were calculated from anticipated residues of commodities known to be treated with simazine, the percent of the crop treated with simazine, and the estimated exposure in mg/kg/day to simazine. Simazine's total estimated dietary cancer risk from all commodities was calculated to be 1.1×10^{-5}.

VII. Summary and Recommendation for Breast Cancer Risk Classification

Based on the lack of available studies that have evaluated the incidence of breast cancer in simazine-exposed populations, limited evidence of mammary carcinogenicity in experimental animal studies, and limited evidence of mechanisms by which simazine can affect breast cancer risk, we conclude there is inadequate evidence to rate simazine as a human breast carcinogen, and recommend that it be classified **in Group 3, *unclassifiable as to its breast cancer risk*** (see Appendix B for the BCERF Breast Cancer Risk Classification System). This evidence is summarized below.

- **Human Studies:** Case-control studies have not been published that have evaluated the effect of exposure to simazine on the risk of developing breast cancer in women. Therefore, we could not adequately assess whether or not simazine directly causes breast cancer in humans.

- **Animal Experimental Studies:** There is limited evidence that simazine is a mammary carcinogen in experimental animals. This

evidence is based on one animal study which reported a significant elevation of fibroadenomas and adenocarinomas only in the female SD rats receiving the highest dose of simazine (1000 ppm). A significant increase in the incidence of these mammary tumors was not reported in the 10 and 100 ppm simazine treated groups compared to 0 ppm controls (McCormick and Arthur, 1988; Stevens et al., 1994).

• **Related Mechanisms:** There is no compelling evidence to show that simazine is a co-carcinogen, tumor promoter, or increases normal or neoplastic cell proliferation rate in human breast cells or tissue. There is no evidence that simazine is estrogenic (Connor et al., 1996; Soto et al., 1995; Tennant et al., 1994; Tennant et al., 1994). The mechanism by which simazine induces mammary tumors when fed at high levels to SD female rats has not been established, though it has been suggested that *s*-triazine compounds may alter hormonal profiles in the SD female rat that may influence the development of mammary tumors in this strain (Chapin et al., 1996; Eldridge et al., 1994; Stevens et al., 1994; Wetzel et al., 1994).

Author's Comment: While there is inadequate evidence to rate simazine as a human mammary carcinogen, this compound does have a high usage in the U.S., especially on fruit crops, including citrus, grapes, and apples. Simazine is commercially available as a pre-mix with atrazine, another *s*-triazine for which there is sufficient evidence of mammary tumor induction in experimental animals. It is likely that these two compounds are metabolized similarly, and may have commonalties with regard to mode of action to induce mammary cancer in animal models. Therefore, there may be additive effects in regard to their exposure when used simultaneously in weed control. Individuals that are involved in the manufacturing of *s*-triazines; handle, mix or apply simazine-containing products; work in fields or orchards treated with simazine; or consume water from highly contaminated rural wells that are near pesticide mixing operations, have the highest likely hood of being exposed to simazine and its breakdown-products.

VIII. Research Gaps and Recommendations for Further Research

• Case-control human epidemiological studies are needed to determine if there is a higher incidence of cancers of the breast or ovaries in females exposed occupationally to simazine and atrazine. This includes women employed in manufacturing facilities, as pesticide applicators, and other agricultural workers exposed to these triazine pesticides. These studies are needed to confirm or negate the original findings of Donna, et al., suggesting that these triazines are ovarian carcinogens and to determine if either triazine poses a risk for cancer in other hormonally-sensitive tissues such as the breast. If a relationship is found between simazine exposure and ovarian/breast cancer risk in occupationally exposed opulations, then studies would need to be extended to other populations with exposures to simazine. This would include those who have lived on orchards or farms that have used simazine, those who have handled or laundered clothing contaminated with simazine, and those who have lived in areas with historical problems with simazine-contaminated drinking water supplies.

• Studies are needed to further characterize the nature of and persistency of simazine transformation products in soils and foods, and whether any of these breakdown products have the potential to induce adverse health effects.

