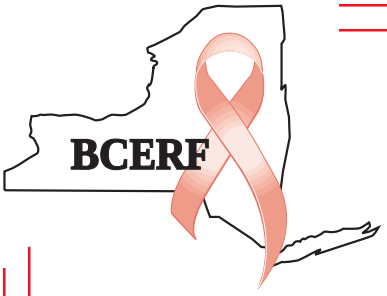

Cornell University
Program on Breast Cancer and Environmental Risk Factors in New York State (BCERF)*

Critical Evaluation # 6
October, 1998

Critical Evaluation of Cyanazine's Breast Cancer Risk

by

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

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Critical Evaluation of Cyanazine's Breast Cancer Risk

Authors' Note: The reader is encouraged to read Appendix B before 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: Cyanazine

B. Chemical Name: 2-(4-chloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropionitrile (IUPAC name) 2-[[4-chloro-6(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropanenitrile (C.A. name) (Tomlin, 1994)

C. Chemical Formula: $C_9H_{13}ClN_6$ (Meister, 1998)

D. CAS Registry number: 21725-46-2 (Meister, 1998)

E. Chemical Structure: (WSSA, 1994)

Cyanazine

F. Trade names*: Bladex® (American Cyanamid Co.; DuPont Agricultural Products; Griffin Corp.); Fortrol® (American Cyanamid Co.); and Cy-Pro® (Griffin Corp.) (Meister, 1998).

G. Trade Names* of Mixtures: Bellater®, cyanazine + atrazine (American Cyanamid Co.); Extrazine II®, cyanazine + atrazine (DuPont Agricultural Products); Cynergy®, cyanazine + atrazine (Griffin Corp.); and Cy-Pro®, atrazine + cyanazine (Griffin Corp.) (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 listed are those currently in use in 1998.

H. Major Transformation Products:

1. Photolysis: There is some degradation of cyanazine by photolysis (USEPA, 1994; WSSA, 1994). This is in contrast to other triazines, atrazine and simazine, which are resistant to photodegradation (USEPA, 1994).

2. Soil: While hydroxy cyanazine is produced rapidly in soils of low pH, and slowly in soils of high pH (WSSA, 1994), this is not the primary route of degradation and occurs to a minor extent. The primary route of degradation is by microbes in the soil that convert the nitrile to an amide group to form cyanazine amide (2-chloro-4-[1-carbamoyl-1-methylethylamino]-6-ethylamino-s triazine) with oxidation to the carboxylic acid, followed by hydrolysis of the ring chlorine to a hydroxyl (Beynon et al. 1972b; 1974; Kolpin et al., 1997a; WSSA, 1994). Unlike other triazines simazine and atrazine, whose major degradation products in soil include deethylated products, *N*-dealkylation of amino-ethyl side chain of cyanazine occurs to a limited extent in soil (Beynon et al., 1972b).

3. Plants: Degradation products in corn plants grown in cyanazine treated soil, include both the amide, and hydroxyl acid forms, as well as transformation products formed by the loss of the *N*-ethyl group to form dealkylated products (Beynon et al., 1972b).

4. Aquatic Ecosystems: The main transformation product of cyanazine detected in water of an aquatic ecosystem is the *N*-deethylated form (2-[4-chloro-6-amino-*s*-triazin-2-ylamino)-2-methylpropionitrile). Other transformation products include cyanazine amide, and the *N*-deethylated form of cyanazine amide (Yu et al., 1975).

5. Metabolism in Mammals:

The metabolism and excretion of cyanazine and its metabolites has been studied in the rat (Crayford and Hutson, 1972; Hutson et al., 1970). The primary urinary metabolites are formed via *N*-deethylation, and conjugation with glutathione to form *N*-acetylcysteinyl metabolites (mercaputuric acids) (Crayford and Hutson, 1972; Hutson et al., 1970). The major urinary metabolites detected in the urine of cyanazine treated rats were *N*-acetyl-S-[4-amino-6-(1-methyl-1-cyanoethylamino)-*s*-triazinyl-2]-L-cysteine and 2-chloro-4-amino-6-(1-methyl-1-cyanomethylamino)-*s*-triazine. Other metabolic pathways include the dechlorination of

cyanazine to yield a 2-hydroxy triazine. The hydroxyl triazine 2-hydroxy-4-(ethylamnio)-6-(1-carboxy-1-methylethyl-amino)-*s*-triazine has been identified as the major fecal metabolite excreted in rats (Crayford and Hutson, 1972).

II. History of Use and Usage:

A. History of Use:
Cyanazine is a pre- and post-emergent herbicide used to control annual grasses and broad leaf weeds. It is in the *s*-triazine family of herbicides. Cyanazine was first registered for use by the Shell Chemical Company in 1971 (Stevens and Sumner, 1991). In the United States (U.S.), over 90% of its use is on corn (Gianessi and Puffer, 1991; USEPA, 1994), usually as a pre-emergence herbicide (Tomlin, 1994). Some of its highest use is in the corn-belt states of the Midwest. It has been estimated that 20% of the corn grown in Iowa is treated with cyanazine, or cyanazine in combination with other herbicides (USEPA, 1994). Cyanazine has also been used as a post-emergence herbicide to control weeds in barley, cotton and wheat crops (Tomlin, 1994). Other uses have included control of broadleaf weeds in oilseed rape, soybeans, sugarcane, potatoes, peas and in forestry (Tomlin, 1994; WSSA, 1994). Until cancellation of its registration in 1999 (see 'Regulatory Status' section), cyanazine will continue to be used as a pre-and post-emergent herbicide to control annual grasses and broadleaf weeds (USEPA, 1996a).

B. Current Usage:
Cyanazine ranked as the 5th most used herbicide in the U.S. during 1990-93. During this time, use on cropland was 32 million pounds (lbs) of active ingredient (AI) per year (Gianessi and Anderson, 1995b). Cyanazine ranked third in herbicide usage in New York State (NYS) with 647 thousand lbs of AI used annually during 1991-93 (Gianessi and Anderson, 1995a).

III. Current Regulatory Status

A. Regulatory Status:
Cyanazine has been classified by the U.S. Environmental Protection Agency (EPA) as a Restricted Use Pesticide (RUP) since 1984 (USEPA, 1994). Its RUP classification was based on the detection of cyanazine in ground and surface water. In 1988, because of evidence of developmental toxicity in experimental animals, the EPA required the use of protective equipment to be worn by applicators during mixing and loading operations (Meister, 1998; USEPA, 1994).

In 1994, the EPA placed cyanazine and two other *s*-triazine herbicides, simazine and atrazine, under Special Review because of concerns about potential cancer risks and exposure through food and water, and risks related to occupational exposure to applicators during mixing and loading (USEPA, 1994).

On August 2, 1995, the DuPont Company, then the primary manufacturer and registrant of cyanazine, voluntarily proposed to amend its cyanazine registrations to incrementally reduce cyanazine maximum application rates in 1997, 1998, and 1999, and to terminate the production of cyanazine for use in the U.S. by December 31, 1999. Existing stocks of cyanazine end use products released for shipment up to December 31, 1999 could be distributed, sold and used through September 30, 2002. Upon accepting these terms from the registrants (DuPont and Griffin Corp.), the EPA terminated its Special Review of cyanazine on July 25, 1996 (USEPA, 1996a).

