Public Health Goal for
SIMAZINE
In Drinking Water

Prepared by

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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PREFACE

Drinking Water Public Health Goals

Pesticide and Environmental Toxicology Section

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), amended 1999, requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and publish PHGs for contaminants in drinking water based exclusively on public health considerations. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based upon currently available data and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs published by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs published by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic

cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR SIMAZINE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) has established a public health goal (PHG) of 0.004 mg/L (4 μg/L, or 4 ppb) for simazine in drinking water. Simazine is a widely used selective herbicide with a high potential to leach into ground water and run off into surface waters. It is readily absorbed from the gastrointestinal tract and excreted via urine (≥ 70 percent) and feces (≥ 20 percent). It is metabolized via stepwise oxidative P-450 dealkylation to mono and didealkylated metabolites without disrupting the triazine ring. The PHG is based on a no-observed adverse-effect level (NOAEL) of 0.5 mg/kg for reduced body weight observed in female Sprague-Dawley rats administered simazine in the diet at 0, 10, 100 or 1,000 ppm for 24 months. There is suggestive evidence that simazine is a carcinogen, based on a finding of mammary gland carcinogenicity and ovarian hyperplasia and adenoma in a single study in female Sprague-Dawley rats and data on mammary carcinogenesis for related triazine herbicides. Simazine is a weak mutagen. Reduced body weight is a consistent finding following simazine exposure in a 13-week dog study, a two-year mouse study, the rat and rabbit developmental studies and a rat reproductive study. The calculation of the PHG incorporates an adult body weight of 70 kg, drinking water consumption of 2 L/day, a 20 percent relative source contribution, and a combined uncertainty adjustment factor of 1000 (10-fold for inter-species variation, 10-fold for intra-species variation, and 10-fold to account for uncertainties associated with simazine carcinogenicity).

The oral LD_{50} of simazine in rats and mice is greater than 5,000 mg/kg. Sheep appear to be more sensitive to the toxic effects of simazine than the other experimental animals tested. Signs of toxicity in sheep include incoordination, tremor, weakness, cyanosis, and clonic convulsions. In a 24-month Sprague-Dawley rat study, reduced body weight was observed in both males and females, along with changes in hematological parameters such as reduced red blood cells, hemoglobin and hematocrit levels, and an increase in platelet counts at the highest dose level (1000 ppm in the diet), as well as mammary gland carcinomas at the 100 and 1,000 ppm dose levels in female rats. A hormonal mode of carcinogenic action is suggested by the recent data on chloro-s-triazines. In addition, a direct genotoxic mechanism, a genotoxic mechanism via estradiol metabolites, hormonal alterations due to reduced body weight, or a combination of these factors may also contribute to the mammary gland carcinogenicity observed in female Sprague-Dawley rats.

The maximum contaminant level (MCL) for simazine in drinking water for both California and the U.S. Environmental Protection Agency (U.S. EPA) is 4 ppb.

INTRODUCTION

Simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) is a selective pre-and post-emergence herbicide for control of broad leaf and grassy weeds in various crops such as corn, almonds, grapes and oranges, and in non-cropped area such as rights-of-way. Simazine inhibits photosynthesis. Average rates of 1 to 2.5 pounds per acre are usually applied by ground boom application, but higher application rates may be used in non-selective conditions. At present, only one dry flowable and two flowable liquid formulations are registered in California. Presently, atrazine and simazine are under “Special Review” by the U.S. EPA because of potential carcinogenic risk from these s-triazine pesticides.

For the development of a PHG for simazine, two types of data were reviewed: data published in the open literature for the past ten years and data submitted to the California Environmental Protection Agency's Department of Pesticide Regulation (DPR) for the registration of simazine as a pesticide. The latter was initially reviewed by DPR (DPR, 1993) and brief summaries of the relevant toxicity data are provided in this document.

CHEMICAL PROFILE

Structural chemical names for simazine include 2-chloro-4,6-bis(ethylamino)-1,3,5-triazine and 6-chloro-N,N-diethyl, 1,3,5-triazine-2,4-diamine. The chemical formula for simazine is $C_7H_{12}C1N_5$; its chemical structure is presented in Figure 1.

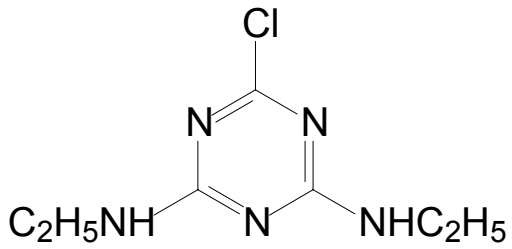

Figure 1. Chemical structure for simazine.

Production and Uses

Simazine is a selective pre-emergence herbicide for control of most annual grasses and broadleaf weeds. It is also used as an algaecide for aquatic weed control. It can be formulated with other herbicides and fertilizers. At higher rates of application, it is used for non-selective weed control in industrial areas and along highways. The annual U.S. production of simazine was reported to be five million pounds in 1975 (NAS, 1977). In California, a total of 847,742 pounds of simazine was used in 1995, mainly on almonds, avocados, grapes, oranges, olives, walnuts, lemons and rights-of-way (DPR, 1995). Simazine formulations are available as wettable powders, water dispersible granules, and in a liquid and granular form.

In 1984, U.S. EPA issued a registration standard for pesticide products containing triazines (atrazine, simazine and cyanazine) because of potential ground water contamination. Cyanazine has since been withdrawn voluntarily by the registrant. Atrazine and simazine are still in use. Both induce mammary tumors in rats and are classified by U.S. EPA as Group C, possible human carcinogens. They are metabolized to similar degradation products, exhibit comparable fate in the environment and may be used as alternatives to each other. Atrazine is considered a representative for the s-triazines and, therefore, the majority of the toxicity studies available are for atrazine. This health risk assessment on simazine draws on the available literature on atrazine in understanding the toxicity and its basis. OEHHA has recently completed a risk assessment of atrazine in support of a PHG for the chemical (OEHHA, 1999).

Physical and Chemical Properties

Important physical and chemical properties of simazine are provided in Table 1.

Table 1. Physical and Chemical Properties of Simazine[1]

Property	Value or Information
Molecular weight	201.69
Color	white crystalline
Physical state	solid
Melting point	225 to 227°C
Solubility	water 3.5 ppm at 20° C, methanol 400 ppm, petroleum ether 2 ppm
Density	1.302 g/cm^3
Partition coefficients	
Log K_{ow}	1.94 to 2.26
Log K_{oc}	2.14
Vapor pressure	6.1×10^{-9} mm Hg @ 20°C
Henry's law constant	3.4×10^{-9} atm-m^3/mol @ 20°C (calculated)

[1]Adapted from DHS, 1988; U.S. EPA, 1990.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

Simazine has low water solubility and low vapor pressure. Therefore, it does not volatilize into air. Simazine might be adsorbed onto soil particles and become airborne.

Soil

About 15 percent of the parent compound remained at one year after initial application and no residues could be detected after 16 months (Talbert and Fletchall, 1965). Simazine is more persistent in soils than atrazine and other triazines such as propazine, ipazine and chlorazine (Beatley, 1964; Switzer and Rause, 1964).

Under anaerobic conditions, [^{14}C]simazine was reported to degrade in loamy sand soil with half times ($t_{1/2}$) of 8 to 12 weeks (Keller, 1978). Degradation products included 2-chloro-4,6-bis(amino)-s-triazine, 2-hydroxy-4,6-bis(ethylamino)-s-triazine and 2-hydroxy-4-ethylamino-6-amino-s-triazine. Adsorption to soils is affected by the content of organic matter in the soil, the clay content and the soil ion exchange capacity (Helling, 1971; Helling and Turner, 1968; Talbert and Fletchall, 1965). Monsanto (cited by U.S. EPA, 1987) reported a $t_{1/2}$ for decomposition of 36 and 234 days for simazine in loamy sand and silt loam soils, respectively. Simazine decomposition in soils is affected by moisture content and temperature (Walker, 1976). The rate of simazine decomposition in soil is faster in acid than in alkaline soils. Photodegradation and volatilization do not contribute significantly to this process. Simazine is hydrolyzed by a non-biological reaction to form a nonphytotoxic metabolite, hydroxysimazine. Dealkylation by free radical reactions is also involved in simazine environmental degradation. Soil

persistence of simazine makes it useful in long-term weed control programs. Usually crops can be planted one year after simazine application at the selective rates (two to four pounds per acre).

In a series of unpublished studies, Martin et al. (1975) and Mattson et al. (1969) detected 2-chloro-4-ethylamino-6-amino-s-triazine, a degradation product of simazine, in soil up to 12 inches deep after simazine application in field studies. In the field, simazine $t_{1/2}$ was reported to be 30 to 139 days (Walker, 1976; Martin et al., 1975; Mattson et al., 1969).

Water

U.S. EPA has analyzed data on triazine pesticides detected in raw and finished surface water primarily in the 12–state mid-western cornbelt region of the United States where the majority of the annual triazine use occurs (U.S. EPA, 1994). These data include results from field monitoring studies and literature reviews, and data submitted under the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Atrazine, simazine, and certain degradation products were frequently detected in the same water samples, reflecting the pattern of herbicide usage in the studied watersheds. Atrazine and simazine follow similar degradation pathways. Both parent compounds form hydroxyl analogues (dechlorination) and dealkylated chloro-degradation products. Two of these chloro-degradation products are identical for simazine and atrazine. Based on the environmental fate and toxicity of these compounds, the cumulative effect on the environment of the triazine herbicides is assumed to be additive.

Ground water monitoring data submitted by pesticide registrants, state government agencies and the U.S. Geological Survey (USGS), as well as data collected by U.S. EPA's National Pesticide Survey of Drinking Water Wells and the Office of Pesticide Programs (OPP) have also been analyzed by U.S. EPA (U.S. EPA, 1994). In OPP's database for pesticides in ground water, simazine was the eighth most prevalent pesticide in 19 out of 30 states in which samples were collected. Seven percent of the samples that contained residues exceeded the federal simazine MCL of 4 ppb.

Simazine was the most frequently detected pesticide in California's Well Inventory Database. Residues of simazine have been reported in 296 wells from 20 counties in California at concentrations ranging from 0.02 to 49.2 μg/L (U.S. EPA, 1994).

Food

Human exposure to simazine can also result from ingesting foods containing residues remaining in or on treated crops such as corn, nuts, and fruits. In addition, dietary exposure may occur by consuming products from animals that feed on simazine-treated crops. The major contributors to simazine exposure in the diet are oranges and apples (U.S. EPA, 1994). U.S. EPA estimated a total dietary exposure of 1.1×10^{-4} mg/kg-day. Tolerances for simazine residues in or on certain raw agricultural commodities have been established. The values range from 0.02 ppm in animal fat and meat through 0.25 ppm in fruits (Code of Federal Regulations, 1984). Health Canada's estimate of theoretical maximum daily intake is 0.2 mg/kg-day based on residue tolerance limits (Health Canada, 1986).