• Further studies are needed to determine the mechanism by which simazine-induces mammary tumors in female SD rats and the relevance of these mechanisms to humans.

• Studies are needed to develop biomarkers for simazine, its breakdown products, and metabolites in humans, in order to more accurately assess exposures in human populations.

IX. Summary of Studies Currently Being Conducted:

The following studies were abstracted from the CRISP database, which lists studies funded by federal agencies (i.e. NIH, EPA, USDA), or where obtained through personal communications with the principal investigators.

Agricultural Health Study; joint intramural research, NCI and NIEHS

Dr. Michael Alavanja, Project Officer, NCI
(personal communication with Dr. Alavanja)

This 10-year prospective study, which is its fourth year, will follow 90,000 farmers, commercial pesticide applicators, and spouses of farmers and applicators in Iowa and North Carolina. The survey will document pesticide usage by questionnaire, and in a subset of the population, actual pesticide exposures will be measured in the urine and blood using validated biomarkers. Information will also be gathered on home use of pesticides, as well as agricultural uses of pesticides. This study is unique, since it will include one of the largest cohorts of female pesticide applicators ever followed, as well as including the female spouses of farmers and pesticide applicators. Approximately 58,000 men and 32,000 women are enrolled in this study. Case-control breast cancer, and ovarian cancer studies as well as other case-control studies of cancer are planned.

Biomarkers of Exposure to Hazardous Substances
Dr. Bruce D. Hammock, University of California at Davis
(adapted from 1997 CRISP database)

This study will include developing rapid immunochemical assays to detect pesticides, and environmental breakdown products of pesticides. Triazines are one group of pesticides that have been targeted for development and validation of these immunoassays. The researchers have also proposed to develop assays to assess human exposure to triazines by measuring triazine metabolites, including triazine mercapturate.

Studies of Manufacturing Workers Exposed to Triazines
Ciba-Giegy Corp (personal communication; letter from Mr. Kerry Miller, Regional State Gov. Relations Manager for Ciba, dated October 21, 1996).

Epidemiological studies are ongoing to monitor workers exposed to simazine at production facilities. However, since entrance of women into these facilities is relatively recent, it is possible that this small female cohort may not have been monitored for a sufficient period of time to date to warrant valid conclusions.

X. Bibliography

Adams, N. H., Levi, P., and Hodgson, E. (1990). *In vitro* studies of the metabolism of atrazine, simazine, and terbutryn in several vertebrate species. J. Agric. Food Chem. *38*, 1411-1417.

Balaguer, P., Joyeux, A., Denison, M. S., Vincent, R., Gillesby, B. E., and Zacharewski, T. (1996). Assessing the estrogenic and dioxin-like activities of chemicals and complex mixtures using *in vitro* recombinant receptor-reporter gene assays. Can. J. Physiol. Pharmacol. 74, 216-222.

Bartowiak, D., Newhart, K., Pepple, M., Troiano, J., and Weaver, D. (1995). Sampling for Pesticide Residues in California Well Water; 1995 Update of the Well Inventory Data Base (Sacremento, CA: California Environmental Protection Agency, Dept. of Pesticide Regulation).

Biradar, D. P., and Rayburn, A. L. (1995a). Chromosomal damage induced by herbicide contamination at concentrations observed in public water supplies. J. Environ. Qual. *24*, 1222-1225.

Biradar, D. P., and Rayburn, A. L. (1995b). Flow cytogenetic analysis of whole cell clastogenicity of herbicides found in groundwater. Arch. Environ. Contam. Toxicol. *28*, 13-17.

BNA (1997). Drinking Water; Changes predicted in final regulation to protect ground water from herbicides. Environ. Rep. *28*, 317-318.

Bradway, D. E., and Moseman, R. F. (1982). Determination of urinary residue levels of the *N*-dealkyl metabolites of triazine herbicides. J. Agric. Food Chem. *30*, 244-247.