B. Drinking Water Standards and Health Advisories:
1. MCL: The EPA has set the Maximum Contaminant Level (MCL) for cyanazine in drinking water at 0.001 mg/ L (USEPA, 1996b). 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 cyanazine in drinking water are as follows:

10 kg child:
- One-day = 0.1 mg/L
- Ten-day = 0.1 mg/L
- Longer term = 0.02 mg/L

70 kg adult
- Longer term 0.07 mg/L
- Lifetime = 0.001 mg/L

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

C. Food Residue Tolerances:
The EPA sets food residue tolerances for allowable levels of cyanazine residues in food. The Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) are the federal agencies responsible for monitoring the levels of cyanazine residues in domestic and imported foods and animal feeds. Current EPA tolerances for cyanazine are as follows: corn for fodder or forage, 0.2 ppm; sweet corn, 0.5 ppm; cottonseed and sorghum, 0.05 ppm; and wheat as grain, straw or fodder, 0.1 ppm (USEPA, 1998).

IV. Evidence of Overall Carcinogenicity (non-Breast sites)

A. Human Studies:
Studies conducted in agricultural workers or in manufacturing workers exposed to cyanazine, or triazines that may have included cyanazine, have not found an increased risk for cancer at non-breast sites. These studies are presented below.

1. Case-Control Studies of Agricultural Workers
A study of the risk of leukemia and exposure to specific pesticides was conducted on 578 white men in Iowa and Minnesota who were diagnosed with leukemia (Brown et al., 1990). The controls (n = 1245) were population based, and matched for age by five year age group, state of residence and vital statistics at the time of the interview. Exposure to pesticides was estimated by means of a standardized questionnaire. There was no increased risk of leukemia among men reported having mixed, handled, or applied cyanazine (Odds Ratio [OR]=0.9; 95% Confidence Interval [CI] 0.5-1.6), based on 38 cases and 108 controls (Brown et al., 1990).

A population-based case control study conducted on men residing in Kansas reported a significant association between the use of triazines (atrazine, cyanazine, metribuzin, prometrone, propazine, terbutryn) and the risk of non-Hodgkin's lymphoma (NHL) (OR= 2.5; 95% CI 1.2-5.4), based on 14 cases and 43 controls (Hoar et al., 1986). However, when the use of other herbicides such as phenoxyacetic acid herbicides (i.e. 2,4-D) were controlled for, the risk of NHL associated with triazine exposure was no longer significant (OR=1.9; 95% CI 0.4-8.0). Cases and controls (three controls per case) were matched for vital status and age ($\pm$ 2 years), but no other cancer risk factors. This study also used questionnaires in their interviews of cases, controls, or next of kin, to obtain information on specific pesticides used, years and number of acres treated, application method, and use of protective equipment (Hoar et al., 1986). There was no other attempt to quantify pesticide exposure other than use of the questionnaire.

A case-controlled study of white men with non-Hodgkin's lymphoma (NHL) and risk associated with specific exposures to agricultural pesticides, was conducted in Iowa and Minnesota by Cantor et al. (1992). History of pesticide use, first and last year of use, and whether the respondent had ever personally mixed, applied or handled certain pesticides was obtained by administering a questionnaire. Information was also obtained on socioeconomic characteristics, medical and occupational history, and known or suspected risk factors for NHL. Population-based controls were matched by five-year age group, vital status as time of the interview, and state of residence. Among those who had ever handled or used cyanazine, the risk of NHL was not significantly elevated in NHL cases (n=27) compared to controls (n=64), with a reported OR of 0.9 (95% CI 0.6-1.5). The ORs were adjusted for age, state, and cancer risk factors, including history of cigarette smoking and family history of lymphopeietic cancer (Cantor et al., 1992).

The relationship between exposure to specific pesticides and the risk of multiple myeloma (MM) was evaluated in 173 white men and 650 controls residing in Iowa (Brown, et al., 1993). While cases and controls were matched for age (by five year age groups), and vital status, cases and controls were not matched for geographic area of residence or occupation. A questionnaire was used to obtain information on general farm activities, use of specific pesticides, and whether the subject had personally handled or applied the pesticide. The risk of MM for mixing, handling, or applying cyanazine was not significantly elevated (RR=1.2; 95% CI 0.06-2.4), based on a small sample of 11 cases and 51 controls. Though this study did not detect a significantly increased risk for any specific pesticide and MM, the sample size was relatively small, and may not have been large enough to detect risks associated with the use of specific pesticides.

While there are other reports of exposure to *s*-triazine herbicides and risk of cancer at various sites, none of these studies specifically estimated exposure to cyanazine. It is not known if the "triazine" exposure cited in these studies was to a combination of triazines, or to one triazine in particular. Therefore, these studies are of limited value in evaluating the cancer causing potential of cyanazine. But, since the three triazines (atrazine, simazine and cyanazine) are of similar chemical structure, structure activity relationships may exist. Also, cyanazine is commonly used in combination with atrazine as a herbicide to control weeds on corn crops. For this reason, we have briefly summarized below studies that have evaluated cancer risk or cancer mortality with exposure to "triazines". These are studies where the authors have estimated exposure to triazines collectively, and have not provided data on exposure to a specific *s*-triazine herbicide(s).

A small, international nested case control study failed to find a positive association between the risk of NHL in male manufacturing workers exposed to triazines, or in pesticide applicators that had used triazines (OR=0.7; 95% CI 0.1-3.1). In the same study, there was no excess risk of soft tissue sarcoma in men exposed to triazines occupationally (OR=0.7; 95% CI 0.04-11.8). In this study, pesticide exposures were estimated for cases and controls from information found in individual job records, work histories, and company exposure questionnaires (Kogevinas et al., 1995).

A case-control study in Iowa did not find a significant elevation in the risk of MM in white Iowa males (OR=1.29; 95% CI not provided) exposed to triazines (Burnmeister, 1990). Cases were based on diagnosed MM identified by the state Health Registry of

Iowa, and pathology specimens were evaluated to confirm the diagnosis. Controls were matched for five-year age group, sex, and for deceased cases, year of death. Exposures to specific pesticides were estimated by questionnaire (next of kin for deceased subjects), however little information was provided on the types of questions used to determine and assess the extent or duration of exposure to triazine herbicides.

There is comparatively little information on cancer risks in women with agricultural exposures to triazine. In a population case-control study conducted in eastern Nebraska, risk of NHL in women who had lived or worked on farms that had used triazine herbicides was not significantly increased (OR=1.2; 95% CI 0.6-2.6), based on 12 cases and 38 controls (Hoar Zahm et al., 1993). Risk was slightly elevated, but was not significantly significant in those who had personally handled triazines (OR=2.2; 95% CI 0.1-31.5). However, it should be noted that this was based on a very small number of subjects; one case and two controls (Hoar Zahm et al., 1993).