METABOLISM AND PHARMACOKINETICS

Absorption

Animals

^{14}C-Simazine was administered orally as a single dose to Sprague-Dawley rats (five/sex/group) at 0.5 or 200 mg/kg. A third group was administered 0.5 mg/kg-day of unlabelled simazine for 14 days followed by a single dose (0.5 mg/kg) of ^{14}C-simazine. At the low dose level, 51 to 62 percent of the administered dose was eliminated in the urine and 12 percent was found in the tissues. At the high dose, 22 percent of the administered dose was found in the urine and two percent was found in the tissues. In the third group, 59 to 66 percent of the dose was eliminated in the urine and eight percent was found in the tissues. Based on urine and tissue levels, the absorbed dose may be about 70 percent of the total (Orr and Simoneaux, 1986).

Distribution

Seven days after the administration of a single oral dose of 0.5 or 200 mg/kg-day simazine to Sprague-Dawley rats, the highest concentration was observed in red blood cells (RBC) with lesser amounts in liver. The RBC concentrations were 16.3 to 19.9 ppm at the high dose and 0.18 to 0.23 ppm at the low dose. Residue levels were slightly higher in male rats than in female rats. Low levels of simazine ranging from 0.0 to 0.16 ppm at the low dose and from 0.78 to 5.2 ppm at the high dose were detected in the heart, lung, spleen, kidney, liver, brain, plasma, and bone (Orr and Simoneaux, 1986).

Metabolism

White female rats were administered a single oral dose of 0.5 mg/kg of ^{14}C ring labeled simazine (Simoneaux and Sy, 1971). Metabolites were analyzed in 24-hour collected urine by thin-layer chromatography and electrophoresis. The metabolites identified were hydroxysimazine, 2-hydroxy-4-amino-6-ethylamino-s-triazine and 2-hydroxy-4,6-diamino-s-triazine. These metabolites accounted for 6.8, 6.1 and 14.0 percent of the administered radioactivity, respectively. Approximately 50 percent of the radioactivity in urine was not identified (Simoneaux and Sy, 1971).

Charles River rats (male, 300 grams) were administered by gavage 1.0 mL of peanut oil containing 0.005, 0.55 or 50 mg/mL simazine (equal to 0, 0.017, 1.7, 17 or 167 mg/kg-day) twice in 24 hours. Urine samples (24-hour intervals) were analyzed by gas chromatography for the presence of mono and di-N-dealkylated metabolites. The di-N-dealkylated metabolite (2-chloro-4,6-diamino-s-triazine) appeared to be the major product, ranging from 1.6 percent at the 1.7 mg/kg dose to 18 percent at the 167 mg/kg dose, while mono-N-dealkylated metabolites ranged from 0.4 percent at the 1.7 mg/kg dose to three percent at the 167 mg/kg dose. This suggests that the rate of metabolism may be dose-dependent (Bradway and Moseman, 1982).

Excretion

Male and female Sprague-Dawley rats administered 0.5 mg/kg of ^{14}C simazine by the oral route excreted 51 and 63 percent of the administered dose, respectively, in the urine and 19 and 13 percent in feces, by seven days after dosing. In rats given 0.5 mg/kg of simazine orally for 14 days followed by a single dose of ^{14}C simazine, male and female rats eliminated 59 and 66 percent of the administered dose in urine and 25 and 18 percent in feces, respectively. Rats given 200 mg/kg of ^{14}C-simazine orally excreted about 21 percent of the dose in the urine and about two percent in the feces (Orr and Simoneaux, 1986, cited by U.S. EPA, 1990).

Simazine metabolites were reported in the urine of sheep for up to 12 days following a single oral dose of 250, 500, 630, 700, or 1,000 mg simazine/kg (50 percent active ingredient). Equivalent concentrations of the metabolites in urine ranged from 6 ppm (low dose) to 70 ppm (high dose) from two to ten days following administration of simazine (Hapke, 1968, as cited by U.S. EPA, 1990).

TOXICOLOGY

Toxicological Effects in Animals

Acute Toxicity

A summary of acute toxicity data on simazine as tested in laboratory animals is presented in Table 2. The oral LD_{50} of simazine in the rat and mouse is greater than 5,000 mg/kg and the inhalation LC_{50} (four hours) in the rabbit is greater than 4.28 mg–hr/liter. In primary eye irritation studies in rabbits, the U.S. Department of Agriculture (USDA, 1984) reported that simazine caused transient inflammation of the conjunctivae. The National Academy of Science (NAS, 1977) listed the acute dermal lethal dose as greater than 8 g/kg. The reported toxic effects following acute exposure were chromorhinorrhea, chromodacryorrhea, perineal and/or abdominal staining, reduced activity, emaciation, and ataxia, before death.

Table 2. Summary of Acute Toxicity Data for Simazine in Laboratory Animals

Species	Test Methods	Effect Level	References
Rat	Single oral dose	LD_{50} > 5,000 mg/kg	Martin and Worthing, 1977
Mouse	Single oral dose	LD_{50} > 5,000 mg/kg	USDA, 1984
Rabbit	Single oral dose	LD_{50} > 5,000 mg/kg	USDA, 1984
Rabbit	Inhalation (4-hr)	LD_{50} > 4.28 mg·hr/L	Foster, 1994
Rabbit	Primary skin irritation, open	500 mg caused mild effect	Ciba-Geigy, 1977
Rabbit	Primary eye irritation	71 mg caused transient inflammation of conjunctivae	
Rabbit	Primary eye irritation	80 mg caused moderate irritant effect	Ciba-Geigy, 1977

Subchronic Toxicity

Sheep and cattle appear to be more susceptible to poisoning by simazine than most common laboratory animals. Repeated administration of simazine to sheep resulted in death after a total dose of 1,400 mg/kg (100 mg x 14 times) given by drench application or 1,000 mg/kg (100 mg x 10 times) orally administered in capsules (Palmer and Radeleff, 1969). In another study (Hapke, 1968) it was shown that a single oral dose of 500 mg/kg was usually fatal to sheep within 5 to 16 days after ingestion. The animals that survived the exposure were symptomatic for two to four weeks after treatment and showed loss of appetite, increased water consumption, incoordination, tremor, and weakness. Some of the sheep were cyanotic and clonic convulsions followed.

Cattle administered seven oral doses of 100 mg simazine/kg-day became moribund and were eventually sacrificed. Gross necropsy showed congestion of lungs and kidneys, swollen, friable livers and small hemorrhagic spots on the surface of the lining of the heart. Smaller doses were associated with transient body weight depression, depending upon the dosing schedule (Palmer and Radeleff, 1964, as reviewed by DHS, 1988).

Allender and Glastonbury (1992) reported a case of simazine toxicosis in sheep. Affected sheep had generalized muscle tremor which progressed to mild tetany, followed by collapse of the hind legs. Death occurred within two to three days of the appearance of clinical symptoms. On postmortem, acute myocardial degeneration was observed in a 38-month old ewe. Necropsy indicated congested liver and enlarged and edematous retropharyngeal lymph nodes. Histopathological examination revealed myocardiopathy and mild chronic focal non-suppurative encephalitis in all sheep examined. The levels of simazine found in liver, brain, and fat varied from 0.2 μg/gram (liver) to 2.50 μg/gram (fat).

Simazine given by gastric tube to rats at the rate of 15 mg/kg-day produced sporadic degeneration of hepatocytes during the first three days. Liver samples were taken under ether anesthesia from animals treated for 3 and 28 days, 24 hours and 14 days after the last dose. The condition did not progress and a process of adaptation was observed (Oledzka-Slotwinska, 1974).

Simazine (98.1 percent) was administered in the diet of Charles River albino mice for 28 days at concentrations of 0, 10, 30, 300, 1,000 or 4,500 (equal to 0. 1.5, 4.5, 15, 45, 150, 450, 1,500 and 4,500 mg/kg-day based on the standard daily feed consumption rate of 15 percent of the body weight). The NOAEL was 450 mg/kg-day based on tremor, generalized weakness and marked reduction in body weights and deaths at the two higher doses of 1,500 and 4,500 mg/kg-day (Ciba-Geigy, 1976).

Simazine was fed in the diet to ten Sprague-Dawley rats/sex/group at concentrations of 0, 200, 2,000 or 4,000 ppm (equal to 0, 10, 100 and 200 mg/kg-day based on the standard feed consumption rate of 5 percent of bw) for 13 weeks (Tai et al., 1985a). Hematological and clinical chemistry parameters and urinalysis were reported for blood and urine samples collected from animals prior to study termination. All animals were necropsied and tissues were examined for histopathological changes. Significant dose-related reductions in feed intake, mean body weight and weight gain occurred in all treated groups. At 13 weeks, various dose-related clinical effects were noted. These included changes in hematological parameters (decreased mean erythrocyte and leukocyte counts and increased neutrophil and platelet counts), clinical chemistry parameters (decreased mean concentrations of blood glucose, sodium, calcium, blood urea nitrogen (BUN), lactic dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase (SGOT) and creatinine, with increased cholesterol and inorganic phosphate concentrations) and urinalysis determinations (elevated ketone levels and decreased protein levels). Relative and absolute weights of adrenal, brain, heart, kidney, liver, testes, and spleen were increased and ovary weight was decreased. Necropsies revealed no gross lesions attributable to simazine ingestion. A dose-related increased incidence of renal calculi and renal epithelial hyperplasia was detected microscopically in treated rats, primarily in the renal pelvic lumen, but only rarely in the renal tubules. Microscopic examinations revealed no other lesions that could be attributed to simazine ingestion. The majority of the alterations in clinical chemistry values may have been related to reduced feed consumption. Since these dietary levels of simazine seriously affected the nutritional status of treated rats, the results of this study are of limited value for quantitative risk assessment (Tai et al., 1985a).

Simazine (97.5 percent) was administered to seven to eight month old beagles (four/sex) for 13 weeks at concentrations of 0, 200, 2,000 or 4,000 ppm (equal to 0, 5, 50 and 100 mg/kg-day) (Tai et al., 1985b). All dogs were observed daily for signs of toxicity. Blood and urine were collected from all dogs at pre-dose and at days 44 and 92 of the study for hematological, clinical chemistry and urinalysis determinations. At the end of the study, all dogs were necropsied for gross and histopathological examination. In the 4,000 ppm dose group, alopecia, tremor, dermatitis (males), and emesis were observed. Similar to the rat study, reduced feed consumption affected the nutritional status of the animals and hence reduced the value of this study. Due to the seriously affected nutritional status of the test animals, the results of this study were also considered of limited value (Tai et al., 1985b).

Simazine (50 percent active ingredient) was administered orally to 21 sheep at 0, 1.4, 3.0, 6.0, 25, 50, 100 or 250 mg/kg-day for various durations up to about 22 weeks (Dshurov, 1979). Fatty and granular liver degeneration, diffuse granular kidney degeneration, neuronophagia, diffuse glial proliferation and

degeneration of ganglion cells in the cerebrum and medulla were found. In sheep that died, spongy degeneration, hyperemia and edema were observed in the cerebrum; the degree of severity was related to the dose of simazine and the duration of exposure. The thyroid showed hypofunction after daily doses in the range of 1.4 to 6.0 mg/kg given for periods of 63 to 142 days. The most severe antithyroid effect followed one or two oral doses of 250 mg/kg, which in one sheep produced parenchymatous goiter and a papillary adenoma. Parenchymatous goiter was also seen in sheep administered simazine orally at 50 or 100 mg/kg once per week for approximately 22 weeks. Based on these data, a lowest-observed-adverse-effect-level (LOAEL) of 1.4 mg/kg can be identified. However, it is not clear from the study details whether the authors corrected for the 50 percent formulation when providing the dosage levels (Dshurov, 1979, as cited by U.S. EPA, 1990).

In a 21-day subacute dermal toxicity study in rabbits, Ciba-Geigy (1980) reported that 15 dermal applications of technical simazine at doses up to 1 g/kg produced no systemic toxicity or any dose-related alterations of the skin.

Chronic/Carcinogenicity Studies

Rat

Simazine (96.9 percent) was administered in the diet to Crl:CD Sprague-Dawley rats at 0 (90/sex), 10, 100 (80/sex) or 1,000 ppm (90/sex) for 104 weeks (Ciba-Geigy, 1988a). The NOAEL for this study was 10 ppm (equal to 0.5 mg/kg) based on decreased body weights at the 100 and 1,000 ppm dose levels in females. Also, decreased body weight gains and feed consumption were observed in both sexes at the 1,000 ppm dose level. The mortality rate was high for all groups, but was significantly different from controls only at the 100 ppm dose level for female rats. A decrease in RBC, hemoglobin (Hb) and hematocrit (HCT) was observed in females in the 1,000 ppm dose group. In male rats, there was an increase in relative weights of the brain, liver, testes/epididymis, and a decrease in heart and relative heart weights at 1,000 ppm. In female rats, there was a relative increase in brain, kidney, and liver weights at 1,000 ppm. The incidences of mammary carcinoma were significantly increased at 100 and 1,000 ppm in females and the incidence of fibroadenoma was significantly higher at 1,000 ppm compared to controls (Table 3). A significantly higher incidence of cystic glandular hyperplasia, an increased incidence of a rare kidney tubular adenoma (2/70), and an increased incidence of pituitary carcinoma were also observed at the 1,000 ppm dose level.

Table 3. Summary of Mammary Lesions in Female Sprague-Dawley Rats Fed Diets Containing Simazine for Two Years[1]

Histopathological lesions	Concentration (ppm)			
	0	10	100	1,000
Adenoma[2]	2/70[3]	4/70	1/70	5/70
Carcinoma	14/70	13/70	19/70*[4]	35/70**[4]
Fibroadenoma	22/70	27/70	19/70	40/70**
Total tumors[5]	39/90	33/88	31/80	61/80
Cystic glandular hyperplasia	51/70	50/70	53/70	65/70**

[1]Adapted from U.S. EPA's review of the Ciba-Geigy study (1988a).
[2]Interim sacrifice and recovery group are not included in the estimation of tumors.
[3]Number of animals with specified observation/total number of tissue examined.
[4]Indicates significance at * ($p<0.05$) and ** ($p<0.01$).
[5]As reported by Stevens et al. (1994) including interim sacrifice and recovery group animals.

An adverse effect disclosure statement was submitted by Ciba-Geigy (July 24, 1992). In the letter it was stated that in June 1989, Ciba-Geigy initiated two new oncogenicity studies on simazine using female Sprague-Dawley rats derived from the F_{2b} generation of the rat reproduction study (DPR, 1993). These animals were exposed to simazine in utero and for 24 months post-partum at dietary levels of 0, 10, 100 or 500 ppm. In addition, an age-matched group of control Sprague-Dawley female rats was employed in the study. The following two separate studies were performed: Study 1: treated and control female rats were allowed to mate with untreated male rats; they then delivered and nursed the pups through lactation day 21. Study II: animals in this group were treated the same as those in Study I, except they were not mated. The results observed after histological examination are presented in Table 4. No other information is available on this data. While ovarianhyperplasia and adenomas are elevated at the highest dose level, these results are not supported by any other rat or mouse study.

The disclosure statement also stated that the incidence of ovarian tumors was not elevated in the combined study previously submitted and reviewed by DPR (DPR, 1993) in which animals were dosed up to 1,000 ppm. Therefore, the ovarian findings in the two studies described above constituted a new potential adverse effect (DPR, 1993).

Mouse

Simazine (96.5 percent) was administered in the diet to Crl:CD 1 (ICR) BR mice at 0 (90/sex/group), 40, 1,000 (80/sex/group) or 4,000 (90/sex/group) ppm for 95 weeks (Ciba-Geigy, 1988b). The NOAEL was 40 ppm (6 mg/kg-day) based on decreased body weight gain and decreased feed and water consumption observed in both males and females at 1,000 and 4,000 ppm. Females also had transitory increases in brain weight, relative brain, liver, and kidney weights at 1,000 and 4,000 ppm and relative adrenal and heart weights, and an increase in relative lung and thyroid/parathyroid weights at 4,000 ppm. There was no increased frequency of tumors in any organ or tissue (DPR, 1993).

Table 4. Ovarian Neoplasia/Hyperplasia Incidence in Female Sprague-Dawley Rats Following Simazine Exposure

Lesions/ Tumors	Feeding level (ppm)				
	0[a]	0[b]	10	100	500
Nulliparous females	12/50	9/25	0/50	21/50	31/50*
Hyperplasia (Sertoli cells)	0/50	0/50	0/50	1/50	5/50
Sertoliform Adenoma					
Primiparous females					
Hyperplasia (Sertoli cells)	17/50	7/25	14/48	14/47	28/49
Sertoliform Adenoma	0/50	0/25	0/48	0/47	1/49

[a]The test and control groups were derived from the F_{2b} litter of the two generation reproduction study.
[b]This control group was comprised of age-matched Charles River Sprague-Dawley female rats.
*Statistically significant compared with the two controls ($p<0.05$).

Dogs

The following two studies in dogs are discussed for non-cancer toxicity findings, as they are of insufficient duration for the study of carcinogenicity.

Simazine (96.5 percent) was administered in the diet for 52 weeks to four Beagles/sex/dose at concentrations of 0, 20, 100 or 1,250 ppm (equal to 0, 0.5, 2.5 and 31.3 mg/kg based on the standard conversion factor of 0.025) (Ciba-Geigy, 1988c). The NOAEL was 20 ppm (0.5 mg/kg) based on reduced body weight gain at 100 ppm, decreased RBC, Hb and HCT, and an increase in platelet counts (DPR, 1993).

In a second experiment, simazine (97.5 percent) was administered in the diet to four dogs/sex/group at 0, 200, 2,000 or 4,000 ppm for 13 weeks. The NOAEL was 200 ppm (5.0 mg/kg) based on reduced body weight and feed consumption at 2,000 and 4,000 ppm. No clear target organ was identified (Ciba-Geigy, 1985, as reviewed by DPR, 1993).

Genetic Toxicity

Simazine was found to be negative in many of the *in vivo* and *in vitro* test systems evaluating gene mutation, chromosomal aberration, and DNA damage conducted for plant pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines for genetic toxicity. Positive results were reported in many independently conducted studies found in the open literature. Some of the tests are reviewed in this section.

Simazine has been tested in a variety of microbial mutagenicity assay systems. None of the data show simazine to cause mutagenic effects in standard prokaryotic test systems. These include tests employing the following organisms: *Salmonella typhimurium* (Simmons et al., 1978; Commoner, 1976; Eisenbeis et al., 1981), *Escherichia coli* (Simmons et al., 1978; Fahring, 1974), *Bacillus subtilis* (Simmons et al., 1978), *Serratia marcescens* (Fahring, 1974), and *Saccharomyces cerevisiae* (Simmons et al., 1978).

Simazine (96.9 percent) was tested in the Ames test at 0 (vehicle = DMSO), 10, 25, 50, 100 or 250 μg/plate on *Salmonella typhimurium* strains TA98, TA1OO, TA1535, TA1537 and TA1538 with and without rat liver S-9. No mutagenicity was observed with any tester strain at any dose level tested. Positive controls functioned as expected (Ciba-Geigy, 1988b as reviewed by DPR, 1993).

Simazine (99.6 percent) was administered in one oral dose (gavage) at various levels to eight mice (Tif:MAGF, SPF)/sex/group. Part I: Cells were harvested at 16, 24 and 48 hours for control (0.5 percent carboxymethyl cellulose (CMC)) and simazine (5,000 mg/kg-limit test). Part 2: Cells were harvested at 24 hours for control (0.5 percent CMC) and simazine (1,250, 2,500 or 5,000 mg/kg-limit test) treatments. One thousand polychromatic (PCE) and normochromatic erythrocytes (NCE) each were scored/animal (five/sex/group) for micronucleus assessment. The PCE/NCE ratio/animal was determined by counting 1,000 erythrocytes. There was no increase in the number of micronuclei in polychromatic erythrocytes relative to negative controls (DPR, 1993).

Simazine (99.6 percent) was applied to primary cultures of human lymphocytes for three hours at 0 (vehicle, DMSO), 6.25, 12.5, 25, 50 or 100 μg/mL with and without activation to test for chromosomal aberrations. No increase in chromosomal aberrations was observed with simazine-treated cells when compared to controls. Positive controls functioned as expected (DPR, 1993).

Simazine (99.6 percent) was administered orally to six Chinese hamsters/sex/group at 0, 1,250, 2,500 or 5,000 mg/kg. One thousand cells in each of three/sex/group were analyzed for micronuclei at 24 hours only, after the second dose. If the effect on cell cycling is not known (the report gives no indication), the laboratory animals should be sacrificed over a 12 to 72 hour period to ensure the detection of micronuclei which are detectable at six and normally for 12 hours before and after the time of peak frequency. No information on PCE/NCE or mitotic index is given. No adverse effect was noted, but the study design was inadequate because of inadequate sampling times (DPR, 1993).

Simazine (96.9 percent) at concentrations of 0 (DMSO or culture medium), 1.57, 4.72, 14.17, 42.5, 85 or 170 μg/mL was tested in primary cultures of rat hepatocytes. The treatment period was 16 to 18 hours in both the original and confirmatory tests. Analysis was performed by autoradiography (three slides/dose, 50 cells scored/slide). Simazine did not induce DNA damage to primary hepatocytes. Positive controls functioned as expected (DPR, 1993).

Simazine induced mutations in the sex-linked recessive lethal test employing the fruit fly *Drosophila melanogaster* (Valencia, 1981). In the study reported by Murnik and Nash (1977), simazine increased X-linked lethal mutations when injected into male *Drosophila melanogaster*, but failed to do so when fed to larvae.

Simazine has been shown to be positive (Simmons et al., 1978) and negative (Waters et al., 1982) for unscheduled DNA synthesis (UDS) in the human lung fibroblast UDS assay.

Simazine (99.6 percent) was tested for DNA repair in primary rat hepatocyte cultures exposed to 0, 0.4, 2, 10 or 50 μg/mL for five hours in the presence of 3H-TdR. No increase in UDS as evidenced by grains/nucleus was reported (DPR, 1993). Similarly, no increase in UDS was observed in human fibroblast cultures treated with simazine (99.6 percent) at levels of 0, 0.2, 1, 5 and 25 μg/mL without activation for five hours.

Simazine failed to produce chromosomal effects as indicated by sister-chromatid exchange (Waters et al., 1982), the mouse micronucleus assay (Waters et al., 1982) and the mouse dominant lethal assay (Ciba Geigy, 1977).

Riccio et al. (1981) tested 11 pesticides for genotoxic activity in two yeast assay systems utilizing the diploid strains D3 and D7 of *Saccharomyces cerevisiae* in the presence and in the absence of a rat liver metabolic activation system. Simazine was negative for all genetic endpoints in both assay systems.

Simazine is mutagenic in plants. It induces chromosome breaks and aberrations, and increases aneuploidy and polyploidy in barley seeds (Wuu and Grant, 1966; Wuu and Grant 1967; Stroev, 1970). Plewa et al. (1984) evaluated genotoxic properties of 11 herbicides and 13 combinations of commercial grade herbicides with *Salmonella typhimurium* and *Saccharomyces cerevisiae* directly and following plant and animal activation. Simazine was tested only in situ with the pollen waxy locus assay of Zea maize, giving positive results. Simazine is a plant-activated promutagen according to this paper.

A water-soluble extract from maize plants exposed to three s-triazine herbicides (atrazine, simazine and cyanazine) has been shown to be mutagenic in strain TA 100 of *Salmonella* (Means et al., 1988). No mutagenic activity was observed in any control plant extracts using either water or a variety of organic solvents. Gel permeation studies of the extracts suggest that the mutagen(s) are small molecules (MW < 1,000).

Ghiazza et al. (1984) studied the effects of simazine on sister-chromatid exchange (SCE) frequency in human lymphocytes following exposure to 0.001, 0.01 or 1 ppm simazine *in vitro*. A significant increase in SCE was observed at 1 ppm (5.07 ± 1.19 compared to 3.51 ± 1.14 in controls). Simazine also induced sister chromatid exchange in human lymphocytes and induced mutation in mouse lymphocytes (Clayton and Clayton, 1994 as cited by Micromedex, 1998).

Biradar and Rayburn (1995) studied the effects of atrazine, simazine, and bentazon on chromosomal damage in Chinese hamster ovary (CHO) cells by flow cytometry. The cell cultures were exposed to atrazine, simazine, or bentazon at concentrations of 0.014, 0.080 or 0.005 μM, respectively, for 48 hours. The authors suggested that U.S. EPA deemed these concentrations safe for drinking water. A known clastogen (Ara-C) was used as a reference for comparing the magnitude of chromosomal damage caused by herbicides. Chromosomal damage was measured by the coefficient of variation and percent chromosomes present in the larger chromosome distribution peaks. Exposure to atrazine increased the coefficient of variation of the largest chromosome distribution peak, suggesting clastogenicity. The negative control, atrazine and Ara-C's coefficient of variations were 3.73, 3.93 and 4.18, respectively. Atrazine concentrations higher (0.023 to 0.092) than the contamination limits exhibited a true clastogenic nature like Ara-C (4.16 compared to 4.18 in positive controls).

Developmental Toxicity Studies

Rat

Simazine (98.2 percent) was administered by gavage to 25 mated (presence of sperm, day 0 of gestation) CR1 COBS CD (SD) (BR) rats/group at 0 (vehicle, 2.0 percent carboxymethylcellulose), 30, 300 or 600 mg/kg during days 6 to 15 of gestation (Ciba-Geigy, 1986). The maternal NOAEL was 30 mg/kg-day based on decreased weight gain and feed consumption at 300 and 600 mg/kg-day dose levels. The developmental NOAEL was 30 mg/kg-day based on lack of ossification in head, teeth, centrum/vertebra, and rudimentary 14th rib at 300 and 600 mg/kg-day.

Pregnant rats were exposed daily to chloroform vapors or aerosols of ethylenethiourea, thimet, simazine, or bromacil during days 7 to 14 of gestation (Dilley et al., 1977). The highest concentration of simazine was 317 $\pm$ 89 mg/m^3. All animals were sacrificed on day 20 of gestation and the dams and fetuses were examined for gross changes. Fetuses were fixed in Bouin's solution or alcohol and examined later for teratology. Simazine did not cause any prenatal changes. None of the compounds was teratogenic at the concentrations used, under these experimental conditions.

Rabbit

Simazine (97 percent) was administered by gavage to 19 rabbits/group at 0, 5, 75 or 200 mg/kg-day during days 7 to 19 of gestation (Ciba-Geigy, 1984). The maternal NOAEL was 5 mg/kg based on decreased weight gain, anorexia, and nervous tremors at the 75 and 200 mg/kg dose levels. The developmental NOAEL was 5 mg/kg based on late resorptions at 75 and 200 mg/kg and reduced fetal weight at the 200 mg/kg dose level. A decreased number of viable fetuses was observed at the mid- and high-dose levels, but these were thought to be due to maternal toxicity at these levels. The author suggested that at doses of 75 mg/kg-day and higher, simazine was toxic to fetuses and dams, but was not embryotoxic nor teratogenic.

Sheep

Necrotic changes in germinal epithelium of the testes, and disturbances in spermatogenesis were observed in sheep which were fed simazine (50 percent active ingredient) at concentrations of 6 mg/kg for 142 days or 25 mg/kg for 37 to 111 days. As the simazine used was only 50 percent pure, and the chemical composition is ill-characterized, the study cannot be utilized for the purpose of risk assessment (Dshurov, 1979, as cited by U.S. EPA, 1990).

Reproductive Toxicity

Simazine (96.9 percent) was administered in the diet to Sprague-Dawley rats (30/sex/group) at 0, 10, 100 or 500 ppm (equal to 0, 0.5, 5 and 25 mg/kg-day) for two generations (Epstein et al., 1991). The systemic parental NOAEL was identified as 10 ppm based on decreased body weight gain at 100 ppm and decreased feed consumption at 500 ppm in both male and female rats of both generations. The sporadic decreased feed consumption noted at 100 ppm was not considered to be compound related. The reproductive toxicity NOAEL was greater than 500 ppm. There were no effects on the reproductive system at any dose levels tested (DPR, 1993).

Hormonal effects

Ovarian hormones are known to play a role in the development of mammary tumors. In order to better understand the role and possible mechanism of ovarian hormones in simazine-induced mammary tumors, we have reviewed several of the studies conducted on a representative s-triazine (atrazine) that were recently submitted in support of atrazine registration as a pesticide active ingredient (DPR, 1998). The effects of atrazine and other triazines on the estrous cycle, estrogen mediated parameter responses, estrogen receptor binding, hormonal induction and metabolism have been the subject of several studies (Wetzel et al., 1994; Stevens et al., 1994; Tennant et al., 1994a,b). The following is a brief summary of the key studies as well as related studies found in the open literature on the endocrine mode of action for chloro-s-triazine induced mammary tumors. These studies also support OEHHA's concern for the potential carcinogenicity of simazine. Overall the data suggest that high doses of atrazine disrupt the estrous cycle and induce mammary tumors. It binds weakly to the estrogen receptor, alters a few estrogen-mediated parameters and has no direct agonist or antagonist activity on the estrogen receptor. At

high doses, it reduces LH and estradiol by influencing the hypothalamus-pituitary control mechanism. These effects are expected to be shared with the other closely-related triazine herbicides.

Atrazine was fed in the diet to Fischer 344 rats at levels of 0, 10, 70, 200 or 400 ppm, and to Sprague-Dawley rats at levels of 0, 70 or 400 ppm (Wetzel *et al.*, 1994). There were 70 female rats in each dose group. Ten rats per group were sacrificed at 1, 3, 9, 12, 15 and 18 months, and all remaining animals at 24 months. Various parameters such as estrous cycle, plasma hormone levels and tumor type were determined. Atrazine-fed female Sprague-Dawley rats spent more days in estrous as compared to controls. This was statistically significant after 9 and 18 months in the 400 ppm dose group and after one and nine months in the 70 ppm dose group. Plasma estradiol concentrations were also significantly increased at three months in female rats fed 70 or 400 ppm atrazine, but there was no time or dose dependent effect. Plasma prolactin levels measured at nine months were increased at the highest dose only. No effect was observed on estrous cycle or estradiol concentration in the Fischer 344 rats. In Sprague-Dawley rats, tumor latency for mammary and pituitary gland tumors during the first year was shortened at the 400 ppm level, but the incidence was not increased. Also, there was an increased incidence of galactoceles (nonneoplastic masses in the mammary gland) in the Sprague-Dawley rats fed 400 ppm of atrazine, compared to the control female rats. The overall incidence, however, over the two years was similar to controls and not statistically significant. This may be due to a high background rate of these tumors in Sprague-Dawley rats. Body weight gain was also lower at the 400 ppm level. The results suggest a possible effect of atrazine on tumor induction.

In Fisher-344 rats, reduced body weight gain was observed in the 200 and 400 ppm dose groups. No other effects were reported. The incidences of mammary and pituitary tumors were comparable across groups and no evidence of an effect on time-to-tumor was noted. Based on these findings the authors hypothesized that high-dose atrazine administration in Sprague-Dawley females is related to an acceleration of age-related endocrine changes leading to an earlier onset and/or increased incidence of mammary tumors. The authors further suggested that this is due to atrazine interference with normal estrous cycling, thus promoting prolonged exposure to endogenous estrogen. It is noteworthy, however, that the estrous cycle was also prolonged at the 70 ppm dose level, although there was no concomitant increase in or earlier onset of mammary tumors in this dose group (Wetzel et al., 1994). The conclusions drawn by the authors do not appear to be well supported by their data. Because of the high background tumor incidence and small number of animals used in this study, it is difficult to draw conclusions about the effects at low dose levels.

It has been hypothesized that mammary tumors in Sprague-Dawley rats induced by simazine, atrazine and other s-triazines develop as a result of endocrine-mediated effects. Stevens et al. (1994) compiled data from previously conducted carcinogenicity studies to substantiate this hypothesis. Atrazine and simazine, and to a lesser extent propazine and terbuthylazine, have been shown to induce mammary tumors in female Sprague-Dawley rats. The 2-thiomethyl-s-triazines (ametryn, prometryn, terbutryn and 2-methoxy-s-triazines) indicated weak to no induction of mammary tumors. Hormonal data were given only for simazine at week 104 of the study, which indicated marked changes in hormonal profile, but significant differences were observed only at the highest dose of 1,000 ppm. The reported values for various hormone levels in control and 1,000 ppm dose levels, respectively, were: estradiol, 12±6 ng/mL compared to 2 ±1 ng/mL; prolactin, 29 ± 18 ng/mL compared to 204 ± 147 ng/mL; progesterone, 39 ±26 ng/mL compared to 11 ± 9 ng/mL; growth hormone, 11 ± 2 ng/mL compared to 37 ± 16 ng/mL.

The effects of simazine, atrazine, and the common metabolite diaminochlorotriazine (DACT) were studied on estrogen-mediated parameters using several rat uterine model systems (Tennant et al., 1994a). For the effect on uterine weight, ovariectomized Sprague-Dawley rats were orally administered up to 300 mg/kg-day of atrazine, simazine or DACT for one to three days. On days two and three, half of each group of rats (three or four) received estradiol by injection. Dose-related decreases in uterine wet weights were obtained in rats treated with estradiol and atrazine as compared with estradiol treated controls. No

effect was observed on uterine wet weight with atrazine alone as compared with vehicle control. For thymidine uptake studies, immature females were given 0, 1, 10, 50, 100 or 300 mg/kg-day atrazine, simazine or DACT for two days. On day two, all animals received estradiol by injection. After 24 hours, all animals were killed and uterine slices were incubated with ^{3}H-thymidine. Thymidine incorporation into uterine slices was decreased at the 50, 100 and 300 mg/kg-day dose levels. For uterine progesterone receptor binding studies, ovariectomized rats were dosed for two consecutive days with 50 or 300 mg/kg-day of atrazine, simazine or DACT. Each dose was followed by subcutaneous injection of estradiol. Parallel groups were treated with 0 or 300 mg/kg-day of atrazine, simazine or DACT without estradiol. Net progesterone receptor binding was reduced significantly in high dose animals in the atrazine and simazine treated groups and non-significantly in the DACT treated group subjected to estradiol treatment, compared to the group treated with estradiol alone. The authors concluded that triazine displayed very low antagonistic potency against estradiol function and they postulated that atrazine may operate through cellular interactions unrelated to these hormone effects.

The effects of simazine, atrazine, or DACT were studied on the binding of estradiol to the rat uterine estrogen receptor (ER) (Tennant et al., 1994b). Under equilibrium conditions none of the three triazines competed against the binding of radiolabeled estradiol to the ER. In ovariectomized rats, a dose of 300 mg/kg-day of atrazine, simazine or DACT for two days reduced ER binding capacity by approximately 30 percent. The authors suggested that triazines bind weakly to the ER, and other molecular interactions may play a part in the triazine effect on target tissues.

The effect of atrazine on ovarian function was studied in Long-Evans hooded (LE-hooded) and Sprague-Dawley rats (Cooper et al., 1996a). Atrazine was administered by gavage to females displaying regular four-day estrous cycles for 21 days at doses of 75, 150, and 300 mg/kg-day. In both strains, a dose of 75 mg/kg-day disrupted the four-day ovarian cycle; but no distinct alteration in ovarian function (i.e., irregular cycles but not persistent estrus or diestrus) was observed. At 150 mg/kg-day atrazine induced repetitive pseudopregnancies in females of both strains. At 300 mg/kg-day, repetitive pseudopregnancies were induced in the Sprague-Dawley females, but the ovaries of the LE-hooded females appeared regressed and the smear cytology was indicative of the anestrus condition. These data demonstrate that atrazine, and probably other s-triazines like simazine, can disrupt ovarian function and bring about major changes in the endocrine profile of female rats.

Atrazine was administered orally to Crl:CD Sprague-Dawley BR female rats (90/group) for 28 to 31 days prior to ovariectomy and continued for an additional ten days at 0, 2.5, 5, 40, or 200 mg/kg-day (Morseth, 1996 as reviewed by DPR, 1996). The rats were evaluated for variations in estrous cycle stages by vaginal smear analysis during weeks two to four of the treatment. Seven days after ovariectomy, rats were implanted with a silastic capsule designed to deliver estradiol levels ≥12 pg/mL. On the tenth day after ovariectomy, blood samples were taken at intervals (20 to 25 samples/interval) for assays of estradiol (to verify capsule implantation), luteinizing hormone (LH), and prolactin. Prior to ovariectomy, estrous cycling was disturbed, most remarkably by prolonged periods of diestrus at 40 and 200 mg/kg-day. Ovariectomized rats provided with estradiol-releasing implants had a remarkable decrement in LH peak levels at both the 40 and especially at the 200 mg/kg-day dose levels, a possible delay in timing of prolactin peak levels. Data are consistent with the hypothesis that the primary toxic action of atrazine leads to delays of ovulation (hence prolonged estrus) by disturbing the releases of LH and the prolactin surge. The author suggested that the data support a "threshold." However, the wide spacing of dose levels and the high degree of variability in response do not allow a definitive conclusion from these results.

Atrazine (200 mg/kg intraperitoneal for three days) suppressed the estrogen-induced surge of LH and prolactin in ovariectomized rats (Cooper et al., 1996b). However, the pituitaries of atrazine treated rats did release LH in response to gonadotropin-releasing hormone (GnRH). Using this model, the authors reported a dose- and time-dependent disruption of pulsatile LH release in rats exposed to 0, 75, 150, and

300 mg/kg atrazine. The authors concluded that atrazine disrupts the central nervous system control of pituitary function.

Atrazine (97 percent) or DACT (97.4 percent) was administered to groups of 15 female Crl:CD®BR rats for at least two weeks at dose levels of 100, 200, or 300 mg/kg-day (Morseth, 1990 as reviewed by DPR, 1996). Initially the high dose level was 400 mg/kg-day, but this was reduced to 300 mg/kg-day for both test compounds on day four due to excessive toxicity. Two groups of 15 animals each served as controls; one received only cornstarch suspension vehicle and the other (a positive control for prolactin secretion) received an intraperitoneal dose of metoclopramide 20 minutes before sacrifice. Rats were sacrificed at the time of first determination of diestrous stage after at least 14 daily treatments, cycle stage being determined by vaginal cytology. Serum collected at sacrifice was assayed for prolactin, LH, FSH, progesterone and estradiol. The majority of atrazine treated rats at dose levels of 200 to 300 mg/kg-day as well as DACT treated rats at dose levels of 100 to 300 mg/kg-day had clinical signs of "thin" or "few or no feces" and a dose-related decrease in body weight. There was also a reduction in thymus weight in all groups. Coincident with the above signs of general toxicity, there were possible hormone level changes, particularly decreases of LH, progesterone and estradiol in the 200 to 300 mg/kg-day DACT treated rats. In general, the high variability evident in hormone levels, coupled with high general toxicity in groups appearing to manifest hormone level changes, makes this study of very limited value for assessing intrinsic effects of test articles on hormone control (DPR, 1996).

Estrogenic activities of simazine or atrazine were assessed using an environmental estrogen (estradiol) bioassay which consists of a Gal-human estrogen receptor chimerical construct (Gal-EGO) and a Gal regulated luciferase reporter gene (17m5-G-Lucia) which have been integrated into HeLa cells. A dose-dependent induction in luciferase activity was observed following treatment of the cells with 17β-estradiol. No significant induction was observed in reporter gene activity following treatment with chloro-s-triazines, suggesting that chloro-s-triazines do not interact with the ER (Balaguer *et al.*, 1996).

The effects of simazine and atrazine were studied on estrogen receptor-mediated responses following *in vivo* and *in vitro* exposure (Connor et al., 1996). After exposure for three days to atrazine at 50, 150 or 300 mg/kg-day, uterine wet weights, progesterone receptor (PR) binding activity and uterine peroxidase activity were measured. No treatment-related effect was observed on any of the parameters studied. However, both compounds inhibited basal cytosolic PR binding and uterine peroxidase activity in a dose-independent fashion. For *in vitro* responses, cell proliferation and gene expression were measured in the MCF-7 human breast cancer cell line and the growth was measured in the estrogen-dependent recombinant yeast strain PL3, which requires the presence of an estrogen substance and functional ERs in order to grow on selective media. No effects were observed on basal or estradiol induced MCF-7 cell proliferation or on the formation of the PR-nuclear complex. No agonist or antagonist effects were observed on estradiol induced luciferase activity. The estrogen-dependent PL3 yeast strain did not grow on minimal media supplemented with atrazine or simazine in place of estradiol. The authors concluded that these results indicate that the ER does not mediate the estrogenic and antiestrogenic effects elicited by these chemicals.

The effects of simazine, atrazine, deisopropyl atrazine, or cyanazine on ER-mediated responses were studied in yeast expressing the human estrogen receptor (hER) and an estrogen-sensitive reporter gene (β-galactosidase) (Tran et al., 1996). In the presence of an estradiol concentration (20 nM) that induced maximal reporter gene activity in yeast, atrazine did not inhibit reporter activity. However, all s-triazines decreased reporter activity in a dose-dependent manner in the presence of a submaximal concentration of estradiol (0.5 nM). The estradiol-dependent activity of a mutant hER lacking the amino terminus was not inhibited by atrazine in yeast. Competition binding assays indicated that all the s-triazines including simazine displaced radiolabeled estradiol from recombinant hER. These results suggest that the ability of s-triazines to inhibit estrogen receptor-mediated responses in yeast occurred through an interaction with hER and was dependent on the concentration of estradiol.

The effects of lindane, atrazine, and prometryn were studied on the formation of the ER complex (Tezak et al., 1992). For the *in vivo* studies, 21 day old rats were administered lindane at 30 or 60 mg/kg; atrazine at 30, 60, or 120 mg/kg; or prometryn at 120 mg/kg for seven days. Animals were killed after 28 days for *in vivo* and *in vitro* studies. Both *in vivo* and *in vitro*, all the pesticides significantly inhibited the formation of the estradiol ER complex in rat uterus cytosol. The inhibition was non-competitive; the pesticides decreased the number of binding sites but not the affinity of the ER for estradiol.

The influence of s-triazine compounds (atrazine, prometryn and de-ethylatrazine) was studied *in vivo* and *in vitro* on testosterone metabolism and binding of 5α-dihydrotestosterone (5α-DHT) to its receptor in the rat prostate (Kniewald et al., 1995). Both atrazine and prometryn reduced 5α-DHT formation. In addition, both significantly decreased the number of binding sites for 5α-DHT on the receptor molecule following *in vivo* or *in vitro* exposure, but the Kd value was not changed. The authors suggested that the inhibition of the enzymatic activities responsible for testosterone conversion and steroid hormone-receptor complex formation was non-competitive and reversible and that s-triazine compounds acted as antiandrogens.

Kniewald et al. (1997) evaluated the appearance of immunoreactive cells for LH and follicle stimulating hormone (FSH) at the rat adenohypophysial (AH) level with and without atrazine treatment. Atrazine (120 mg/kg) was administered daily intraperitoneally for seven days to females and per 72 hours (every third day) for 60 days to males. The authors state that exposure to atrazine induces sterility in rats expressed by permanent diestrous in females and significant changes in spermatogenesis in males as observed by electron microscopy. However, no data or references were provided to support this statement. The presence of LH and FSH reactive cells were determined in 6 μM sections from AH stained by the avidin-biotin–peroxidase complex method and counter stained with hematoxylin. Atrazine treated males had 17 and 24 percent lower LH and FSH immunoreactive cells, respectively, as compared with controls. Atrazine treated females had the same percentage of LH and FSH reactive cells in AH as controls in diestrous. The authors conclude that luteinizing releasing hormone (LHRH) from hypothalamus stimulates synthesis of FSH more than LH within the AH and that this increased FSH is responsible for the absence of cycle in the female rats.

The effects of atrazine on androgen converting enzymes and protein synthesis were studied in male porcine pituitary adenohypophysial gland (Kniewald et al., 1994). The pituitaries were removed from six-month-old pigs castrated at the age of three months. Fresh tissues were incubated with ^{14}C-testosterone and enzyme activities responsible for testosterone conversion were measured and expressed as pg of steroid/mg tissue. Atrazine was added to the incubation mixture at various concentrations. Atrazine at 0.175 to 0.7 μmol significantly inhibited 5α-reductase, which converts testosterone to DHT (24 ± 2 pg/mg compared to 32 ± 3.1 pg/mg in control) and 17β-hydroxysteroid dehydrogenase, which converts testosterone to 5α-androstane-3α, 17β-diol (A-diol) (66 ± 5.4 pg/mg compared to 109 ± 8.2 pg/mg in control). Atrazine also inhibited protein synthesis in pituitary cytosol. Electrophoretic analysis of cytosolic protein indicated that while other major bands remained unchanged, the purified fraction at 28.2 kilodaltons (kD) was increased. This was identified after gel filtration and 2-D SDS PAGE as prolactin. These results may suggest a possible mechanism for the effects of atrazine on androgen control of reproduction.

The effect of atrazine was studied on the thyroid gland in female albino rats administered atrazine orally at 0.2 LD_{50} for periods of 6 or 12 days (Kornilovskaya et al., 1996). At the termination of dosing, the anesthetized animals were killed and blood was drawn for the determination of serum triiodothyronine (T3) and thyroxin (T4). A dose-dependent decrease in serum T3 concentrations was observed in all the treatment groups (control: 0.57 nmol/L; atrazine for six days: 0.35 nmol/L; atrazine for 12 days: 0.21 nmol/L). No effects were observed on the concentration of thyroxine. Histologically, the thyroid epithelium featured small cuboidal cells and occasional structures of the follicle confluence within epitheliomas. There was also an increase in the number of follicle-building thyroid cells and follicular

volume and a decrease in nuclear volume. The authors suggested that the observed thyroid hyperplasia might be due to the activation of the hypothalamus-pituitary axis due to a decrease in T3 levels (Kornilovskaya et al., 1996).

The ability of various xenobiotics including atrazine to inhibit the binding of the [^{3}H] physiological ligand (present at a concentration of 7 nM) to the rabbit uterine cytosol androgen and estrogen receptors, to rat androgen-binding protein, and to human sex hormone-binding globulin (hSHBG) was examined *in vitro*. Atrazine caused a statistically significant inhibition of specific binding of [^{3}H]5α-DHT to the androgen receptor. The binding of [^{3}H]5α-DHT to rat androgen-binding protein was inhibited 40 percent by atrazine. There was no inhibitory effect of atrazine on the binding of 5α-DHT to hSHBG (Danzo, 1997).

The metabolism of estradiol, using a radiometric assay that measures the relative formation of 16-α-hydroxyestrone (16-alpha-OHE-1) and 2-hydroxyestrone (2-OHE-1) from specifically tritiated estradiol in (ER+) MCF-7 cells, was studied in the presence of various environmental xenobiotics (Bradlow et al., 1995). The ratio of 16-alpha-OHE-1/2-OHE-1 observed after treatment with the known rodent carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) was compared with the ratio after treatment with DDT, atrazine, γ-benzene hexachloride, kepone, coplanar PCBs, endosulfans I and II, linoleic and eicosapentenoic acids, and indole-3-carbinol (I3C). All pesticides tested including atrazine significantly increased the ratio of 16-alpha-OHE-1/2-OHE-1 metabolites to values comparable to or greater than those observed with DMBA treatment. In contrast, the antitumor agent I3C increased 2-OHE-1 formation and yielded ratios that were a third of those found in unexposed control cells, and 1/10th of those found in DMBA-treated cells. The authors suggested that atrazine and other xenobiotics might increase the risk of breast cancer by altering the ratio of 16-alpha-OHE-1/2-OHE-1. The estradiol metabolite 2-hydroxyestrone (2-OHE-1) inhibits breast cell proliferation, while 16-alpha-hydroxyestrone (16-alpha-OHE-1) enhances breast cell growth, increases unscheduled DNA synthesis, increases oncogene and virus expression, and increases anchorage-independent growth.

Summary of studies presented

Effects on estrous cycle

Simazine was not specifically tested for effects on the estrous cycle, but the marked increased incidence of mammary carcinomas, results much like those characteristic of atrazine, is well documented. In Sprague-Dawley female rats, atrazine exposure at about 20 mg/kg-day increased the percentage of days in estrous and increased the incidence and shortened the onset of mammary tumors. The estrous cycle was also prolonged at the 3.5 mg/kg-day dose level, but there was neither an increase in nor an earlier onset of mammary tumors in this dose group. In another study conducted in Long-Evans hooded (LE-hooded) and Sprague-Dawley rats, 75 mg/kg for 21 days disrupted the four-day ovarian cycle, but no distinct alteration occurred in ovarian function (i.e., irregular cycles but not persistent estrus or diestrus in both strains of rat). At 150 mg/kg-day, atrazine induced repetitive pseudopregnancies in females of both strains. At the 300 mg/kg-day dose, repetitive pseudopregnancies were induced in the Sprague-Dawley females, but the ovaries of the LE-hooded female appeared regressed and the smear cytology was indicative of the anestrus condition. A recent study showed that a dose of 20 mg/kg-day atrazine given to ovariectomized female Sprague-Dawley rats did not increase or cause earlier onset of mammary tumors. The genotoxic carcinogens DMBA and MNU both induced an increased incidence and earlier onset of mammary tumors in this experimental model.

Effects on estrogen mediated parameters

The effects of simazine and other chloro s-triazines including the representative atrazine were studied on estrogen-mediated parameters following *in vivo* and *in vitro* exposure. *In vivo*, dose-related decreases in uterine wet weights were obtained in rats treated with estradiol and atrazine, but not with atrazine alone. In addition, thymidine incorporation in uterine slices was decreased at 50, 100 and 300 mg/kg-day doses of atrazine. For *in vitro* responses, no effects were observed on basal or estradiol-induced MCF-7 cell proliferation or on the formation of the PR-nuclear complex formation. No agonist or antagonist effects were observed on estradiol-induced luciferase activity. The estrogen-dependent PL3 yeast strain did not grow on minimal media supplemented with atrazine or simazine in place of estradiol. In an estrogen (estradiol) bioassay wherein binding of ligand to ER regulated luciferase reporter gene (17m5-G-Lucia) activity, a dose-dependent induction in luciferase activity was observed following treatment of the cells with 17β-estradiol, but not with atrazine and simazine. In yeast expressing the human estrogen receptor (hER) and an estrogen-sensitive reporter gene (β-galactosidase), atrazine did not inhibit reporter gene activity. However, atrazine decreased reporter activity in a dose-dependent manner in the presence of a submaximal concentration of estradiol (0.5 nM).

Effects on receptor binding

Simazine has not been tested in receptor binding assays. However, competition binding assays indicated that atrazine displaced the radiolabeled estradiol from recombinant hER. Net progesterone receptor binding was reduced significantly in high-dose animals in the atrazine-treated groups subjected to estradiol treatment and non-significantly in estradiol-pretreatment rats. In the absence of estradiol treatment, lesser but nevertheless statistically significant reductions in progesterone receptor binding was observed. In another study, atrazine alone for one to three days inhibited basal cytosolic PR binding. In rat prostate, atrazine inhibited 5α-dihydrotestosterone (5α-DHT) formation and decreased the number of binding sites for 5α-DHT on the receptor molecule following *in vivo* or *in vitro* exposure to atrazine, but the Kd value was not changed. Atrazine also inhibited the binding of tritiated physiological ligands, [^{3}H]5α-DHT and ABP (40 percent) to androgen receptors. There was no inhibitory effect of atrazine on the binding of 5α-DHT to hSHBG in rat prostate.

Effects on hormone induction and metabolism

In determining the effects of chloro s-triazines on hormonal induction, simazine was not tested. However, the representative s-triazine compound atrazine has been studied in this context. Reduced levels of LH, progesterone and estradiol were observed in a recent rat study. While the exact mechanism of hormonal disruption by atrazine is not known, the alteration in estrous cycling is considered to be due to the disruption in hypothalamic-pituitary regulation. Atrazine inhibited 5α-reductase (which converts testosterone to DHT) in male porcine pituitary adenohypophysis, and 17β-hydroxysteroid dehydrogenase (which converts testosterone to 5α-androstane-3α, 17β-diol (A-diol)). Atrazine also inhibited prolactin synthesis in pituitary cytosol. A dose-dependent decrease in serum T3 concentrations was observed in all the treatment groups. No effects were observed on the concentration of thyroxine. Atrazine increased the ratio of 16-α-hydroxyestrone (16-alpha-OHE-1) and 2-hydroxyestrone (2-OHE-1) from specifically tritiated estradiol in (ER+) MCF-7 cells to values comparable to or greater than those observed with DMBA treatment. In contrast, the antitumor agent I3C increased 2-OHE-1 formation and yielded ratios that were a third of those found in unexposed control cells and one-tenth of those found in DMBA-treated cells.

Proposed hypotheses for chloro-s-triazine carcinogenicity

Hormone-Mediated Effects

It has been hypothesized that atrazine (a representative chloro-s-triazine) induces mammary tumors by disrupting the normal secretory activity of the hypothalamic-pituitary-ovarian axis. Either directly or indirectly, atrazine affects the hypothalamus leading to decreased secretion of hypothalamic norepinephrine (NE). NE in turn modulates the secretion of gonadotropin from the hypothalamus (GnRH). GnRH is responsible for inducing the pituitary release of LH. The LH surge provides a signal to the ovaries promoting ovulation. Insufficiency of the LH surge leads to a failure of ovulation. The ovaries of females with decreased LH will continue to secrete estrogen in an attempt to signal a LH surge by the pituitary glands. Ovarian derived estrogen feeds back to prolactin secreting cells in the pituitary to secrete prolactin and stimulate their proliferation. Estrogen and prolactin derived from the hyperplastic pituitary act on the mammary glands to eventually increase the risk of mammary tumor formation. Exposure of the pituitary to increased estrogen results in an increase in prolactin secretion and pituitary weight, and eventually, in increased adenomas. The role of prolonged exposure to estrogen and prolactin in mammary carcinogenesis in rats is well-documented (Nandi et al., 1995; Russo and Russo, 1996).

Chloro-s-triazines (atrazine, simazine, propazine and terbuthylazine) have consistently produced mammary tumors in female Sprague-Dawley (SD) rats. No dose related increase was observed in male SD rats. The data from long term bioassays demonstrate that there is a decrease in time to tumor for mammary tumors in the female SD rats after atrazine exposure (Stevens et al., 1994). Mammary tumors were also observed in F344 rats exposed to atrazine in one study (Pinter et al., 1990). This was a life-long study and the results are considered equivocal because of the poor design. In humans, evidence from epidemiological studies is not sufficient to evaluate if s-triazines are carcinogenic; however, there is suggestive evidence of a possible association of triazine exposure with non-Hodgkin lymphoma (NHL) and hormonally induced prostate, breast, and ovarian cancer (reviewed in OEHHA, 1999; U.S. EPA, 1999).

Mammary tumors are a common and expected occurrence in aging female SD rats and are associated with aging of the reproductive system. In aging female SD rats, characteristic patterns of reproductive parameter changes occur at 9 to 15 months of age. This includes a breakdown of hypothalamic regulation of the estrous cycle, which is characterized by the gradual transition of a four- or five-day estrous cycle to an irregular cycle and eventually to a state of persistent estrous. This persistent estrous is associated with a moderate estrogen and prolactin level and low progesterone levels. Excessive estrogen and prolactin secretion can create an endocrine milieu conducive to mammary gland and pituitary gland cell proliferation and mammary tumor formation. Increased pituitary weight, pituitary hyperplasia, and pituitary adenomas are characteristic features of enhanced estrogens, and all have been reported to be increased in aged female rats that undergo constant estrous as a primary concomitant of reproductive aging (Baird et al., 1990; Blankenstein et al., 1984). The basic premise here is that chlorotriazines hasten the normal aging process and cause an earlier onset of an endocrine environment favorable to the development of mammary tumors in the SD strain of rats. Support for this hypothesis is provided by the absence of mammary tumors in ovariectomized rats treated with atrazine. It must be noted that mammary tumors in rats are strongly hormone dependent for both induction and growth, and ovariectomy has been shown to cause regression of about 80 percent of 7,12-dimethylbenz(a)anthracene (DMBA) induced tumors in treated rats (reviewed in Russo and Russo, 1996).

U.S. EPA (1999) reviewed recently published and unpublished studies from their National Health Effects Research Laboratory. These studies suggest that high doses of atrazine (75 to 300 mg/kg-day) given for a short duration of time (one to three exposures) reduce hypothalamic norepinephrine levels. Also, these high doses were shown to reduce both serum LH and prolactin surges in ovariectomized Long-Evans rats (Cooper, 1996a, 2000). These studies provide evidence that atrazine may affect the level of hypothalamic catecholamines. Evidence for an attenuation of LH surges and an early onset of prolonged and or

persistent estrous is provided in several studies (reviewed in OEHHA, 1999). However, contradictory results have been reported. Cooper et al. (1996b) stated that "The results of these experiments demonstrate that the chlorotriazine, atrazine, can disrupt regular ovarian functions in the two strains of rats. ... However, our results do not support their [Eldridge et al., 1994] conclusion that atrazine increased the number of days spent in vaginal estrus. Furthermore, the pattern of hormone secretion observed in the animals receiving the two higher doses of atrazine in this study would not support the hypothesis that atrazine induces changes in the endocrine milieu that are conducive to the development of mammary gland tumors. The prolonged periods of diestrus observed...were associated with elevated serum progesterone and low estradiol concentrations. Combined, the present results suggest that atrazine exposure induces a repetitive pseudopregnant condition."

Pregnancies or repetitive pseudopregnancies are not considered endocrine environments that support the development of mammary tumors. Estrogen stimulation of the pituitary causes an increase in the secretion of prolactin, increased pituitary weight, hyperplasia and pituitary adenomas. Increased prolactin is closely associated with the development of mammary tumors, especially fibroadenomas (Baird et al., 1990). There is no data to support a high prolactin level in any of the studies on chlorotriazines. In fact, the results of the hormone measurement did not reveal any significant dose related alteration in serum hormone levels compared to the control. An attempt is made to use histomorphological evidence (e.g., acinar development, dilated duct, and increases in the incidence and severity of galactoceles) as an indication of an early onset of increased prolactin production. There is evidence to support an increase in pituitary weight in only one study with no dose response. There is only one study on serum estradiol level. Although this study showed an early onset of increased estradiol levels at 4.23 mg/kg, there is no clear dose response relationship. (reviewed by U.S. EPA, 1999; OEHHA, 1999).

Atrazine disrupts the rat estrous cycle (well supported) which may lead to premature reproductive senescence (less clear). This creates a hormonal milieu conducive to mammary and pituitary tumor formation in SD rats (Cooper, 1983). While there is little direct evidence to support the last link associated with mammary tumor formation (pituitary weight increase, increased hormone level, fibroadenoma of the pituitary), overall, the data suggest a potential estrogenic mechanism for the mammary tumors.

Genotoxicity

Only a few of the recent genotoxic studies, including studies submitted by the registrant to Department of Pesticide Regulation (DPR) on atrazine and simazine, are positive for mutagenicity. Chlorotriazines are consistently positive in plant mutagenicity assays, but are sporadically positive in non-plant mutagenicity assays. In a series of tests reported by Adler (1980), atrazine was negative in the majority of *in vitro* tests, but was positive in a series of *in vivo* tests. In recent years, positive genotoxic effects have been observed in the *in vivo* micronucleus assay, *in vitro* clastogenicity tests in Chinese hamster ovary cells, and for gene mutation and p^{53} protein expression in human peripheral blood lymphocytes. Overall, the evidence from a variety of in vitro and in vivo studies suggests a direct mutagenic potential for simazine. For an extensive review of these studies see OEHHA (1999) and U.S. EPA (1999).

Genotoxicity of hormonal metabolites

The metabolism of estradiol, using a radiometric assay that measures the relative formation of 16-α-hydroxyestrone (16-α-OHE-1) and 2-hydroxyestrone (2-OHE-1) from specifically tritiated estradiol in (ER+) MCF-7 cells, was studied in the presence of various environmental xenobiotics (Bradlow et al., 1995). The ratio of 16-α-OHE-1 to 2-OHE-1 observed after treatment with the known rodent carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) was compared with the ratio after treatment with DDT, atrazine, γ-benzene hexachloride, kepone, coplanar PCBs, endosulfans I and II, linoleic and

eicosapentenoic acids, and indole-3-carbinol (I3C). All pesticides tested including atrazine significantly increased the ratio of 16-α-OHE-1 to 2-OHE-1 metabolites to values comparable to, or greater than, those observed with DMBA treatment. In contrast, the antitumor agent I3C increased 2-OHE-1 formation and yielded ratios that were a third of those found in unexposed control cells and 1/10th of those found in DMBA-treated cells. The authors suggested that atrazine-like xenobiotics might increase the risk of breast cancer by altering the ratio of 16-α-OHE-1 to 2-OHE-1. The estradiol metabolite 2-OHE-1 inhibits breast cell proliferation while 16-α-OHE-1 enhances breast cell growth, increases unscheduled DNA synthesis and oncogene and virus expression, and increases anchorage-independent growth. There are no data on the ratio of estradiol metabolites after simazine treatment.

In recent toxicity studies, atrazine has been shown to increase aromatase, the enzyme that converts androgen to estrogen, in various human cell lines in a dose and time dependent manner (Stoker et al., 2000). Estrogen and its metabolites have been shown to cause DNA damage (Roy and Liehr, 1999).

Mammary tumors, like other tumors, are a result of a series of molecular genetic events involving the activation of oncogenes and inactivation of tumor-suppressor genes (p53 protein). Expression of these genes has been observed in rats (c-Ki-ras) and humans (erbB, erbB2 and erbB3). There are no studies to indicate the absence of these oncogenes in atrazine-induced mammary tumors.

Other effects of chloro-s-triazine

Recent pre-and post puberty studies in Wistar male (Stoker et al., 2000) and female rats (Laws et al., 2000) suggest that atrazine delays the development of puberty in both sexes. All chlorotriazines (atrazine, simazine, cyanazine as well as three triazine metabolites) appear to share a common endocrine disrupting ability (Cooper et al., 2000).

Conclusion

In conclusion, there is suggestive evidence of carcinogenicity. The treatment with simazine leads to an increased incidence of mammary carcinomas, ovarian hyperplasia, and adenomas in female SD rats. Such findings were not made in the experiments in female mice. The mechanism of tumorigenesis in rats is unclear. Tumors may be related to the disruption of the hypothalamic–pituitary-ovarian axis. The dose response for the key hormonal events in simazine-induced carcinogenesis may involve additive effects of chemicals working by similar modes of action, hormonal alterations due to reduced body weight, and the potential for direct genotoxicity or genotoxicity via estradiol metabolites. These are all areas for study that would result in a better elucidation of the dose response relationship for simazine and for triazine herbicides in general .

Toxicological Effects in Humans

No cases of systemic human poisoning from simazine have been reported in the published literature. However, according to Soviet reports (Elizarov, 1972; Yelizarov, 1977) there were 124 cases of contact dermatitis noticed among workers manufacturing simazine and propazine. Mild cases lasting three or four days involved pale pink erythema and slight edema. Serious cases lasting seven to ten days involved greater erythema and edema and a vesiculopapular reaction that sometime progressed to the production of bullae.

Exposure to triazine herbicides has been associated with non-Hodgkin's lymphoma (NHL) (Zahm et al., 1993), ovarian cancer (Donna et al., 1989), breast cancer (Kettles et al., 1997) and prostate cancer (Mills et al., 1998). These studies have recently been reviewed by U.S. EPA (1999).

A recent report reviewed the available epidemiological studies relating triazine herbicide to various types of cancer (non-Hodgkin's lymphoma, Hodgkin's disease, leukemia, multiple myeloma, soft tissue sarcoma, colon cancer and ovarian cancer). The authors concluded that the epidemiological data were inadequate for establishing a causal association with triazines for Hodgkin's disease, leukemia, multiple myeloma, soft tissue sarcoma, colon cancer and ovarian cancer. For non-Hodgkin's lymphoma, available studies did not demonstrate the type of dose response or induction time pattern expected if atrazine were the causal factor involved (Sathiakumar and Delzell, 1997, reviewed in OEHHA, 1999). There are no data on simazine alone.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

The most sensitive endpoint for noncarcinogenic effects of simazine is reduced body weight and body weight gain in female rats in a two-year feeding study (Ciba-Geigy, 1988a). The NOAEL for this effect is 10 ppm (equal to 0.5 mg/kg based on the standard feed consumption rate of 5 percent of the body weight/day). At the next higher dose of 100 ppm, significantly reduced mean body weight was observed at different times throughout the study and at study termination when compared to controls. At the highest dose of 1,000 ppm, mean body weights, RBC, Hb and HCT were reduced and white blood cells were increased in both male and female rats. Reduced body weights were also observed in the two-year mouse feeding study (NOAEL 6 mg/kg-day), in a one-year dog study (NOAEL 0.5 mg/kg-day), in a rabbit developmental study (NOAEL 5 mg/kg-day), and in the two-generation rat reproduction study (NOAEL 0.5 mg/kg-day). The long-term rat study (Ciba-Geigy, 1988a) was selected for the calculation of the health-protective level based on noncarcinogenic effects.

Carcinogenic Effects

U.S. EPA has proposed a general procedure in deriving MCLGs for group C carcinogens in water. Either an RfD approach should be used (as with a noncarcinogen), but an additional uncertainty factor of one to ten (usually ten) would be applied to account for the limited evidence of carcinogenicity, or a quantitative method (cancer potency estimation with low dose extrapolation) should be used and the MCLG set in the 10^{-5} to 10^{-6} cancer risk range (U.S. EPA, 1996b). According to these proposed guidelines for carcinogenic risk assessment, the type of extrapolation employed for a given chemical is based on the data supporting linearity or non-linearity or a biologically based or case-specific model. When insufficient data are available supporting either approach for a cancer risk estimate, the default is to use a linear extrapolation.

For the linear approach, either a q_1* value or a carcinogenic slope factor (CSF), as suggested in the proposed cancer guidelines, can be used. The level of evidence for simazine carcinogenicity is suggestive, with mammary and pituitary carcinogenicity, and ovarian hyperplasia and adenoma observed in female rats. OEHHA has calculated a health-protective value for simazine using a cancer slope factor approach for comparative purposes.

Estimation of cancer potency and LED_{10}

The most relevant data for estimating the LED_{10} are based on the increased incidence of mammary tumors in Sprague-Dawley rats (Ciba-Geigy, 1988a) which demonstrated a statistically significant increase in mammary tumors at the mid- and high-dose levels. The multistage model is fit to the carcinogenicity

dose-response data and the 95 percent upper confidence limit on the linear term (q_1*) is calculated using the standard likelihood procedures as employed by U.S. EPA. An alternative potency estimate using a carcinogenicity slope factor (CSF) proposed by U.S. EPA in its 1996 proposed guidelines was also calculated by linear extrapolation below the LED_{10} dose. LED_{10} is the lower confidence limit on a dose associated with 10 percent extra risk. ED_{10} was calculated using the linearized multistage model and $(\text{body weight})^{3/4}$ interspecies scaling. A good fit criterion of $p > 0.05$ was adopted for the Chi-square test. The LED_{10} value and other potency estimates are given in Table 5. The earlier quantitative potency estimate derived by U.S. EPA from this study using the linearized multistage model is 0.12 $(\text{mg/kg-day})^{-1}$. OEHHA's cancer potency estimates (q_1*) and CSF are 0.104 and 0.092 $(\text{mg/kg-day})^{-1}$, respectively. The LED_{10} value is 1.08 mg/kg-day. The potency estimates and the LED_{10} value were calculated using Tox-Risk (version 3) software (K.S. Crump Division, Clement International Corp., Ruston, LA).

Table 5. LED_{10} and Potency Estimates for Simazine Based on Rat Mammary Tumors

q_1* $(\text{mg/kg-d})^{-1}$	Chi-square	P	MLE_{10}[a] $(\text{mg/kg-d})^{-1}$	LED_{10}[a] (mg/kg-d)	CSF $(\text{mg/kg-d})^{-1}$	U.S. EPA q_1* $(\text{mg/kg-d})^{-1}$
0.104	0.58	0.75	1.60	1.08	0.092	0.120

[a]MLE and LED are given as dietary concentration on a 100 percent food basis in Tox-Risk. They were converted to mg/kg-day assuming 1.5 kg diet/day for a 70 kg human.

CALCULATION OF PHG

Noncarcinogenic Effects

Calculation of a public health-protective concentration (C, in mg/L) for a chemical in drinking water for noncarcinogenic endpoints follows the general equation:

$$C = \frac{\text{NOAEL/LOAEL x BW x RSC}}{\text{UF x L/day}}$$

where,

NOAEL/LOAEL	=	no-observed-adverse-effect-level or lowest-observed-adverse-effect-level,
BW	=	adult body weight (default of 70 kg for male, 60 kg for female, 10 kg for child),
RSC	=	relative source contribution (defaults of 0.2 to 0.8),
UF	=	uncertainty factors (typical defaults of ten to account for inter-species extrapolation, ten for uncertainty from use of a subchronic study, and ten for potentially sensitive human subpopulations), and
L/day	=	water consumption rate: 2 L/day for a 60 to 70 kg adult, 1 L/day for 10 kg child.

This calculation is based on the assumption that a 70 kg adult consumes two L/day of water and that the simazine contribution from water is 20 percent (RSC = 0.2). For simazine, the NOAEL is 0.5 mg/kg-day for reduced body weight based on a two-year rat feeding study (Ciba-Geigy, 1988a). In this case, a cumulative uncertainty factor of 1,000 is used, which includes factors of 10 for inter- and intra-species uncertainties, plus an extra factor of 10 for the uncertainties associated with the suggestive evidence of carcinogenicity.

$$C = \frac{0.50 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{1000 \times 2 \text{ L/day}}$$

$$= \textbf{0.0035 mg/L = 3.5 ppb = 4 ppb (rounded)}$$

Carcinogenic Effects

The following general equation for carcinogenic chemicals could also be used to calculate a public health-protective concentration (C) for simazine in drinking water:

$$C = \frac{BW \times R}{q_1^* \text{ or CSF} \times L/day}$$

where,

BW	=	adult body weight (a default of 70 kg),
R	=	*de minimis* level for lifetime excess individual cancer risk (a default of 10^{-6}),
q_1^* or CSF	=	cancer slope factor; q_1^* is the upper 95 percent confidence limit on cancer potency slope calculated by the LMS model; CSF is a potency derived from the lower 95 percent confidence limit on the 10 percent tumor dose (LED_{10}) above controls, where CSF = 10 percent/LED_{10}; both potency estimates are converted to human equivalent [in $(mg/kg\text{-}day)^{-1}$] using $BW^{3/4}$ scaling, and
L/day	=	daily volume of water consumed by an adult (a default of 2 L/day).

For simazine, q_1^*, CSF and the LED_{10} values of 0.104 and 0.092 $(mg/kg\text{-}day)^{-1}$ and 1.08 mg/kg-day were calculated from the mammary tumor data in rats from the two-year dietary carcinogenicity study of Ciba-Geigy (1988a). The potency estimates q_1^*, as used by convention by U.S. EPA, and CSF as

recommended in the proposed cancer guidelines (1996), are used for comparative purposes. An RSC is not included in the calculation of a health-protective concentration for a carcinogen because the use of the low dose extrapolation is considered adequately health-protective without the additional estimate of relative source contribution.

$$\text{C using } q_1^* = \frac{1 \times 10^{-6} \times 70 \text{ kg}}{0.104 \text{ (mg/kg-day)}^{-1} \times 2 \text{ L/day}} = \textbf{0.00034 mg/L} = \textbf{0.34 ppb}$$

$$\text{C using CSF} = \frac{1 \times 10^{-6} \times 70 \text{ kg}}{0.092 \text{ (mg/kg-day)}^{-1} \times 2 \text{ L/day}} = \textbf{0.00038 mg/L} = \textbf{0.38 ppb}$$

Conclusions

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water and for preparing foods and beverages. It is also used for bathing or showering and in washing and other household uses, resulting in potential dermal and inhalation exposures. In the case of simazine, use of an RSC of 0.2 for drinking water is assumed adequate to account for any exposure through foods and beverages which results from the pesticidal uses of simazine. Exposure through other household uses of drinking water are considered to be negligible, because simazine is non-volatile and should be poorly taken up through skin.

The estimated health-protective simazine concentrations in drinking water calculated using various approaches are summarized in Table 6.

Table 6. Simazine Concentrations Based on Various Approaches

Endpoints	Approach	Concentration (ppb)
Non-cancer	NOAEL plus UF	4
Cancer, linear	q1*	0.34
Cancer, linear	CSF	0.38

There is a 10-fold difference between the calculated values based on the cancer endpoint using the linear approach versus the non-cancer endpoint using the uncertainty factor approach with an extra 10-fold because of concerns about the tumors. Currently, there is only suggestive evidence that simazine is a carcinogen. OEHHA considers a concentration of 4 ppb (rounded) for simazine based on a NOAEL of 0.5 mg/kg for reduced body weight observed in the two-year carcinogenicity study in Sprague-Dawley rats (Ciba-Geigy, 1988a) to be adequately protective against adverse effects, and the most appropriate value for a PHG.

RISK CHARACTERIZATION

The most consistent, lowest-dose effect of simazine in long-term oral exposures is reduced body weight gain. There is also suggestive evidence of mammary and pituitary carcinogenicity and ovarian hyperplasia and adenoma in female rats but not in mice.

The finding in female rat of mammary tumors is consistent with findings of mammary tumors observed with other s-triazines. Recently, a mode of action for mammary tumors in rats involving changes in endocrine function (and potentially a threshold) was suggested for atrazine, a representative for all s-triazines (Stevens et al., 1999). Atrazine is not directly estrogenic. It decreases luteinizing (LH) hormone levels in rats and disrupts the estrous cycle. There is evidence to suggest a hypothalamus-pituitary level control for these effects. The reduced decrease in T3 levels observed in rats also suggests involvement of hypothalamus-pituitary control. While there are a number of studies on the intermediate steps (LH hormone levels and estrous cycle) to suggest an endocrine mode of action for the mammary tumor development, the primary mechanism of the toxic effect for triazines has not been identified. More than one mode of action cannot be ruled out at this time. In addition, the recent studies on pre-and post puberty effects in male (Stoker et al., 2000) and female Wistar rats (Laws et al., 2000) suggest that atrazine delays the development of puberty in both sexes. There are no long-term studies in young animals to provide a fuller perspective on the consequences of the delayed puberty. While it might be presumed that a similar mode of action is operative for simazine, there are no data to evaluate.

Breast cancer is the leading cause of death among American women regardless of race and ethnicity. In 2001, about 21,000 new breast cancer cases can be expected in California, of which about 4,205 will be fatal (ACS, 2000). The United States age-adjusted mortality rates per 100,000 person-years for breast cancer ranged from 27 among white females to 29 among black females. Neither the main cause nor the mechanism of breast cancer in humans is known. Therefore, an additional uncertainty factor of ten to account for the potential for risk of mammary tumor is applied.

The PHG is based on exposure to simazine from drinking water. Because of the physical and chemical properties of simazine, an insignificant amount of inhalation and dermal exposure is expected from bathing and showering, although there are no data on dermal absorption for simazine. A varied dose-dependent dermal absorption of 2 to 20 percent based on rat studies wherein high concentrations of atrazine (an s-triazines representative) in acetone or tetrahydrofuran was used (DPR, 1998) is not appropriate in estimating the dermal exposure to simazine from bathing and showering. Contact time for the material in water would be variable, but less than in the rat studies.

The RSC of 20 percent (0.2) used for simazine in drinking water represents the default value for pesticides that are intended for application to foods. OEHHA has not attempted a rigorous evaluation of actual contributions from food versus water. This is partly because of the lack of data on concentrations of the active simazine metabolites in these sources, but also because of potential co-exposures to other chlorotriazines and their metabolites. Use of this health-protective assumption of 80 percent contribution from other sources plus the combined uncertainty factor of 1,000 should provide protection of the entire population, including possible sensitive subgroups, from exposure to simazine and its metabolites in drinking water.

OTHER REGULATORY STANDARDS

U.S. EPA's MCL for simazine is 4 µg/L (4 ppb). This MCL was calculated based on the RfD of 0.005 mg/kg-day derived from a NOAEL of 0.5 mg/kg-day for decreased body weight from a two-year oncogenicity study in rats. The California MCL is also 4 ppb and is based on the same oncogenicity study and approach used by U.S. EPA. This MCL was derived before the peer review committee for simazine, under the Office of Pesticide Programs, determined it to be a group C carcinogen and recommended that the carcinogenic risk be quantified. Health Canada (1986) established an interim maximum acceptable concentration of 10 ppb for simazine in drinking water. This is based on a NOAEL of 5 mg/kg-day from a two-year dog study with an uncertainty factor of 4,000. OEHHA's PHG of 4 ppb is based on the NOAEL of 0.5 mg/kg-day for reduced body weight observed in female Sprague-Dawley rats, as in the previous U.S. EPA and California risk assessments.

Simazine has not yet been considered for listing under Proposition 65. However, it is in the Proposition 65 prioritization tracking database for future consideration for listing as a chemical known to cause cancer or reproductive toxicity by the State's qualified experts (OEHHA, 1997).

U.S. EPA's pesticide office used the linearized multi-stage model to extrapolate simazine tumor responses observed at the high dose to predict tumor response at low doses (U.S. EPA, 1994). This model assumes that there is no threshold for carcinogenic effects. U.S. EPA's pesticide office calculated a cancer potency (q_1*) of 0.12 $(mg/kg\text{-}day)^{-1}$. Based on this model, the MCL of 4 ppb is associated with an estimated upper-bound cancer risk level of about 10^{-5} for drinking water, assuming a person consumes two liters of water per day containing simazine at this level over a 70-year lifetime (U.S. EPA, 1994). U.S. EPA's more recent triazine assessments have not utilized this approach, instead recommending non-linear extrapolations based on non-cancer endpoints (U.S. EPA, 1999).

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