Chapin, R. E., Stevens, J. T., Hughes, C. L., Kelce, W. R., Hess, R. A., and Daston, G. P. (1996). Symposium overview: Endocrine modulation of reproduction; paper presented, Eldridge, J.C., Stevens, J.T., Wetzel, L.T., Tisdel, M.O., Brechenridge, C.B., McConnell, R.F., and Simpkins, J.W. Atrazine: Mechanisms of hormonal imbalance in female SD rats. Fundam. Appl. Toxicol. *29*, 1-17.

Chen, Y., Duh, J., and Wang, Y. (1983). The influence of climate and soil properties on the degradation of simazine in soils in Taiwan. Proc. Natl. Sci. Counc. Repub. China *7*, 36-41.

Connor, K., Howell, J., Safe, S., I., C., Liu, H., Berhane, K., Siarretta, C., and Zacharewski, T. (1996). Failure of chloro-*s*-triazine-derived compounds to induce estrogenic responses *in vivo* and *in vitro*. (abstract) (New Orleans: 1996 American Chemical Society Meeting).

Cooper, R. L., Stoker, T. E., Goldman, J. M., Parrish, M. B., and Tyrey, L. (1996). Effect of atrazine on ovarian function in the rat. Reprod. Toxicol. *10*, 257-264.

Crosignani, P., Donna, A., and Berrino, F. (1990). Re: "Triazine herbicides and ovarian epithelial neoplasms" [letter]. Scand. J. Work Environ. Health *16*, 446-447.

Donna, A., Betta, P.-G., Robutti, F., Crosignani, P., Berrino, F., and Bellingeri, D. (1984). Ovarian mesothelial tumors and herbicides: A case-control study. Carcinogenesis *5*, 941-942.

Donna, A., Betta, P. G., Gagliardi, F., Ghiazza, G. F., Gallareto, M., and Gabutto, V. (1981). Preliminary experimental contribution to the study of possible carcinogenic activity of two herbicides containing atrazine-simazine and trifuralin as active principles. Pathologica *73*, 707-721.

Donna, A., Crosignani, P., Robutti, F., Betta, P. G., Bocca, R., Mariani, N., Ferrario, F., Fissi, R., and Berrino, F. (1989). Triazine herbicides and ovarian epithelial neoplasms. Scand. J. Work Environ. Health *15*, 47-53.

Dunkelberg, H., Fuchs, J., Hengstler, J. G., Klein, E., Oesch, F., and Strüder, K. (1994). Genotoxic effects of the herbicides alachlor, atrazine, pendimethaline, and simazine in mammalian cells. Bull. Environ. Contam. Toxicol. *52*, 498-504.

Dynamac Corp. (1990). Drinking Water Criteria Document for Simazine (Rockville, MD: Criteria and Standards Division, Office of Drinking Water, U.S. Environmental Protection Agency).

Eldridge, J. C., Tennant, M. K., Wetzel, L. T., Breckenridge, C. B., and Stevens, J. T. (1994). Factors affecting mammary tumor incidence in chlortriazine-treated female rats: Hormonal properties, dosage, and animal strain. Environ. Health Perspect. *102 (Suppl 11)*, 29-36.

Fink, R. J. (1975). The effect of simazine on the reproductive capability of mallard ducks. Toxicol. Appl. Pharmacol. *33*, 188-189.

Flury, M. (1996). Experimental evidence of transport of pesticides through field soils - A review. J. Environ. Qual. *25*, 25-45.

Gianessi, L. P., and Anderson, J. E. (1995a). Pesticide Use in New York Crop Production (Washington, D.C.: National Center for Food and Agricultural Policy).

Gianessi, L. P., and Anderson, J. E. (1995b). Pesticide Use in U.S. Crop Production (Washington, D.C.: National Center for Food and Agricultural Policy).

Gianessi, L. P., and Puffer, C. (1991). Herbicide Use in the United States: National Summary Report (Washington, D.C.: Resources for the Future).