A case-control population study conducted in Kansas evaluated exposure to pesticides and colon cancer risk (Hoar et al., 1985). Cases were obtained from the Kansas cancer registry from 1976-82, and controls were selected from the general population by random digit dialing, from Medicare files, and from state mortality files for deceased cases. The risk of developing colon cancer in those who reported employment on a farm was not significantly elevated (OR=1.6; 95% CI 0.8-3.5), based on 57 cases and 662 controls. Very few of the colon cancer cases (n=2) and controls (n=43) reported exposure to triazines (OR=1.4; 95% CI 0.2-7.9). This is too small a sample size for any meaningful conclusion on the use of triazines and the risk of colon cancer. No information was provided on how exposure to specific pesticides was assessed or estimated in cases and controls (Hoar et al., 1985).

2. Cohort Mortality Studies of Manufacturing Workers:

There are two reports on cancer mortality in men exposed to triazine through employment in plants manufacturing triazines (Sathiakumar et al., 1992; Sathiakumar et al., 1996). Unfortunately, no information was provided on the specific triazines manufactured at these plants. In the 1992 study, mortality was evaluated in 4,434 men employed in an agrochemical plant in Alabama. Subjects had to be employed at least one month between the years 1951-87 to be included in the study. Mortalities were compared U.S. death rates to compute standardized mortality ratios (SMR = observed deaths X 100/expected deaths). The agricultural products produced in the plant included organochlorine (DDT) and organophosphate insecticides; triazine herbicides (specific type of triazine not provided), fungicides, miticides, and micronutrients. While deaths from cancer of the buccal cavity and pharynx (SMR=388; 95% CI 125-905), esophagus (SMR=417; 95% CI 112-1,076), and lung (SMR=150; 95% CI 94-227) were all elevated. However, there was no attempt to control for potential confounding factors, including the use of tobacco products. Since there was no attempt to control for exposure histories to specific pesticides, it is not possible to determine if triazine exposure played a role in the increased cancer risk of these manufacturing workers.

The 1996 study evaluated a cohort of 2,683 men who had a definite or probable exposure to triazines, and 2,234 men with possible triazine exposure; no women were included in this study (Sathiakumar et al., 1996). Cancer mortality in the cohort was compared to U.S. cancer death rates or to local state cancer mortality statistics to compute SMRs. Men in the definite/probably exposed group had a SMR of 90 (95% CI 43-166) for all cancer deaths, indicating lower cancer death rate than the general population. There was some evidence of increased mortality from NHL, with three deaths observed and 0.78 expected (SMR=385; 95% CI 79-124). But, two of the three the men that had died from NHL had been employed in triazine related jobs for less than one year. In those possibly exposed to triazines, there was not a statistically significant excess of cancer deaths (SMR = 120; 95% CI 80-172). However, this cohort may have been too young (only 13% were older than 45 years) or had too short a duration of follow-up (only 27% had 20 or more years of follow-up) to detect excess cancer mortality due to triazine exposure (Sathiakumar et al., 1996). Future studies should include women in the cohort, should attempt to characterize the type of triazine exposure, and should include controls from similar types of occupations who have not been exposed to triazines.

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

While there is little information on cancer incidences or mortalities in populations exposed to cyanazine, the available published studies have not found a relationship between occupational cyanazine exposure and NHL (Hoar et al., 1986; Cantor et al., 1992), leukemia (Brown et al., 1990) or multiple myeloma in men (Brown et al., 1993). Other case-control studies on triazine exposure that may have included exposure to cyanazine also do not support a causal relationship between triazine exposure and NHL in men or women (Hoar Zahm et al., 1993; Kogevinas et al., 1995; Sathiakumar et al., 1996); or multiple myeloma (Burnmeister, 1990), soft-tissue sarcoma or colon cancer (Hoar et al., 1985) in men. It should be noted that most of the studies that have evaluated cancer risk in agricultural workers exposed to cyanazine have used questionnaires to estimate exposures. There have been no attempts to assess exposure by means of biomarkers, and recall bias may have influenced the accuracy of the estimated exposures to specific classes of pesticides.

B. Experimental Animal Studies (non-breast sites):
A published two-year feeding study evaluating cyanazine's carcinogenicity was conducted by the Shell Chemical Company (Walker et al. 1974). Two unpublished studies evaluating cyanazine's oncogenic potential in mice (USEPA, 1991) and rats (Bogdanffy, 1990) were cited and summarized in the EPA Special Review Document for triazines (USEPA, 1994).

1. Mice:
The results from the unpublished mouse oncogenesis study indicated that dietary administration of cyanazine did not increase the incidence of tumors in treated CD-1 mice compared to controls (USEPA, 1994; USEPA, 1991). It was not possible to evaluate the adequacy of this study as a cancer bioassay, because the details on the experimental design of this study were not available in the summary.

2. Rats:
A 2-year feeding study on the toxicological effects of cyanazine in rats was conducted by the manufacturers of cyanazine, Shell Chemical Company (Walker et al., 1974). Cyanazine was fed in the diet at 6, 12, 25 and 50 ppm to male and female CFE rats (24 rats per dose group per sex) for two years. Controls consisted of 48 males and 48 females. A brief narrative stated that feeding cyanazine for up to two years "influenced neither the type nor the incidence of tumors found in the rats." However, no information was presented on the incidences and tumor types observed in the treated and the control animals. The description of the experimental did not give any information on the histopathological procedures used in this study. Although the results stated that mortality was "similar" in control and treated groups, no actual data was presented on the incidence of unscheduled deaths or mortality rates, in treated and control animals. The lack of sufficient detail in both the experimental design and the results of this study make the interpretation of the adequacy of this study as a cancer bioassay difficult. This study did use fewer animals, 24 in each treatment group, compared to the 50 animals per dose used by the National Toxicology Program in cancer bioassays. This limits the ability to detect statistical differences in the incidences of tumors in treated and control groups.

Sprague-Dawley (SD) rats, 52 of each sex per dose, were fed technical cyanazine at 0, 1.5, 5, 25 or 50 ppm in the diet for 2 years. The highest dose was near the maximum tolerated dose (MTD) as evidence by a decreased gain of body weight gain (14%) in the males and females during the first 3 months of the study. Female and male SD rats fed up to 50 ppm cyanazine in the diet for two years did not have a treatment related increase in non-mammary tumors in either sex (Bogdanffy, 1990; USEPA, 1994). (Note: an increased incidence of malignant mammary tumors was observed in the rats receiving 25 or 50 ppm cyanazine in their diets compared to controls. These results are discussed in section V. B. of this Critical Evaluation).

C. Current Classification of Carcinogenicity by Other Agencies

1. IARC: Not listed.

2. NTP: Not listed in 1998 Annual Report on Carcinogens (USDHHS, 1998).

3. EPA: Rated as Group C, possible human carcinogen, based on induction of mammary tumors in female rats (USEPA, 1994).

V. Critical Evaluation of Evidence for Breast Carcinogenicity

A. Human Studies:
There are no case-control human epidemiological studies that have evaluated breast cancer incidence or mortality in women with exposures to cyanazine.

B. Experimental Animal Studies:
A 2-year feeding study evaluating the toxicology and carcinogenicity of cyanazine was conducted by the Shell Chemical Company (Walker et al., 1974). Two unpublished long-term experimental animal studies evaluating the oncogenic potential of cyanazine (Bogdanffy, 1990; USEPA, 1991) were referenced and summarized in the EPA Special Review document on cyanazine (USEPA, 1994). Complete details of the experimental design, tumor incidence, tumor pathology, survival rates, and other results were not always available for these studies.