Gruessner, B., and Watzin, M. C. (1995). Patterns of herbicide contamination in selected Vermont streams detected by enzyme immunoassay and gas chromatography/mass spectrometry. Environ. Sci. Technol. *29*, 2806-2813.

Harris, C. I. (1967). Fate of 2-chloro-*s*-triazine herbicides in soil. J. Agric. Food Chem. *15*, 157-162.

Hazelette, J. R., and Green, J. D. (1988). Simazine Technical: 95-Week Oral Toxicity/Oncogenicity Study in Mice, April 4, 1988, MRID No. 40614404, Study No. 842121. (Testing Facility: Pharmaceuticals Division, Ciba-Geigy Corp.).

Heindel, J. J., Chapin, R. E., Gulati, D. K., George, J. D., Price, C. J., Marr, M. C., Myers, C. B., Barnes, L. H., Fail, P. A., Grizzle, T. B., Schwetz, B. A., and Yang, R. S. H. (1994). Assessment of the reproductive and developmental toxicity of pesticide/fertilizer mixtures based on confirmed pesticide contamination in California and Iowa groundwater. Fundam. Appl. Toxicol. *22*, 605-621.

IARC. (1991). Simazine. In IARC Monographs on the Evaluation of Carcinogenic Risk to Humans; Occupational Exposures in Insecticide Application and Some Pesticides (Lyon: IARC, World Health Organization), pp. 495-513.

Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Fishbein, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchell, I., and Peters, J. (1969). Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. J. Nat. Cancer Inst. *42*, 1101-1114.

IRIS (1997). Simazine, CASRN 122-34-9, April 1, 1997 (<http://www.epa.gov/ngispgm3/iris/subst/0263.htm>: EPA Integrated Risk Information Service).

Janzowski, C., Klein, R., and Preussmann, R. (1980). Formations of *N*-nitroso compounds of the pesticides atrazine, simazine, and carbaryl with nitrogen oxides. In *N*-Nitroso Compounds: Analysis, Formation and Occurrence (IARC Scientific Publications No. 31), E. A. Walker, L. Griciute, M. Castegnaro and M. Borzsonyi, eds. (Lyon: IARC), pp. 329-339.

Kearney, P. C., and Kaufman, D. D. (1969). Miscellaneous Herbicides. In Degradation of Herbicides, P. C. Kearney and D. D. Kaufman, eds. (New York: Marcel Dekker, Inc), pp. 356-363.

Kleinberg, D. L. (1987). Prolactin and breast cancer. N. Engl. J. Med. *316*, 269-271.

Knuesli, R., Berrer, D., Dupuis, G., and Esser, H. (1969) Chapter 2, *s*-Triazines. In: Degradation of Herbicides, Keary, P. C. and Kaufman, D.D. (eds) (New York: Marcel Dekker, Inc.) pp.51-78.

Kolpin, D. W., Barbash, J. E., Gillion, R. J. (1998). Occurrence of pesticides in shallow groundwater of the United States: Initial results from the National Water-Quality Assessment Program. Environ. Sci. Technol. *32*, 558-566.

Koseki, Y., Cole, D., Matsuzawa, A., and Costlow, M. E. (1987). Prolactin regulation of estrogen and progesterone receptors in normal and neoplastic mouse mammary tissue. Jpn. J. Cancer Res. *78*, 1105-1111.

Lasinski, E., Kapaghian, J., and Green, J. (1987). Gene Mutation Test: Simazine Technical, MRID NO. 40614406, Study No. 87308; 872269 (Unpublished study prepared by Ciba-Geigy Corp.).

McCormick, C. C., and Arthur, A. T. (1988). Simazine -Technical: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats, April 12, 1988, MRID No.406144-05 Study No.2-001109 (Testing Facility: Pharmaceuticals Division, Ciba Geigy Corp.).

Meister, R. T. (1998). Pesticide Dictionary; Simazine. In Farm Chemicals Handbook '98, Vol. 84, R. T. Meister, ed. (Willoughby, OH: Meister Publishing Company), pp. C 353-C 354.