1. Mice:
Cyanazine's oncogenic potential was evaluated in a long-term feeding study in CD-1 mice. Dietary administration of cyanazine did not induce a higher incidence of mammary tumors of any type in treated animals compared to rats fed control diets which did not contain cyanazine (USEPA, 1991 as cited in USEPA, 1994). Because this study was only available in a summary form, details on the experimental design, and execution of the study were not available. Therefore, we could not make a conclusion as to the adequacy of this study as a cancer bioassay.

2. Rats:
As has been previously described, Shell Chemical Company conducted a 2-year feeding study to evaluate the carcinogenicity of cyanazine in CFE male and female rats. We could not evaluate the adequacy experimental design and execution of this study because of the limited information that was provided (Walker et

al., 1974). A narrative of this study stated that the type and incidence of tumors was not influenced by cyanazine treatment, however, no actual data on tumor types or incidences was presented.

A summary of an unpublished study sponsored by the E.I. DuPont de Nemours Company on the toxicity and oncogenicity of cyanazine was cited in the Special Review Document on triazines (USEPA, 1994). Male and female SD rats, 52 per dose group, were fed cyanazine technical at 0, 1, 5, 25, or 50 ppm in the diet for 2 years. The levels chosen for the cancer bioassay are considered to be appropriate, since the high-dose used was determined to be at the maximum tolerated dose (MTD), based on a 14% decrease in weight gain during the first three months of the study. Results from this study indicate a statistically significant increase in malignant mammary gland tumors (adenocarcinomas and carcinosarcomas; incidence of each tumor type not stated in the summary) in the female rats fed diets containing 25 and 50 ppm cyanazine. The incidence of the malignant mammary tumors in the cyanazine treated animals was outside historical control range (Bogdanffy, 1990 as cited in USEPA, 1994).

The triazines simazine and atrazine also induce a significant increase in mammary tumors in female SD rats, though at considerably higher doses than for cyanazine. Atrazine induced a significantly higher incidence of benign mammary tumors at 70 and 500 ppm, and benign and malignant mammary tumors at 1000 ppm in female SD rats in a 2-year feeding study (Mayhew et al., 1986; Stevens et al., 1994). In a similar 2-year chronic feeding study, mammary fibroadenomas and adenocarcinomas were significantly increased in female SD rats fed diets containing 1000 ppm simazine compared to 0 ppm controls (McCormick and Arthur, 1988; Stevens et al., 1994). The induction of mammary tumors in cyanazine, simazine, and atrazine treated female SD rats suggests a structure-activity relationship between these three triazines, and possibility a similar mechanism of action (USEPA, 1994).

While there have not been any studies published that have tried to determine the mechanism by which cyanazine induces breast tumors in experimental animals, other researchers have suggested that other *s*-triazines, such as atrazine, may induce changes in the estrous cycle, the hormonally controlled reproductive cycle in rodents. It has been suggested that changes in the estrous cycle, such as prolonged estrus, may result in elevations in serum estrogen levels which may play a role in the increased incidence, and earlier appearance of mammary neoplasms in *s*-triazine treated SD female rats (Chapin et al., 1996; Eldridge et al., 1994; Stevens et al., 1994; Wetzel et al., 1994). Some investigators have not been able to substantiate this hypothesis. Cooper et al. (1996) administered atrazine to female SD rats by gavage at 75, 150 and 300 mg per kg per day for 21 days. While animals in the 75 mg/kg group did have a disruption of their estrous cycles, the pattern observed was irregular cycles with no evidence of persistent estrus. At the two higher doses of atrazine (150 and 300 mg/kg), animals displayed periods of prolonged vaginal diestrus, and elevated serum progesterone levels which suggested the animals were pseudopregnant. Levels of serum estrogen in the 150 and 300 mg/kg/day atrazine treated animals displaying patterns of persistent diestrus were not elevated, and were classified as "minimal" by the authors. The number of days animal spent in estrus were significantly depressed ($p<0.05$) in the female SD rats treated with 300 mg atrazine/kg. The levels of atrazine used in this study were in excess of the levels used to induce mammary tumors in long term feeding studies (400 to 1000 ppm = 20 to 50 mg/kg/day). Others have hypothesized that *s*-triazines may induce premature reproductive aging, that may result in a decreased latency for the development of mammary tumors in the female SD rat which normally has a high spontaneous rate of mammary tumors (Chapin et al., 1996). No studies have been conducted to determine if cyanazine induces changes in reproductive aging, the estrous cycle, or levels of ovarian or gonodotropic hormones in the female rat.

C. Other Relevant Data on Breast Cancer Risk

1. Evidence of Estrogenicity:

There is no evidence that cyanazine is estrogenic. Cyanazine was determined not to be estrogenic in an *in vitro* assay which measures the ability of a chemical to stimulate cell proliferation in the MCF-7 estrogen-dependent breast tumor cell line (Soto et al., 1995). Cyanazine also was not found to be estrogenic in an *in vitro* assay using yeast transfected with the human estrogen receptor (hER) and an estrogen-sensitive reporter. Competition binding assays demonstrated that cyanazine displaced radiolabeled estradiol (tritiated estradiol-17-β) from the recombinant hER (Tran et. al., 1996). There were no studies located that tested if cyanazine directly binds to the hER.

2. Reproductive Effects:

Reproductive and developmental effects of a mixture of pesticides and fertilizers, based on levels found in Iowa groundwater, was assessed in multigenerational studies in SD rats and CD-1 Swiss mice (Heindel et al., 1994). The mixture included cyanazine administered in the drinking water at 0.07, 0.78, and 7.6 μg/kg bwt per day. These concentrations corresponded to 1X, 10X, and 100X, respectively, of median levels of cyanazine found in Iowa groundwater. Assessment of fertility and reproductive performance in exposed F_0 and F_1 generations, and measures of sperm function, were assessed using the National Toxicology Program's Reproductive Assessment by Continuous Breeding protocol. Reproductive endpoints evaluated included measures of percentage abnormal sperm and spermatid head count; testicular and

epididymal histology; live pups per litter; number of litters per mating pair; live pup weight; number of reabsorptions per liter; and sex ratio of pups. Developmental endpoints included maternal body weight gain and food consumption, and pup external, skeletal, visceral, craniofacial, brain, and cardiovascular malformations. No adverse effects were observed on any reproductive or developmental endpoints in any of the pesticide treated groups (Heindel et al., 1994). It should be noted that the levels of pesticides used in this study were not at MTDs, since the purpose of the study was to see if there were adverse effects at levels of the pesticides similar those known to occur in groundwater.

One study has examined whether there is an association between levels of contamination of pesticides in drinking water, including atrazine, cyanazine, alachlor and metolalchlor, and the incidence of intrauterine growth retardation (IUGR), birth weight, and prematurity in communities in Iowa (Munger et al., 1997). An area served by the Rathburn Rural Water System (RRWS) was found to have higher levels of these herbicides in the drinking water supply and had a greater risk of IUGR (Relative Risk, RR=1.8; 95% CI = 1.3-2.7) than other southern Iowa communities served by a different water system where the levels of these pesticides were lower. The association for IUGR was highest for atrazine (r=0.31, p=0.001); it was also elevated for metolachlor (r=0.28, p=0.004); cyanazine (r=0.24, p=0.02); and chloroform (r=0.18, p=0.07). Though this study is suggestive, it does not provide evidence for a casual relationship between IUGR and the levels of pesticide contamination. No individual estimates of exposure to the pesticides were made, and some of the effects observed could be due to different socio-economic characteristics in the different communities. For instance, smoking, prenatal care, weight gain in the mother, and age of the mother (i.e. teenage mothers) are predictive of intrauterine weight gain and prematurity. The Rathburn community had higher rates of maternal smoking, poor prenatal care, less education, and lower median income than some areas of southern Iowa (Munger et al., 1997); no information was available on the age or weight gain of the mother. This ecologic study of triazine levels in water and IUGR rates should be followed up by case-control studies that more carefully control for confounding variables.

3. Mutagenicity:

Assays of the potential for cyanazine to induce genetic damage in a variety of test systems have provided inconsistent results. Two unpublished studies have been cited by the EPA in the Special Review Document on triazines. The first study found cyanazine to test positive in a mouse lymphoma assay and an unscheduled DNA synthesis (UDS) assay, while another study indicated negative results in a UDS assay in rat spermatocytes (USEPA, 1994).

In the peer-reviewed literature, a recent study evaluated cyanazine's ability to induce DNA damage by examining UDS in human peripheral blood lymphocytes, cytogenetic changes using the sister-chromatid exchange assays, and structural chromosome aberrations in the bone marrow of rats. Cyanazine was non-genotoxic in all test systems (Hrelia et al., 1994). A lack of chromosomal damage was also demonstrated in human lymphocytes exposed to non-cytotoxic levels of cyanazine (1 μg/ml) (Roloff et al., 1992). The ability of cyanazine to affect cell transformation activities of BALB/c 3T3 cells has been evaluated in the presence (with S-9 mix) and absence of bioactivation. A significant increase in cell transformation of cyanazine treated cells was not observe in the presence of absence of the S-9 mixture (Perocco et al., 1993).

In one of the only studies that has evaluated cyanazine's genotoxicity in whole animals, male Fischer 344 rats, and female B6C3F1 mice were fed a mixture of pesticides in the drinking water for 91 days to simulate the pesticides found in the groundwater of Iowa (alachlor, atrazine, cyanazine, metribuzin, metolachlor, and ammonium nitrate). The drinking water mixture included cyanazine at 0, 1X (0.4 ppb), 10X (4.0 ppb) and 100X (40 ppb) of the median concentration found in Iowa groundwater. Spleens were removed, and cultured for analysis of sister-chromatid exchange, chromosome aberrations, and micronuclei in cyctochalasin B-induced binucleated cells. Cytogenetic damage was not demonstrated in any of the tests for the Iowa groundwater mixture (Kligerman et al., 1993). Cyanazine also did not induce chromosomal damage in Chinese Hamster Ovary (CHO) cells exposed to levels of cyanazine found in Illinois drinking water (12 μg/L) (Taets et al., 1998).

Commercial and technical grades of cyanazine have been tested for genotoxicity in *Salmonella typhimurium* and *Sacchromyces cerevisiae* with liver microsome activation (S-9), plant activation or no activation (Plewa et al., 1984). Cyanazine tested negative in the D-4 strain of *Sacchromyces cerevisiae* with or without activation. Cyanazine tested positive, but only after plant activation, in strains of *Salmonella typhimurium* (TA 1535, 1537, 1538, and 100) (Plewa et al., 1984). Mutagenic activity of cyanazine was also observed in the TA100 strain of *Salmonella typhimurium* exposed to an extract from cyanazine-treated corn plants (Means et al., 1988). In contrast, other studies have found no evidence of cyanazine mutagenicity in various strains of *Salmonella typhimurium*, however, these studies did not use a plant activation system, but used the S-9 rat liver microsome activation system to test cyanazine's mutagenicity (Eisenbis et al., 1981; Lusby et al., 1979). These studies indicate that cyanazine does not appear to be mutagenic after metabolic activation using liver extracts, but does demonstrate mutagenicity after plant activation in strains of *Salmonella typhimurium*.

Other studies have demonstrated increased mutation rates in cyanazine-treated corn. The frequency of reverse mutations at the *wx* locus was significantly increased ($p<0.001$) in pollen grains of the inbred W22 strain of *Zea mays* grown on soil plots pretreated with 3.58 or 4.80 kg cyanazine/ha (Plewa and Wagner, 1981; Plewa et al., 1984). Pollen grains of corn plants grown in plots treated with cyanazine plus the herbicide metolachlor (2.7 kg AI/ha, and 2.3 L AI/ha, respectively) had an increased rate ($p=0.05$) of *waxy* forward mutations (Rodriguez et al., 1998). However, this study did not determine if the increased mutation rate was due solely to cyanazine or to an interaction with metolachlor.

In summary, while there is some evidence of cyanazine's genotoxicity, other studies have not found evidence of a mutagenic or genotoxic effect. There is evidence for genotoxicity of cyanazine in a mouse lymphoma assay and an UDS assay (USEPA, 1994), in plant activated strains of *Salmonella* (Means et al., 1988; Plewa et al, 1984), and in *Zea mays* (Plewa and Wagner, 1981; Plewa, et al., 1984; Rodriguez et al., 1998). Others researchers have not been able to demonstrate a genotoxic effect of cyanazine in treated bacteria, yeast, rodent or human cell lines, or in laboratory animals (Eisenbis et al., 1981; Hrelia et al., 1994; Kligerman et al, 1993; Lusby, et al. 1979; Perocco et al., 1993; Roloff et al., 1992; Taets et al., 1998; USEPA, 1994).

VI. Other Relevant Information

A. Environmental Fate:

1. Soil:

As mentioned previously, while some degradation of cyanazine occurs by photolysis, the primary route of degradation of cyanazine is by microbes in the soil that convert the nitrile to an amide group (cyanazine II), with possible further oxidation to the carboxylic acid (cyanazine III), followed by hydrolyis of the ring chlorine to the hydroxyl (cyanazine IV) (Beynon et al., 1972b; WSSA, 1994). Dealkylation of cyanazine only occurs to a limited extent in soil (Benyon et al., 1972b).

In field and laboratory experiments, the half-life of cyanazine has been reported in the range of 6 to 35 days depending on organic matter content, temperature, soil type, the moisture content of the soil, and aerobic/anaerobic conditions (Beynon et al., 1972a; Muir and Baker, 1978; WSSA, 1994; Yoo et al., 1981). The half-life is shorter at pH lower than 5.5, and longer with soil pH above 7.5 (WSSA, 1994). Although degradation of cyanazine in soil samples does occur when incubated under laboratory conditions at 5^{o} C, the rate of degradation is enhanced in samples incubated at higher temperatures (20^{o} to 50^{o} C) (Majka and Lavy, 1977). The half-life for cyanazine in soils under aerobic conditions is between 17 to 25 days, which is considerably shorter than half lives of atrazine and simazine, at 150 and 110 days, respectively. Under anaerobic conditions, the half-life of cyanazine is longer, on the order of 108 days, compared to up to 2 years for atrazine and simazine (USEPA, 1994). Therefore, it appears that of the three major triazines, cyanazine has the fastest rate of biological degradation.

However, it should be noted that there was little recent information available on the levels or the persistency of cyanazine degradates in soil. Beynon et al. (1972b) followed the degradation of ^{14}C-labeled cyanazine in soils at 114 to 168 days after treatment under greenhouse conditions. The major degradation products were formed by the loss of the nitrile group to form cyanazine amide (cyanazine II); its acid (cyanazine III); and the dechlorinated form of the acid (cyanazine IV). Dealkylated products were formed to a minor extent, but the half-lives of the degradation products were not calculated. Muir and Baker (1978) monitored the levels of cyanazine and three degradation products, cyanazine II; cyanazine IV; and cyanazine V, in soil 12 months after cyanazine application. Persistence was followed for three separate years, 1973, 1974 and 1975. The half-life for cyanazine ranged from 10.8 to 24.1 days. Cyanazine IV had little degradation during the cold winter months, and was considered to be the most persistent cyanazine metabolite under the conditions of this study. Twelve months after cyanazine

2. Surface Water:

A summary of surface water monitoring studies in the Midwestern corn belt conducted in the late 1980s and early 1990s was compiled by the EPA as a part of their report that initiated the special review of triazines (USEPA, 1994). Concentrations of cyanazine in some sections of the Midwest, including the Hoover Reservoir in Ohio, the Rathburn Reservoir and West Lake in Iowa, Perry and Turtle Creek Reserves in Kansas, and Otter Lake in Illinois have reported levels that exceeded several µg per L year round. Median concentrations of cyanazine in the surface waters of the corn belt region of the Midwest were in the 0.45 to 4.4 µg per L range, while maximum concentrations ranged from 5.6 µg per L to as high as 86.1 µg per L. Peak concentrations of cyanazine usually were observed between May and early June, frequently following run-off after rainfall (USEPA, 1994). These studies indicated that cyanazine levels in Midwestern surface water had frequently exceeded the MCL of 1 µg/L during the 1980s and early 1990s.

In the 1980s cyanazine was detected in both the raw surface water and in the finished water in several watersheds in Ohio. The average levels of cyanazine in finished tap water samples obtained in 1983 was 0.75 µg per L for Tifflin, Ohio; 0.029 µg per L for Fremont, Ohio; and 0.85 µg per L for Bowling Green, Ohio (Baker, 1983). Graphic plots of cyanazine concentrations in raw and tap water during the year indicated that levels in surface water were frequently found in the range of 0.5 to 2.0 µg per L during

late June through mid-July. Levels in the finished tap water were slightly lower during these times of the year, but similar to the levels contained in raw water (Baker, 1983).

Some of the contamination of surface water supplies in the Midwest are very regional. In Iowa, drinking water supplies from the RRWA system, that obtains water from the Rathburn reservoir on the Chariton River, has had persistently elevated levels of cyanazine in drinking water. Mean levels were reported to be 1.4 µg per L for cyanazine in samples obtained from the RRWA. These levels are above the MCL of 1.0 µg in public drinking water supplies. However, surface water supplies obtained in southern Iowa other than from the RRWA had lower mean levels at 0.7 µg of cyanazine per L. Groundwater from the same region in southern Iowa had no detectable levels of cyanazine (Munger et al., 1997).

Information on levels of cyanazine in NYS watersheds has recently been published by the U.S. Geological Survey. Several studies on the levels of pesticide residues in the surface waters of the Hudson River-Mohawk River Subbasin were conducted in the mid-1990s. In one report (Wall and Phillips, 1997b), three sites were sampled: the Mohawk River at Cohoes; the Canajoharie Creek at Conajoharie; and Lisha Kill at Niskayuna. Most detects of cyanazine were found in samples obtained from May through August. This probably reflects the higher use of cyanazine during the spring and summer growing seasons. The median levels of cyanazine detected in the Mohawk River were 0.02 µg/L and maximum levels were 0.073 µg/L. The Mohawk River receives water from forested, urban, and agricultural sites. The median levels in the Canajoharie Creek during May to August were 0.016 µg/L; maximum levels were 2.1 µg/L. The one sample with the value of 2.1 µg/L was the only sample of the 108 samples from the entire study that had a level of cyanazine that exceeded the MCL of 1 µg per L. The Canajorharie Creek receives water from agricultural areas where pesticides are applied. There were no reports of cyanazine detections in Lisha Kill; most of the water inputs are from urban and residential areas which would be expected to have low use of cyanazine (Wall and Phillips, 1997b).

In a second study conducted by the U.S. Geological Survey (Wall and Phillips, 1997a), water samples were collected from 46 sites on 42 streams and rivers that fed into the Hudson River basin. Cyanazine was detected in 17% of the samples, with median concentrations at 0.0295 µg per L, which is 34 times lower than the MCL for cyanazine in drinking water. The maximum concentration detected for cyanazine was 0.2 µg/L. Samples with positive detections of cyanazine were observed only in samples from watersheds with agricultural or mixed land use, and not from watersheds with urban or forested land uses (Wall and Phillips, 1997a).

Cyanazine has also been detected in surface waters of other Northeastern states. Investigators obtained water samples from multiple sites in the LaPlatte River and Missisquoi River watersheds in Vermont. Samples were taken from the end of May to the end of August during 1992 and 1993. Mean levels of cyanazine ranged from 0.031 to 3.03 µg per L, with maximum levels in the range of 0.7 to 6.9 µg per L. While maximum levels did exceed the MCL for cyanazine in this study, the authors noted that the levels usually dissipated in the rivers within days after the rainfall (Gruessner and Watzin, 1995). No information was provided on the level of cyanazine transformation products.

Residues of agricultural herbicides were monitored during 1986-1990 in water samples taken from the mouth of the Grand, Saugeen, and Thames River in Ontario, Canada. Both storm run-off and base-flow samples were obtained. Few of the samples, only 1.3%, had positive detections of cyanazine residues. This is in contrast to the high rate of detection of triazine herbicide atrazine in 72% of the water samples. However, atrazine was used to a much greater exent that cyanazine. For example, 177,208 kg of atrazine were used in the Grand River Basin in 1988 compared to 20,611 kg of cyanazine. The cyanazine detections were most frequently observed during the months of June and July, with mean residue levels ranging from 0.32 to 0.54 µg/L (Frank et al., 1991).

There were very few reports in the literature which determined the levels of cyanazine degradation products in surface water. Muir and Baker (1976) determined the levels of triazine herbicides and their degradation products in tile-drain water from fields producing corn in the mid 1970s. Levels of the degradation product cyanazine amide (0.45µg/L) were similar to the levels reported for the parent compound, cyanazine (0.5 µg/L).

3. Groundwater:

In the Office of Pesticide Programs Pesticide in Groundwater Database (PGWDB), cyanazine was the fifteenth most detected pesticide nationwide (USEPA, 1994). Of the wells that detected cyanazine, 14% reported levels that exceeded the MCL of 1 µg per L.

One of the states most frequently reporting groundwater contaminated with cyanazine has been Iowa. Cyanazine detections were reported in the groundwater of the Big Springs watershed in Iowa in the 1980s. The maximum concentrations from samples taken in 1981 to 1985 ranged from 0.5 to 4.6 µg per L (Ritter, 1990). Groundwater was also reported to be contaminated with cyanazine near farm chemical dealerships in Iowa in the mid-1980s. Concentrations were as high as 225,000 µg per L in standing water in rinsing and loading areas, and maximum concentrations in affected wells were as high as 36 µg per L (Ritter, 1990). Iowa's State-Wide Rural Well Water survey found cyanazine to be the

fifth most frequently detected chemical of the 27 agents that were monitored in this study. It was estimated that 1.2% of private drinking wells in Iowa were contaminated with cyanazine (USEPA, 1994).

A recent study has examined trends in the frequency of detections, and concentrations of cyanazine in Iowa groundwater samples from the early 1980s to the mid-1990s (Kolpin et al., 1997b). Samples were collected from 1019 wells during the months of April to September during the years 1982 to 1995 for the Iowa Groundwater Monitoring Program. Cyanazine's agricultural use varied during this time period, with a trend toward decreased use. Average use from 1982-1986 was 4.2 thousand kg AI; from 1987-1991 use decreased to 2.65 thousand kg, while use from 1992 to 1995 rose to 3.2 thousand kg. The authors noted that median concentrations of cyanazine in groundwater samples were similar from 1982 to 1995, despite the fact that use of cyanazine in Iowa has decreased about 25% during this time period. The frequency of detection of cyanazine was low at 3.6%. The maximum concentration detected was 4.5 mg / L, but only 0.3 % of the samples exceeded the MCL or HAs set by EPA for public drinking water supplies. In an assessment of frequency of detections in shallow, intermediate, and deep wells, the highest frequency of cyanazine detections were observed in intermediate wells. Although the frequency of detections for cyanazine were low in Iowa groundwater, the authors noted that degradation product of cyanazine, cyanazine amide, is more persistent than cyanazine. They noted that more information is needed on the trends in levels of cyanazine amide in groundwater to assess the relationship between cyanazine use-patterns, and concentrations of the parent compound and its degradation products (Kolpin et al., 1997b).

Few studies have reported the levels of both cyanazine and its major degradation product, cyanazine amide, in groundwater. As a part of the Iowa Ground Water Monitoring Program, residue levels of herbicides and their major degradation products were determined in 106 municipal wells the summer of 1995 (Kolpin et al., 1997a). These wells represented major aquifer types across the state. Cyanazine was detected in 5.7% of the wells, while cyanazine amide was detected three times more frequently in 20% of the wells. The maximum level of cyanazine detected was 0.3 µg/L, compared with 0.58 µg /L of cyanazine amide (Kolpin et al., 1997a). A study of the occurrence of herbicide residues in water samples from 100 wells from mid-western states in 1991 also reported a higher frequency of cyanazine amide detections than cyanazine detections. Cyanazine was detected in 2.3% of the wells, compared with detections of cyanazine amide in 11% of the wells (Kolpin et al., 1996). The most recent studies of cyanazine levels in groundwater have been reported by the U.S. Geological Survey as a part of the National Water Quality Assessment Program (Kolpin et al., 1998). Water samples were collected during 1993-95 in 20 major water basins of the U.S. Both agricultural and urban settings were represented in the 1034 sites sampled. Cyanazine detections were infrequent, with an average of only 2% of the sites with positive detections. Frequency of detections were highest in land-use settings that grew corn or corn and alfalfa (4.2% and 4.6%, respectively). There were no reports of cyanazine exceeding the MCL in this study. Levels of cyanazine amide in the ground water were not determined.

4. Precipitation:
Concern has been expressed regarding the atmospheric transport of pesticides, including cyanazine, and its deposition in precipitation. Some of the highest levels of cyanazine in rainfall have been found in Iowa, one of the highest use states for cyanazine. A study conducted in the late 1980s and early 1990s detected cyanazine in 81 of the 325 rainfall samples (25% detection), with a median concentration of 0.33 µg per L, and mean levels at 0.91 µg per L. The maximum concentration of cyanazine reported in this study was 28 µg per L (Nations and Hallberg, 1992). The highest proportion of cyanazine detections were found in the Big Spring Basin of northeastern Iowa, which is also the area with the greatest use of cyanazine and other corn herbicides atrazine and alachlor. Seasonal patterns indicated that detections of cyanazine in precipitation occurred primarily between April through July. Concern was expressed by the authors because they also found that cyanazine was detected in the rainfall in areas distant from where the cyanazine was applied to field crops. Levels at a distant site near a sensitive ecosystem where a fish hatchery was located were found to be in the range of 0.13 to 0.81 µg per L. The exact mechanism by which cyanazine enters precipitation was not determined, but the authors suggested that volatilization of cyanazine may be the largest source for agricultural pesticides to enter the atmosphere (Nations and Hallberg, 1992).

B. Dietary Cancer Risk:
There is relatively little information on the levels of cyanazine and its transformation products in foods, and related cancer risks. In a model ecosystem, Yu et al. (1975) did not find that cyanazine or its transformation products bioconcentrated in aquatic organisms. Cyanazine and its degradation products were not detected in algae, dahpnia, fish, mosquitoes, or snails. There were only two organisms that had detectable levels of cyanazine or known transformation products. Cyanazine was detected in Elodea at the levels of 0.621 ppm, and *N*-deethylated cyanazine was detected at 0.172 ppm in crab meat.

In its Special Review of triazines, the EPA expressed concern regarding dietary cancer risk posed by ingesting cyanazine treated foods (USEPA, 1994). The EPA has set tolerance levels for the maximum levels of cyanazine 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 cyanazine in food were calculated (see Table 2 in USEPA, 1994). These upper bound cancer risk estimates were calculated from anticipated residues in commodities known to be treated with cyanazine (i.e. corn, sweet corn, cottonseed, sorghum, and wheat) or residues in food products from animals fed cyanazine-treated crops (poultry, beef, milk, and eggs), the percent of the crop treated with cyanazine, and the estimated exposure in mg/kg/day to cyanazine. The total estimated dietary cancer risk of cyanazine was calculated to be 2.9×10^{-5}.

VII. Summary and Recommendations for Breast Cancer Risk Classification:

• **Human Studies:** Case-control human epidemiology studies have not been conducted on whether exposure to cyanazine affects breast cancer risk or breast cancer mortality. Therefore, due to an absence of these types of studies, we could not evaluate whether there is or is not a causal relationship between exposure to cyanazine and the risk of breast cancer.

• **Animal Experimental Studies:** There is limited evidence that cyanazine is a mammary carcinogen in experimental animals. This is based on one unpublished study that demonstrated a significantly higher incidence of malignant mammary tumors in female SD rats fed 25 or 50 ppm cyanazine over two years (Bogdanffy, 1990 as cited in USEPA, 1994). Cyanazine does not appear to induce mammary neoplasms in mice (USEPA, 1991 as cited in USEPA, 1994).

• **Related Evidence:** Related evidence of whether cyanazine affects breast cancer risk is limited. Both *in vivo* and *in vitro* tests indicate that cyanazine is not estrogenic (Soto et al, 1995; Tran et al., 1996). Studies have not tested whether cyanazine disrupts the estrous cycle in experimental animals or otherwise affects gonadotropic or ovarian hormone levels in animal or *in vitro* models. No studies were located that evaluated its ability to affect cell proliferation in normal breast cells or breast tumor cell lines. Although there is some evidence that cyanazine is mutagenic, the evidence is not consistent, since others have not been able to demonstrate a genotoxic effect of cyanazine.

Therefore, based on this evidence, we conclude that there is insufficient evidence to rate cyanazine as a human breast carcinogen, and it should be classified in *Group 3, unclassifiable as to its breast cancer risk* (See Appendix B for an explanation of the BCERF Breast Cancer Risk classification system.

Author's Note: It should be noted that this Critical Evaluation was hindered because of the lack of availability of details on the experimental design, execution, and results of the published and unpublished long-term animal bioassays that have evaluated cyanazine's cancer causing potential. Despite the relative paucity of information on cyanazine, both in terms of it carcinogenicity and its environmental fate, it should be noted that cyanazine, like the *s*-triazines atrazine and simazine, induced malignant mammary tumors in female SD rats. Malignant mammary tumors in cyanazine treated SD rats were induced at much lower dietary levels (25 and 50 ppm) (Bogdanffy, 1990) than levels found to induce mammary tumors in simazine (1000 ppm) and atrazine treated (70-1000 ppm) female SD rats (Stevens et al., 1994; USEPA, 1994). This indicates that cyanazine may be a more potent mammary carcinogen in experimental animals than other triazines.

VIII. Research Gaps and Recommendations for Future Research

• Studies are needed to determine if women occupationally exposed to cyanazine as agricultural workers, farmers, or pesticide applicators, have a higher incidence of breast cancer. If such a relationship if found, then studies should be extended to other populations with potential exposures to cyanazine, such as individuals who have handled and laundered clothing contaminated with cyanazine, or who have live on farms that have had a history of long-term cyanazine use, and who may have been exposed to cyanazine from agricultural applications.

• One of the highest uses of cyanazine has been in the Midwestern corn belt. Some of the highest levels of cyanazine in surface and groundwater and in precipitation have also been detected in this region, especially in Iowa. Given the existence and availability of historical data on water levels of cyanazine, studies should be done to determine if there are any geographical relationships between incidences of breast cancer and areas known to have had high levels of cyanazine in surface or ground water. If there are indications of such a relationship, this would provide the basis to conduct case-control studies to determine if there is a relationship between past exposures to cyanazine from drinking water sources and breast cancer risk.

• More studies are needed on the levels and health affects of cyanazine degradation products. Very little information was available on the levels or persistency of cyanazine degradation products in soils, groundwater, surface water, tap water or in rainfall. It has been assumed that cyanazine is comparatively of

less concern for exposure than other triazines, because of its relatively faster rate of degradation in soils. However, this assumption can only be made if it is shown that its degradation products do not persist or pose no adverse health effects. Therefore, even though cyanazine is being phased out of use, it should be the responsibility of its registrants to show that its degradation products, such as cyanazine amide, are not carcinogenic, or have the capacity to induce other adverse health effects.

• Studies should be conducted to determine the mechanism(s) by which cyanazine induces mammary tumors in SD female rats, and the relevance of these mechanism(s) to humans.

• While cyanazine's production for U.S. markets will be phased out by the year 2002, if it is continued to be produced for export, there will still be the potential for those employed at manufacturing facilities to be exposed to this herbicide. Those who have been employed, and who will be employed in such facilities should be monitored to determine if there are any adverse health effects in men or women with long-term cyanazine exposure.

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)

This 10-year prospective study, which is in its third 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.

Interventions To Reduce Cancer Risk Among Farm Families
Dr. Melissa Perry, Medical College Of Wisconsin
(adapted from author's abstract)

This study proposes to translate prior epidemiologic, laboratory, clinical, and behavior information on cancer risks into a primary prevention program to reduce cancer among farmers and their families. The preventive interventions will target pesticide applicators, most of whom are farmers, and their families through community-based educational programs designed to increase cancer prevention knowledge, risk perception, and self- efficacy in order to create behavior change to reduce cancer risks. Because the majority of applicators are male, and because other family members are likely to be exposed to pesticides by virtue of living in the farm setting, wives and adult daughters of the applicators will also receive an educational intervention. This intervention will be designed to increase knowledge of pesticides risks and increase screening behaviors including breast self exam and mammography among women of recommended age. To be conducted in Vermont.

Reducing Pesticide Exposure In Minority Families
Dr. Linda Mc Cauley, Oregon Health Sciences University
(adapted from CRISP database)

The specific aims of the study are to: (1) compare the levels of pesticides in homes as a function of the type of agricultural crop the parents work with, the types of pesticides commonly used on the crops, proximity of housing to the field and characteristics of the home; (2) evaluate specific health outcomes associated with pesticide overexposure in both workers and their children and to evaluate specific biomarkers; and (3) assess the effectiveness of the Migrant Headstart program as a mechanism for delivering culturally-appropriate environmental health prevention strategies.

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XI. Appendix A. Common Abbreviations, Acronyms and Symbols

ADI	Allowable Daily Intake, set by the World Health Organization
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
CHO	Chinese Hamster Ovary Cells
Cl	chlorine
CRISP	Computer Retrieval of Information on Scientific Projects; database of scientific intra and extra mural projects supported by the Dept. of Health and Human Services (i.e., NIH, EPA, USDA)
DNA	deoxyribonucleic acid
ER	estrogen receptor
FDA	Food and Drug Administration
ha	hectacre
H	hydrogen
HA	The health advisories are nonenforceable limits of the concentration of the chemical in the drinking water that is not expected to cause any adverse noncarcinogenic health effects when consumed for no more than the time period specified, with a margin of safety
hER	human estrogen receptor
IARC	International Agency for Research on Cancer, headquartered in Lyon, France
ICET	Institute for Comparative and Environmental Toxicology
IUGR	Intrauterine growth retardation
kg	kilogram
lbs	pounds
L	liter
µg	microgram
mg	milligram
MCF-7	breast tumor cell line developed at the Michigan Cancer Foundation
MCL	Maximum Contaminate Level; enforceable limit set by the EPA which sets the maximum level of a contaminate in a public drinking water supply
MM	Multiple myeloma
MTD	maximum tolerated dose
n	number of subjects/animals in the group
N	Nitrogen
NA	Not available
NHL	Non-Hodgkin's lymphoma
NCI	National Cancer Institute
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NS	Not statistically significant
NTIS	National Technical Information Service; repository for federal agency technical reports
NTP	National Toxicology Program
NY	New York
NYS	New York State
OD	Odds Ratio
ppm	parts per million
ppb	parts per billion
RR	Relative Risk
RRWS	Rathburn Rural Water System, located in Iowa
RUP	Restricted Use Pesticide
SMR	Observed deaths in exposed population multiplied by 100, divided by expected deaths.
SD	Sprague-Dawley; albino rat strain
TMA	Time-weighted average
U.S.	United States
UDS	unscheduled DNA synthesis
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
WSSA	Weed Science Society of America
wt	weight

Symbols:

α	alpha
γ	gamma
β	beta
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-carcino genicity, 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.