Miles, C. J., and Pfeuffer, R. J. (1997). Pesticides in canals of south Florida. Arch. Environ. Contam. Toxicol. *32*, 337-345.

Minder, C. E. (1990). Re: "Triazine herbicides and ovarian epithelial neoplasms" [letter]. Scand. J. Work Environ. Health *16*, 445-446.

Montgomery, J. H. (1993). Simazine. In Agrochemicals Desk Reference (Boca Raton: Lewis Publishers), pp. 371-377.

Murnik, M.R. and Nach, C. L. (1977). Mutagenicity of the triazine herbicides atrazine, cyanazine, and simazine in *Drosphila melanogster*. J. Toxicol. Environ. Health *3*, 691-697.

Pearson, O. H., Llerena, O., Llerena, L., Molina, A., and Butler, T. (1969). Prolactin-dependent rat mammary cancer: A model for man? Trans. Assoc. Am. Physicians *82*, 225-238.

Plewa, M. J., Wagner, E. D., Gentile, G. J., and Gentile, J. M. (1984). An evaluation of the genotoxic properties of herbicides following plant and animal activation. Mutat. Res. *136*, 233-245.

Pliss, G. B., and Zabezhinsky, M. A. (1970). On carcinogenic properties of symmetrical triazine derivatives. Vorp. Onkol. *16*, 82-85.

Rahman, A., and Holland, P. T. (1985). Persistence and mobility of simazine in some New Zealand soils. N.Z. J. Exp. Agric. *13*, 59-65.

Redondo, M. J. (1997). Dissipation and distribution of atrazine, simazine, chlorpyrifos, and tetradifon residues in citrus orchard soil. Arch. Environ. Contam. Toxicol. *32*, 346-352.

Ritter, W. F. (1990). Pesticide contamination of ground water in the United States - a review. J. Environ. Sci. Health *25*, 1-29.

Sathiakumar, N., Delzell, E., and Cole, P. (1996). Mortality among workers at two triazine herbicide manufacturing plants. Am. J. Ind. Med. *29*, 143-151.

Smith, A. E., Grover, R., Emmond, G. S., and Korven, H. C. (1975). Persistence and movement of atrazine, bromacil, monuron, and simazine in intermittently-filled irrigation ditches. Can. J. Plant Sci. *55*, 809-816.

Soto, A. M., Sonnenschein, C., Chung, K. L., Fernandez, M. F., Olea, N., and Serrano, F. O. (1995). The E-Screen Assay as a tool to identify estrogens: An update on estrogenic environmental pollutants. Environ. Health Perspect. *103*, 113-122.

Stevens, J. T., Breckenridge, C. B., Wetzel, L. T., Gillis, J. H., Luempert III, L. G., and Eldridge, J. C. (1994). Hypothesis for mammary tumorigenesis in Sprague-Dawley rats exposed to certain triazine herbicides. J. Toxicol. Environ. Health *43*, 139-153.

Tennant, M. K., Hill, D. S., and Eldridge, J. C. (1994). Possible antiestrogenic properties of chloro-*s*-triazines in rat uterus. J. Toxicol. Environ. Health *43*, 183-196.

Tomlin, C. (1994). Simazine [No. 627]. In The Pesticide Manual, C. Tomlin, ed. (Surrey, England: British Crop Protection Council), pp. 913-914.

Tran, D. Q., Kow, K. Y., McLachlan, J. A., and Arnold, S. F. (1996). The inhibition of estrogen receptor-mediated responses by chloro-*s*-triazine-derived compounds is dependent on estradiol concentration in yeast. Biochem. Biophys. Res. Commun. *227*, 140-146.

USDHHS (1998). Report on Carcinogens, Eighth Edition Summary, 1998, I. L. Systems, ed. (Rockville, MD: U.S. Dept. of Health and Human Services, and the National Toxicology Program).

USEPA (1990). National Pesticide Survey; Summary results of EPA's National Survey of Pesticides in Drinking Water Wells (U.S. EPA, Office of Water, Office of Pesticides and Toxic Substances).

USEPA (1994). Atrazine, Simazine and Cyanazine; Notice of Initiation of Special Review. Fed. Reg. *59*, 60412-60443.

USEPA. (1996). Drinking Water Regulations and Health Advisories, EPA 822-B-96-002: Office of Water, U.S. Environmental Protection Agency).

USEPA. (1998). Tolerances and Exemptions from Tolerances for Pesticide Chemicals in or on Raw Agricultural Commodities, 40 CFR 180, Subpart A, B, and C. In Code of Federal Regulations, pp. 273-434.

Wall, G. R., and Phillips, P. J. (1997). Pesticides in surface waters of the Hudson River basin, New York and adjacent states, U.S. Geological Survey fact sheet no. FS 238-96.

Wetzel, L. T., Luempert III, L. G., Breckenridge, C. B., Tisdel, M. O., Stevens, J. T., Thakur, A. K., Extrom, P. J., and Eldridge, J. C. (1994). Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats. J. Toxicol. Environ. Health *43*, 169-182.

WSSA (1994). Simazine. In Herbicide Handbook, 7th, W. H. Ahrens, ed. (Champaign, IL: Weed Science Society of America), pp. 270-272.

XI. Appendix A. Common Abbreviations, Acronyms and Symbols

AI	Active Ingredient
BCERF	Program on Breast Cancer and Environmental Risk Factors in New York State, based the Cornell's Center for the Environment, Institute of Comparative and Environmental Toxicology
bwt	body weight
C	carbon
CAS	Chemical Abstract Service
CfE	Cornell University's Center for the Environment
CI	Confidence Interval
Cl	chlorine
cm	centimeter
CRISP	Computer Retrieval of Information on Scientific Projects; database of scientific intra- and extramural projects supported by the Dept. of Health and Human Services (i.e., NIH, EPA, USDA)
DNA	deoxyribonucleic acid
ER	estrogen receptor
FDA	Food and Drug Administration
H	hydrogen
ha	hectare, equivalent to 2.71 acres or 10,000 square meters
HA	The health advisories are nonenforceable limits of the concentration of the chemical in the drink ing 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
hER	human estrogen receptor
IARC	International Agency for Research on Cancer, headquartered in Lyon, France
ICET	Institute for Comparative and Environmental Toxicology
i.p.	interperitoneal
IRIS	Integrated Risk Information System; EPA risk assessment database
kg	kilogram
L	liter
µg	microgram
mg	milligram
MCF-7	Cells derived from human breast tumor developed by the Michigan Cancer Foundation
MCL	Maximum Contaminant Level; enforceable limit set by the EPA which sets the maximum level of a contaminate in a public drinking water supply
ml	milliliter
n	number of subjects/animals in the group
NAQUA	National Water-Quality Assessment Program
ng	nanograms; one billionth of a gram
N	nitrogen
NHL	non-Hodgkin's lymphoma
NCI	National Cancer Institute
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NTIS	National Technical Information Service; repository for federal agency technical reports
NTP	National Toxicology Program
NY	New York
NYS	New York State
OR	Odds Ratio
ppm	parts per million
ppb	parts per billion
RR	Relative Risk
SD	Sprague-Dawley; rat strain
SMR	Standardized Mortality Ratio; SMR= the ratio of "observed" to "expected" deaths,multiplied by 100.
TWA	Time-weighted average
U.S.	United States
USDA	United States Department of Agriculture
USDHHS	United States Department of Health and Human Services
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
wt	weight

Symbols:

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

XII. Appendix B. BCERF Critical Evaluations of Breast Cancer Risk

This includes an overview of the Critical Evaluations and explanation of the BCERF Breast Cancer Risk Classification Scheme (revised 10/98 sms).

The Process:
Starting Point - Existing Critical Evaluations on Evidence of Carcinogenicity

IARC Monographs (**I**nternational **A**gency for **R**esearch on **C**ancer)
NTP ARC (**N**ational **T**oxicology **P**rogram, **A**nnual **R**eport on **C**arcinogens)
ATDSR (**A**gency for **T**oxic **D**isease **S**ubstance **R**egistry)

Conduct **Literature Searches** using databases to obtain historical and the most recent information; i.e. Toxline, Medline, Biosis, Cancerlit

-**Peer-reviewed scientific literature**-available through Cornell libraries and interlibrary loans.
-**Technical Reports**-NTIS-National Technical Information Service
-**TOXNET databases**—USEPA's IRIS database source of oncogenicity and regulatory status information
-**Gray literature**—Studies submitted to U.S. Environmental Protection Agency (EPA) that are not published—i.e. industry generated oncogenicity studies

-Some abstracts of cancer bioassays are on line (IRIS database)
-Request reports from industry
-Request reports from EPA through Freedom of Information Act

The Critical Evaluation includes some general background information, including: chemical name, chemical formula, Chemical Abstract Subject Registry no. (CAS #), chemical structure, trade name(s), trade names of mixtures, metabolites/degradation products, history of use, and current regulatory status.

Evidence of cancer in other (non-breast) organ systems is provided in synopsis form with some critical commentary, along with the current overall carcinogenicity classification by international (IARC) and U.S. Federal Agencies (NTP, USEPA).

Human epidemiological studies, animal studies, and other relevant studies on possible mechanisms of carcinogenesis are critically evaluated for evidence of exposure to agent and breast cancer risk based on "strength of evidence" approach, according to a modification of IARC criteria as listed in the IARC Preamble. (See attached sheets for a more detailed explanation of the BCERF Cancer Risk classification scheme)

The **emphasis of the document** is a critical evaluation of the evidence for breast cancer risk, classification of the agent's breast cancer risk, identification of research gaps, and recommendations for future studies. A section is devoted to brief summaries of new research studies that are in progress. A bibliography with all cited literature is included in each critical evaluation. Major international, federal and state agencies will be provided with copies of our report.

General Outline of BCERF Critical Evaluations-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. Humans 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. BCERF 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 proto-oncogenes or oncogenes; evidence of inactivation of tumor suppressor gene associated with breast cancer; evidence of adverse effect on immune function; or evidence of a structural similarity to a known breast carcinogen (structure-activity relationship).

Group 3: **Not classifiable** as to its breast carcinogenicity to humans. Agents are placed in this category when they do not fall into any other group.

Group 4: **Probably not a breast carcinogen in humans**: This category is used for agents for which there is evidence suggesting a lack of breast carcinogenicity in human studies and in animal studies, together with a lack of related evidence which may predict breast cancer risk. The absence of studies does **not** constitute evidence for a lack of breast carcinogenicity.

BCERF Breast Cancer Risk Classification Scheme, continued

Brief Definitions of Sufficient, Limited, and Inadequate Evidence: (adapted from the IARC Preamble by S.M. Snedeker)
Human Studies

Sufficient evidence of carcinogenicity in humans: Must have established evidence between exposure to the agent and human breast cancer. Case-reports are given the least weight in considering carcinogenicity data in humans—they are suggestive of a relationship, but by themselves cannot demonstrate causality. Consistent, case-control studies which have controlled for confounding factors and have found high relative risks of developing breast cancer in relation to an identified exposure are given the most weight in determining a causal relationship.

Limited evidence of breast carcinogenicity in humans: A positive association has been observed between exposure to the agent and breast cancer, but chance, bias or confounding factors could not be ruled out.

Inadequate evidence of breast carcinogenicity in humans: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association.

Experimental Animal Studies:

Sufficient evidence of breast carcinogenicity in animals: Evidence of malignant tumors or combination of benign and malignant tumors in (a) two or more species of animals, (b) or two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

Limited evidence of breast carcinogenicity in animals: The studies suggest a carcinogenic effect, but are limited for making a definitive evaluation because: (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (c) the agent increases the incidence of only benign neoplasms of lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains of animals.

Inadequate evidence of breast carcinogenicity in animals: The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations.