

Public Health Goal for Atrazine In Drinking Water

Prepared by

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PREFACE

**Drinking Water Public Health Goals
Pesticide and Environmental Toxicology Section
Office of Environmental Health Hazard Assessment
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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) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. 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 solely on health effects without regard to cost impacts 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 adopted by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted 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 ATRAZINE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) has developed a Public Health Goal (PHG) of 0.00015 mg/L (0.15 µg/L or 0.15 ppb) for atrazine in drinking water. The current California MCL is 3 ppb for atrazine in drinking water. The PHG is based on mammary tumors (adenocarcinoma and fibroadenoma) observed in females in a carcinogenicity study in Sprague-Dawley rats (70/sex/dose) fed atrazine at dietary concentrations of 0, 10, 70, 500, or 1,000 ppm for 24 months and using a linear dose response approach with a carcinogenic slope factor (CSF) of 0.23 (mg/kg-day)⁻¹. In F344 rats, a significantly higher incidence of benign mammary tumors was found in the high dose males and a significantly positive trend test was observed for leukemia and lymphoma in females. Atrazine had no carcinogenic effects in a mouse carcinogenicity study. Epidemiological studies are equivocal. The exact mechanism of mammary tumor formation is not known. Atrazine is positive in a number of mutagenicity studies. It disrupts the estrous cycle in female rats, but it is not directly estrogenic. It decreases triiodothyronine (T₃) levels in rats and causes thyroid hyperplasia. Decreases in luteinizing hormone (LH), progesterone and estradiol levels were also observed in rats administered atrazine or its metabolite diaminochlorotriazine. The most sensitive endpoint for non-carcinogenic effects is cardiomyopathy, observed in a one-year dog study. The NOEL for this effect is 0.48 mg/kg. Based on the NOAEL of 0.48 mg/kg, the non-cancer health protective concentration is 0.0034 mg/L (3 ppb). Atrazine is readily absorbed from the gastrointestinal tract and excreted via urine (≥70%) and feces (≥20%). It is metabolized via stepwise oxidative P-450 dealkylation to de-ethyl or de-isopropyl and then to diaminochlorotriazine. The toxicity of these metabolites is essentially similar to the parent compound.

INTRODUCTION

Atrazine (6-chloro-N-ethyl-N²-isopropyl-1,3,5-triazine-2,4-diamine) is a selective pre- and post-emergence herbicide widely used on agriculture crops such as cauliflower, corn, sorghum and sugarcane, and in noncropped areas such as wheat fallow and rights-of-way. Atrazine inhibits photosynthesis. Average rates of 1 to 2.5 pounds/acre are usually applied by ground boom application, but higher concentrations may be used under nonselective conditions. At present only one dry flowable and two flowable liquid formulations are registered in California. Presently, two (atrazine and simazine) triazines are under Special Review by U.S. EPA because of potential carcinogenic risk from exposure to these pesticides.

For the purpose of developing an atrazine PHG, two types of data have been reviewed: data published in the open literature during the past 10 years and data submitted for the registration of atrazine as a pesticide. The latter was previously reviewed by the California Environmental Protection Agency's (Cal/EPA's) Department of Pesticide Regulation (DPR, 1996) and brief summaries of the relevant toxicity data are given here.

CHEMICAL PROFILE

Physical and Chemical Properties

The properties of atrazine are summarized in Table 1.

Table 1. Atrazine Properties

Name	Atrazine
Trade names	Malermais®, X-Siprim®, Vegfru Solaro®
Physical state	crystalline solid, white, colorless
Molecular weight	215.7
Chemical formula	C ₈ H ₁₄ ClN ₅
Melting point	175-177 °C
Density	1.87 g/cm ³ at 20°C
Solubility in water	30 mg/L
Solubility in organic solvent	18,000 mg/L in methanol
Vapor pressure	3 x 10 ⁻⁷ mm Hg @ 20° C
Henry's law constant	3.4 x 10 ⁻⁹ atm-m ³ /mol at 20 °C
Octanol-water partition coefficient (Log K _{ow})	2.61
pK _a	1.7 @ 21 °C
K _{oc}	100 L/kg
Field half life	60 days

Production and Uses

A total of 44,485 pounds of atrazine was used in California in 1993 for corn (62%), landscape (5%), rights-of-way (18%) and Sudan grass (8%) (Pease, 1996). In 1995 the amount of atrazine used in California was 41,241 pounds (DPR, 1995). Atrazine is commonly used in combination with other pesticides, including metochlor, cyanazine, S-ethyl diisobutylthiocarbamate, alachlor, bromoxynil, and sodium chlorate.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Atrazine is a widely used herbicide. Based on water solubility, soil adsorption coefficient, vapor pressure field half-life and the amount use, atrazine and its metabolites are expected to have high contamination potential for ground water. U.S. EPA (1994) has extensively reviewed atrazine occurrence data from information available to the agency from pesticide registrants, states' monitoring programs, the United States Geological Survey (USGS), as well as information from U.S. EPA's National Pesticide Survey of Drinking water Wells (NPS) and the Office of Pesticide Programs (OPP's) Pesticides in Ground Water Data Base (PGWDB).

Air

Atrazine is not expected to become volatilized to a significant degree because of low water solubility and low vapor pressure. However, atrazine may be released into the air during production, formulation and application.

Soil

Atrazine is expected to maintain a high to moderate degree of mobility in soils because of its average sorption coefficient (K_{oc}) values of about 100. Once leached into the subsoil and ground water, metabolism of atrazine slows considerably. After many years of use, atrazine residues may accumulate in subsoils and ground water and ultimately pose risks to humans consuming drinking water from contaminated ground water sources. When metabolite residues are combined with parent residues, estimates of hazard levels to humans drinking contaminated water may be substantially higher.

Under aerobic conditions, atrazine and closely related triazine herbicide simazine have half-lives of 150 and 110 days, respectively, whereas anaerobic conditions extend half-lives to two years for both chemicals. Atrazine and simazine follow similar degradation pathways with both parent compounds forming hydroxy analogues and de-alkylated chloro degradates which may persist in soil and water for many months. These hydroxy degradates tend to be less mobile than the parent or de-alkylated degradates (reviewed by U.S. EPA, 1994).

In a citrus orchard plot dissipation and distribution of atrazine, simazine, chlorpyrifos and tetradifon residues were evaluated in Valencia (Spain) following their controlled addition for agricultural purposes in a Mediterranean red soil. The amounts of applied pesticides were monitored in different soil layers (0-0.05, 0.05-0.22, 0.22-0.42, and 0.42-0.52 m). Degradation half-lives calculated assuming zero-order kinetics were 11 days for atrazine, 12 days for simazine, 10 days for chlorpyrifos, and 18 days for tetradifon. The distribution through the soil profile shows that the pesticide concentrations were always highest in the upper layer (0-0.05 m) of soil, and that atrazine was the most mobile of the four pesticides investigated (Redondo *et al.*, 1997). No leaching of atrazine or its metabolites was observed below soil layers of 15-30.5 cm in California, Minnesota and Tennessee soils (U.S. EPA, 1994). It was observed that the water-holding capacity of a soil affects the rate of degradation of atrazine. For sandy soils with 4%, 35% and 70% water holding capacity, the half-lives were 151, 37 and 36 days, respectively (U.S. EPA, 1994).

Water

Atrazine is considered to be a priority A'chemical for potential ground water contamination by U.S. EPA (U.S. EPA, 1994) and was ranked the highest of 83 pesticides in the Agriculture Canada priority scheme for potential ground water contamination (Health Canada, 1993). In areas where atrazine is used, atrazine or its metabolites are frequently detected pesticides at concentrations above the MCL. Atrazine concentrations fluctuate in various water bodies, depending upon whether sampling occur during the pre-or post-application season and upon the subsequent degree of pesticide runoff. U.S. EPA has established an MCL for atrazine at 3 µg/L (3 ppb) (U.S. EPA, 1996). According to U.S. EPA's Office of Pesticide Programs' (OPP's) 1992 Pesticide in Ground Water Data Base (PGWDB), of 1,512 wells that contained residue of atrazine, 172 wells (11%) were found to have concentrations that exceeded the MCL of 3 ppb, ranging from traces to 1,500 ppb.

Atrazine and the closely related triazine simazine are the most geographically widespread pollutants detected within 23 California counties in 1993. Also, metabolites of atrazine, namely deethyl-atrazine and deisopropyl atrazine were detected. These metabolites were present in the ground water as a result of agricultural applications. Residues of atrazine (parent compound) have been reported in 21 counties at concentrations ranging from 0.02 to 8.5 µg/L (Pease *et al.*, 1995). Some of California's water systems exceed the current MCL for atrazine. In California, 192 wells had detectable atrazine or its metabolites and four were above the MCL of 3 ppb (Pease *et al.*, 1995).

Richard *et al.* (1995) describe an exposure assessment process for large human population using atrazine as an example. An average exposure concentration for atrazine was estimated for a population of three mid-western states (Ohio, Illinois and Iowa) by dividing the population into groups of known size, within which the drinking water exposure is the same or similar. The exposure estimates for each group was based on the best available data. The authors concluded that atrazine exposure through drinking water did not represent a significant human health risk. Most population had exposure to atrazine concentration less than the maximum contamination level (MCL) of 3 ppb. Only 0.05% of the assessed Ohio population and 0.21% of the assessed Iowa population had exposure about the MCL. The majority of the exposed population had exposure of less than 1 ppb.

From a review of U.S. EPA's Environmental Fate and Ground Water Branch's 12 major studies on the concentration of pesticides in raw (eight studies) and finished (four studies) surface water (Nelson and Jones, 1994), the authors concluded that: 1) atrazine, cyanazine, alachlor and metolachlor were the most frequently detected pesticides and were in the highest concentrations in samples of both raw and finished surface water in the 12-state mid-western corn belt, 2) concentrations of these pesticides rapidly increased from pre-application concentrations of low sub-microgram per liter to post application peak concentrations of several to greater than 10 ppb, with one or more peak concentrations occurring between early May and early July, 3) pesticide concentrations at any given location vary from year to year and season to season depending upon rainfall and associated runoff, 4) atrazine concentrations have been reported to remain elevated almost year round during some years for some lakes and reservoirs.

A study of the distribution and transport of atrazine in surface water in the 1,117 square-mile Delaware River Basin in northeast Kansas was conducted from July 1992 through September 1995 (Pope et al., 1997). In this study, nearly 5,000 samples were analyzed by enzyme-linked immunosorbent assay (ELISA) for triazine herbicide concentrations. Time weighted daily mean concentrations during May, June and July commonly exceeded the MCL of 3.0 µg/L. Time weighted, daily mean concentrations equal to or greater than 20 µg/L were not uncommon, but during August to April daily mean concentrations were less than 1.0 µg/L. Time weighted annual mean atrazine concentrations did not exceed the MCL, but were larger during 1994 than 1993 and ranged from 0.36 µg/L to 2.8 µg/L in 1994 and 0.27µg/L to 1.5µg/L in 1993. The authors ascribed these differences to differences in the rainfall amounts and subsequent runoff volumes between the two years.

Monsanto's National Alachlor Well Water Survey (NAWWS) was conducted to estimate the proportion of private and rural domestic wells in the alachlor use area that contain detectable concentrations of alachlor, but also analyzed atrazine, simazine and cyanazine levels. Atrazine was estimated to be present in 12% of wells in the alachlor use area. According to NAWWS data, it is estimated that 2.4 million people are exposed to parent atrazine residues of less than 0.2 µg/L and 184,000 people in this area are exposed to residues greater than or equal to 0.2 µg/L (study detection limit 0.2 µg/L) (cited by U.S. EPA, 1994).

In the Iowa State-Wide Rural Well Water Survey (SWRL) (U.S. EPA, 1994), atrazine was one of the most frequently detected pesticides and most often exceeded the MCL. It was estimated that atrazine (parent compound only) could be detected in 0.6% of wells statewide at concentrations that exceeded the MCL. If data on the detection of chloro-triazine degradates are also included the total number of wells with detection is increased and would likely increase the exposure estimates.

Triazine degradates in groundwater

Only a few studies are available on the occurrence or level of atrazine degradates in ground water. The most significant information on degradation products comes from the Iowa and Wisconsin state surveys reported by U.S. EPA (1994). In the Iowa SWRL, two major atrazine degradates, de-ethyl and deisopropyl, were detected at approximately the same rate (3.5% and 3.4%, respectively) as atrazine itself (4.4%). The degradates were detected in combination with atrazine, but over half of the degradates detections occurred in the absence of detectable parent compound. In the Wisconsin Rural Well Survey, degradates accounted for 67% of the total triazine residues detected. Almost 92% of wells that were resampled in Phase 2 of the study contained a combination of parent and degradates residues. Two atrazine chloro degradates, de-ethyl atrazine and di-aminotriazine, were found with approximately the same frequency as the atrazine parent compound (83 to 88%) at concentrations of up to 8.8 and 9.9 µg/L, respectively. A third chloro degradate, deisopropyl atrazine, was detected less frequently (60.6%) and at lower concentrations (0.1 to 2.6 µg/L).

The distribution of atrazine and its two metabolites in the ground water was studied in the midcontinental United States (Liu et al., 1996). The mean of atrazine residue

concentrations was 53% greater than that of atrazine alone for those observations above the detection limit ($>0.05 \mu\text{g/L}$). The authors suggest that consideration of the concentration of atrazine degradates is necessary to obtain a true estimation of risk of using these aquifers as sources for drinking water.

Ground water exposure

According to U.S. EPA's estimates, based on results of the NPS and the NAWWS surveys, between two million and three million people using ground water as their primary drinking water source are exposed to atrazine at average concentrations of at least $0.2 \mu\text{g/L}$. Based on monitoring studies, U.S. EPA has determined average time-weighted mean concentrations (TWMC) of $0.84 \mu\text{g/L}$ for atrazine and $0.23 \mu\text{g/L}$ for the closely related triazine simazine. The high end or 90th percentile TWMC is $1.88 \mu\text{g/L}$ for atrazine and $0.31 \mu\text{g/L}$ for simazine (U.S. EPA, 1994).

Food

Human exposure to atrazine may also result from ingested residues remaining in or on treated crops such as corn, nuts and fruits. In addition, dietary exposure may occur through consumption of animal products derived from animals which were fed atrazine treated crops. In a Canadian National Surveillance Study from 1984-1989, atrazine was not detected in 1,075 samples of fruits, vegetables, grains dairy products and wine (Government of Canada, 1990) or in a survey of 19,851 samples in the U.S. (Luke et al., 1988). The estimated theoretical maximum dietary intake of atrazine by Health Canada is 0.0003 mg/kg . This was based on negligible residues (0.01 mg/kg food) in all barley, corn, oats and wheat (Health Canada, 1993). U.S. EPA's estimate of carcinogenic risk from exposure to atrazine and its chlorometabolites is 4.4×10^{-5} for all commodities with sugarcane being the largest contributor. Excluding sugarcane the estimated carcinogenic risk is 2.2×10^{-5} with milk, sweet corn, corn, meat and eggs being the major contributors. Excluding sugarcane, the estimated dietary exposure is 0.0001 mg/kg-day .

METABOLISM AND PHARMACOKINETICS

A. Pharmacokinetics

Human

Atrazine exposure was evaluated in six manufacturing workers during its industrial production by means of assessment of ambient exposure and determination of atrazine dealkylated metabolites in urine (Catenacci *et al.*, 1993). The study was conducted in a facility producing technical atrazine with a granulometry above the respirable size. Ambient exposure was estimated by measuring air and skin atrazine concentrations. The total atrazine exposures for each of the six workers ranged from 10 to 700 micromoles during an eight-hour period. The metabolites excreted in the urine accounted for 1 to 2% of the exposure dose. Levels of these compounds were higher in the urine of baggers than in

box operators and were not significantly correlated with the dose of exposure. About 80% of the excreted metabolites were di-dealkylated atrazine, 10% were deisopropylated, 8% were de-ethylated and only 1 to 2% were unmodified atrazine.

A series of urine samples from workers applying atrazine was analyzed using an Enzyme-Linked Immunosorbent assay (ELISA) for atrazine and its metabolites (Lucas *et al.*, 1993). A mercapturic acid conjugate of atrazine was found to be the major urinary metabolite. Levels of this conjugate were at least 10 times greater than those of any of the N-dealkylated products or the parent compound. No hydroxylated product was detected.

In six workers engaged in the manufacture of atrazine, the di-dealkylated metabolites comprised 80% of the urinary metabolites and only 2% was excreted as unchanged atrazine (Barbieri *et al.*, 1992).

The mono-dealkylated metabolites 2-amino-4-chloro-6-isopropylamino-triazine (DEA) and 2-amino-4-chloro-6-ethylamino-triazine (DIA) were observed in urine of six human volunteers for up to 48 hr following a single oral dose of 0.1 mg atrazine/kg bw. Also, the di-dealkylated metabolite, 2,4 diamino-6-chloro-s-triazine (DDA), was present in small amounts for up to 96 hours. These three metabolites accounted for 5.4, 1.4, and 7.7%, respectively, of the atrazine dose, a total of 14.5%. The metabolism of atrazine has been proposed to involve a stepwise oxidative P-450 dealkylation of atrazine to DEA and then to DDA. Atrazine or the dealkylated chloro metabolites may then be conjugated with glutathione leading to the excretion of cysteine conjugates or mercapturic acids in urine. Blood analysis performed on one individual detected measurable levels of DEA and DDA, but not DIA up to 8-24 hours post-dose. Renal excretion of DDA follows a single-compartment first-order model. The half time of renal elimination was 10.7 hr. compared to disappearance in blood of 17.8 hr. DEA and DIA were found to generally follow a two-compartment first-order model. Renal excretion half-lives for DEA were 2.3 ± 0.5 and 8.4 ± 0.7 hrs, while those for DIA were 2.4 ± 1.0 and 36.2 ± 6.2 hrs (CDFA, 1990).

The biotransformation and absorption of atrazine were studied in human skin utilizing a flow through in vitro diffusion system. Aerated receptor fluid was pumped beneath the skin surface at 3 ml/hr (Ademola *et al.*, 1993). The skin absorbed about 16.4% of the applied dose. The highest concentration (12%) of the applied dose was found in the skin supernatant. Two metabolites of atrazine DIA and DDA were found in the receptor fluid and skin supernates. An additional metabolite DEA was found in the skin supernates. Similar to the percutaneous absorption, skin microsomal fraction metabolized atrazine to its deisopropyl and dethylpropyl derivatives. In addition 2-hydroxy derivatives were formed by the skin microsomal fraction.

Rat

¹⁴C-Atrazine was orally administered to rats weighing 310 to 420 g at levels of 0.53 mg/rat containing 3.54 μ Ci of ¹⁴C. Urine and feces were collected daily. The rats were sacrificed on days 2, 4, and 8 and the tissues were collected for radioactivity analysis. After 72 hours, 20.3% of the administered dose was excreted in the feces, 65.5% in the urine, and 15.8 % was retained in the tissue. This suggests that about 80% of the dose is absorbed.

Atrazine (¹⁴C-labelled [98%] or unlabelled) was administered orally to three groups of 5 CRCD rats /sex/dose; 2 rats /sex/dose served as untreated controls. Group 1 was given a single oral dose of 1 mg/kg, group 2 was given a single oral dose of 100 mg/kg, and group 3 was subchronically treated with a single daily dose of 1.0 mg/kg for 14 days with unlabelled atrazine. On day 15 a single oral dose of 1 mg ¹⁴C-atrazine /kg was administered to all treated groups. Data were compared on the basis of sex, dose rate and pretreatment regimen. No significant differences in mean % recoveries were observed between dose groups or between male and female animals within dose groups. Urine was the primary route of excretion. Approximately 74 and 19% of the dose was eliminated in the urine and feces, respectively, within seven days of dosing. Among different tissues, RBCs had the highest concentration of radioactivity. The whole body half-life of elimination was determined as 31.3 ± 2.8 hours; the data indicated that elimination occurred from two body compartments by first order kinetics (Ciba-Geigy, 1987a).

In a subchronic exposure study, atrazine was administered to female Sprague-Dawley rats at levels of 0, 1, 3, 7, 10, 50, or 100 mg/kg bw for ten days. Following cessation of dosing, concentrations declined exponentially with an estimated t_{1/2} of 38.6 hrs. The estimated apparent volume of distribution (Vd) was 4.15 L/kg bw, and the mean steady-state concentration for a 10 mg/kg bw dose was 5.61 mg-equivalents atrazine/L plasma. Atrazine concentrations in red blood cells (RBCs) rose with repeated dosing but failed to achieve a steady-state level within the treatment period. The highest concentrations (0.6% or less of the total dose) were found in RBCs and liver; other tissues contained less than 0.25% of the total dose. Following cessation of treatment the estimated t_{1/2} was 8.14 days. The estimated Vd, t_{1/2}, and predicted mean steady-state concentration for a 10 mg/kg daily dose, were 0.70 L/kg, 5.1 days, and 104.6 mg-equivalents/L cells, respectively (Ciba-Geigy, 1987).

The metabolism of several s-triazine herbicides (atrazine, terbuthylazine, ametryne, and terbutryne) was studied in vitro using liver microsome from rats, pigs and humans (Lang and Boecker, 1996). While all species produced the same types of metabolites, there were species-specific differences in the ratio of the metabolites produced. Of particular interest are differences in the S/R ratio of the hydroxylated metabolite of the isopropyl moiety of atrazine, 1-hydroxyisopropylatrazine. Both the (S) and (R) enantiomer are formed enzymatically in rat and human liver. The (S) enantiomer is predominant in rats and (R) enantiomer is predominant in humans giving an S/R ratio of 76:24 in rats and 28:72 in humans. No difference was observed by pig liver microsomes in their S/R ratio.

Following oral gavage, rats excreted 47% to 76% of the dose in the urine within 72 hr, with corresponding fecal elimination ranging from 49 to 15%, respectively. At 72 hr, 4.7 to 7.2% of the dose remained in the tissues, with the highest concentration present in the erythrocytes. The reason for the wide inter-individual variation is not apparent, but other studies indicate that urinary excretion is in the 70 - 75% range. Elimination patterns following dermal application are even more variable, with urine: fecal ratios ranging from 3:1 to 97:1. Despite this wide-range, there is apparently sufficient biliary excretion to account for the radioactivity in the feces following oral gavage (CDFA, 1990 review).

Atrazine was administered orally to male Fischer rats at levels of 15 and 30 mg/kg-day for 7 days. The rats were sacrificed 24 hr after administration of the last dose, and liver, kidney and brain tissues were collected. Atrazine or its dealkylated metabolites were observed in

the liver, kidney and brain of the treated rats. The authors suggested that s-triazines are metabolized via total N-dealkylation of lateral alkyl amino groups in the 4, 6 position and hydroxylation in the 2 position in the rat (Gojmerac and Kniewald, 1989).

The metabolism of the triazines atrazine, simazine and terbutryn was studied in vitro by using hepatic 1000g supernatants or microsomal system derived from rats (Sprague-Dawley and Fischer), mice, goats, sheep, pigs, rabbits and chickens (Adams *et al.*, 1990). There were species related variations in rates of metabolism and in the ratio of primary metabolites, but no strain or sex related differences were observed. The phase 1 reactions were P-450 mediated, and the principle metabolite was 4- and/or 6-monodealkylated-s-atrazine.

Plant

Atrazine is mainly absorbed through roots but some absorption also occurs through foliage, depending upon the plant species. Three basic reactions have been identified in plant metabolism of atrazine: hydrolysis of the 2-chloro group, N-dealkylation of the side chain, and conjugation of the 2-chloro group with glutathione. The dechlorination reaction is nonenzymatic and is mediated in corn by a natural constituent of corn, 2,4-dihydroxy-7,1,4-benzoxazin-3-one (reviewed by DHS, 1989).

The uptake and metabolism of atrazine has been studied in poplar trees (Burken and Schnoor, 1997). The following metabolites were identified by using UV and radiochromatographic detectors: hydroxyatrazine, de-ethylatrazine, deisopropylatrazine, de-ethylhydroxyatrazine, and didealkylatrazine. Ammeline (2-hydroxy-4, 6 diamino-s-triazine) was also one of the most polar and the least retarded of the analysates. It was observed that the preferential N-dealkylation product was deethylatrazine as opposed to deisopropylatrazine. Deethyl concentrations were 9 times higher than deisopropyl concentrations suggesting that dealkylation of the ethyl side chain was the preferential dealkylation mechanism as opposed to deisopropylation. Also, deethyl was found to be the predominant initial biotic degradation product of atrazine in surface water environments (Kolpin and Kalkhoff, 1993; Lerch and Donald, 1994) and in deeper soil and ground water where denitrifying conditions prevail. This suggests that similarities exist between metabolic pathways occurring in poplar trees and in aerobic biota in surface waters and soils. After uptake, atrazine and its metabolites are confined to the poplar biomass. Plant-bound atrazine residues have been shown to be excreted nearly quantitatively in feces by rats within one day of administration and by sheep within 2 days. It has been suggested that bound residues are associated with lignin thus feeding plant material containing bound residue of pesticides may result in little or no absorption by monogastric species (Khan and Dupont, 1986).

The transformation and distribution of ¹⁴C-atrazine was studied in corn plants (Mathew *et al.*, 1996). The metabolic products observed consisted of hydroxy analogues of atrazine and their dealkylated metabolites. The transformation products varied in leaf stalk and root tissues. Diaminohydroxyatrazine, hydroxyatrazine, and de-ethylhydroxyatrazine were the main ¹⁴C residues. For example, roots contained 25% of the total extractable root ¹⁴C residue as hydroxyatrazine, whereas leaves and stalks had 41% and 42% of their extractable ¹⁴C

residue as hydroxyatrazine. Also, only 4% of extractable stalk ¹⁴C residue was de-ethylhydroxyatrazine, whereas de-ethylhydroxyatrazine residues in leaves and roots accounted for 13 and 16% of extractable ¹⁴C residues.

Summary

Atrazine is well absorbed from the gastrointestinal tract (70-90%). It is metabolized by stepwise oxidative- P-450 dealkylation to mono-, then di-dealkylated metabolites. Humans tend to preferentially dealkylate the ethylamino moiety, while rats preferentially dealkylate the isopropylamino moiety. Atrazine or the dealkylated chloro metabolites may then conjugate with glutathione, leading to the excretion of cysteine conjugates or mercapturic acids in urine.

Elimination kinetics were slightly different between the two species, humans having a single compartment with a t_{1/2} of 11.5 hr, rats having central and peripheral compartments, and a biphasic elimination with t_{1/2} = 6.9 hr for the first phase and t_{1/2} = 31.1 hr for the second phase. Studies of in vitro metabolism using liver microsomes obtained from various species suggests that all species produce similar types of metabolites but differ in terms of the rate and ratio of the metabolites produced. In plants, the major metabolite is hydroxyatrazine. The toxicological significance of these differences is not known.

TOXICOLOGY

Toxicological Effects in Animals and Plants

Acute Toxicity

The acute lethal dose (LD₅₀) of atrazine in various species by different routes is given in table 2.

Table 2. Acute toxicity of atrazine in experimental animals¹.

Species	Administration mode	LD ₅₀ (mg/kg bw)
Rat	oral	1869-3080
Rat	inhalation (1 hr)	0.7 mg/L
Mice	oral	1750
Rabbit	oral	750
Rabbit	dermal	9300 mg/kg (80%)

1. Source: Bergman and Pugh, 1994

The reported toxic effects following acute exposure were: depression, reduced respiratory rate, motor discoordination and clonic and tonic spasm.

Dog

Atrazine (97%) was given to 5-month-old beagles (6 dogs/sex in the control and high dose groups and 4 dogs/sex in the low-and mid dose groups) for one year at dietary levels of 0, 15, 150, and 1000 ppm (equal to male: 0, 0.48, 4.97, and 33.65 mg/kg-day; female; 0, 0.48, 4.97 and 33.8 mg/kg-day). Three animals were killed during the study in moribund condition: one 150 ppm male on day 75; one 1000 ppm female on day 113 and one 1000 ppm male on day 250. Cardiopathy (discrete myocardial degeneration) was the most significant effect observed in animals fed 1000 ppm. Clinical signs associated with cardiac toxicity were: ascites, cachexia, labored/shallow breathing, and abnormal EKG (irregular heart beat and increased heart rate, decreased P-II values, atrial premature complex, atrial fibrillation). These were first observed as early as 17 weeks into the study. Gross pathological examination revealed moderate-to-severe dilation of the right atrium (and occasionally the left atrium), microscopically manifested as atrophy and myelosis (degeneration of the atrial myocardium). Other effects observed were: decreased food consumption and body weight gain at 1000 ppm, decreased red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT), total protein and albumin, as well as an increase in platelet counts, P, Na, glucose and liver and ovary relative weights at 1000 ppm. At 150 and 1000 ppm, females experienced increased heart weights and in both sexes treatment related electrocardiographic changes in the heart accompanied by gross detectable pathology were observed. The NOEL is 15 ppm (0.48 mg/kg) (DPR, 1996).

Developmental and Reproductive toxicity

Animal Developmental Toxicity Studies

Rat

Atrazine (96.7%) was orally administered to a group of pregnant Charles River rats (27/group) at levels of 0, 10, 70, and 700 mg/kg-day on days 6 through 15 of gestation. The maternal NOEL was 10 mg/kg-day based on maternal toxicity including death (21 of 27 dams) at the highest dose of 700 mg/kg-day and reduced weight gains and food consumption at the 70 and 700 mg/kg-day dose levels. Other reported symptoms included reduced food consumption, reduced weight gain, salivation, ptosis, swollen abdomen, oral/nasal discharge and bloody vulva. There were statistically significant increases in both fetal and litter incidences in skeleton variations suggesting delayed ossification in the 70 mg/kg-day and 700 mg/kg-day dose groups. No maternal toxicity was observed in the 10 mg/kg-day or control groups. Therefore, the developmental NOEL is 10 mg/kg-day (Ciba-Geigy, 1984 as reviewed by DPR, 1996).

Rabbit

Atrazine Technical grade (96.3%) was given to pregnant New Zealand White rabbits at daily doses of 0, 1, 5, or 75 mg/kg-day on days 7 through 19 of gestation. The control group received 5 ml/kg-day of 3% cornstarch containing 0.5% Tween 80, which was equivalent to that received by treated rabbits. The maternal NOEL of 5 mg/kg-day was based on reduced weight gain and food consumption in the 75 mg/kg dose group. The developmental NOEL of 5 mg/kg-day was based on increased resorption, decreased fetal weight, and fewer live fetuses at the high dose.

In Vitro Studies

The teratogenic potential of commercial formulations of atrazine (40.8%) and 2,4-D was evaluated using frog embryo teratogenic assay-Xenopus (FETAX) (Morgan *et al.*, 1996) in buffer and natural water. All treatments showed a significant concentration-response effect on exposed embryos, except for the 2,4-D natural water samples. The embryotoxicity and teratogenicity to frog embryos occurred at high concentrations approaching their maximum solubility levels in water for both herbicides. The authors suggest that water contamination by these herbicides may not be of toxicological significance with regard to reproductive or developmental toxicity.

Animal Reproductive Toxicity Studies

Rat

In a two generations, one litter per generation, rat reproduction study, atrazine (97.6%) was administered in the diet to CRCD, VAF/PLUS rats (30/sex/group) at dietary concentrations of 0, 10, 50, or 500 ppm. Body weights and body weight gains were significantly lower in F₀ and F₁ animals throughout the study period in the 500 ppm dose group. Sporadic decreases in body weight were also observed for the 50 ppm dose group, but were not considered to be treatment related. Food consumption was also reduced for males and females during the pre-mating period for both parental generations (F₀ and F₁) and for F₁ females on days 0-7 of gestation. No adverse effects were observed upon reproduction. The parental and reproductive NOELs were 50 and ≥500 ppm, respectively for this study (Ciba-Geigy, 1988, as reviewed by DPR, 1996).

The effects of subacute exposure to atrazine were studied on body weight, ovarian cycling, conception rate and litter size in Fischer rats (Simic *et al.*, 1994). Atrazine was administered orally at 120 mg/kg for 7 days. Reduced body weights were observed during the treatment in both sexes and in females up to two weeks after the final dose. There was a significant increase in the relative weights of pituitary and prostate glands. Atrazine also prolonged the estrous cycle, characterized by extended vaginal diestrus. As a result of disturbed ovarian cycling, the rate of successful mating decreased in the first week after treatment when either both sexes were exposed and mated or exposed females were mated with unexposed males. The litter sizes in the treated groups were similar to the controls.

Genetic Toxicity

Atrazine was negative in the majority of microbial and animal test systems utilized for studying gene mutation, chromosomal aberration and DNA damage following in vivo and in vitro exposure with and without metabolic activation (DPR, 1986). Atrazine's metabolites DDA, hydroxyatrazine, deethylatrazine, and deisopropylatrazine were also negative for gene mutation in the Ames test, for DNA repair in human fibroblasts and rat hepatocytes and for chromosomal aberrations in micronucleus tests (DPR, 1996).

Eleven laboratories assessed the mutagenic effects of various chemicals including atrazine in many different assay systems sponsored by the European Environmental Commission (EEC) (Atrazine was mutagenic in the host mediated assay with yeast and E. Coli and in V79 cells when plant derived metabolic activation was provided. The doses of 100 and 600 mg/kg of atrazine significantly increased the frequency of ampicillin-resistant mutants in Escherichia coli. In the presence of plant metabolic activation system, a significant increase in the mutation rate was observed in 5 genetic loci of Schizosaccharomyces pombe and at one locus in Aspergillus nidulans. Atrazine was also mutagenic in the dominant lethal mutation assay in the mouse at doses of 1500 and 2000 mg/kg given orally, and in mouse bone marrow tests at doses of 1500 and 2000 mg/kg. Atrazine was negative in most in vitro experiments even with mammalian metabolic activation. The author hypothesized that metabolic activation occurs in mammals independently from the liver and possibly in the acidic part of the stomach. These mutagenic effects may also be due to the nitrosamine formation in stomach by atrazine (Cova *et al.*, 1996).

Six positive and 31 negative nonplant genetic studies of atrazine were reviewed to compare two different methods of data assessment (Brusick, 1994). The first method of assessment was based on conventional expert judgment for determining results as positive or negative, considering all variables in the study. The second approach assessed the composite results from the reviewed database. The International Commission developed this method for Protection Against Environmental Mutagens and Carcinogens (ICPEMC). This approach weighs data without a consensus for positive or negative effects. Based on the expert judgment method, the analysis suggests that atrazine may be responsible for chromosomal damage in vivo but only under certain circumstances. Based on the ICPEMS method, the analysis suggests that the intrinsic mutagenic or clastogenic activity for atrazine is quite low.

In a recent study (Gebel *et al.*, 1997) various herbicides (alachlor, atrazine, terbutylazine, glufosinate-ammonium, isoproturon, pendimethaline and trifluralin) were tested for genotoxicity in the mouse bone-marrow micronucleus test (MNT). This study was conducted according to the Organization of European Community Development (OECD) guidelines. Both atrazine and trifluralin caused a significant increase in the number of micronuclei at doses of 1400 mg/kg body weight in female mice. Alachlor, terbutylazine, glufosinate-ammonium, isoproturon and pendimethaline did not have any genotoxic effects in the mouse bone-marrow micronucleus test in either female or male animals.

Biradar and Rayburn (1995a) 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 and 0.005 μM , respectively, for 48 hours. These concentrations are considered safe by the U.S. Environmental Protection Agency (US EPA) for drinking water. A known clastogen (Ara-C) was used as a reference for comparing the magnitude of chromosomal damage caused by herbicides. Measuring the coefficient of variation (CV) and percent chromosomes present in the larger chromosome distribution peaks assessed chromosomal damage. Exposure to atrazine increased the CV of the largest chromosome distribution peak suggesting clastogenicity. The negative control, atrazine and Ara-C's CVs were 3.73, 3.93 and 4.18, respectively. Chromosomes exposed to both simazine and -bentazon did not exhibit chromosomal damage. Atrazine concentrations higher (0.023 to 0.092) than the contamination limits exhibited a true clastogenic nature like Ara-C (4.16 vs. 4.18 in positive controls). Atrazine was also positive in Chinese hamster ovary (CHO) cells in an another study (Biradar and Rayburn, 1995b). In another recent mutagenicity study (Ruiz and Marzin, 1997), atrazine was found negative in the Ames assay and in the SOS chromotest.

Roloff *et al.* (1992) studied the cytogenetic effects of atrazine and linuron alone and in combination following in vivo exposure in mice bone marrow and spleen cells and in vitro exposure in human peripheral blood lymphocytes. Mice were exposed to 20 $\mu\text{g}/\text{ml}$ atrazine, 10 $\mu\text{g}/\text{ml}$ linuron or a combination of 10 $\mu\text{g}/\text{ml}$ atrazine and 5 $\mu\text{g}/\text{ml}$ linuron in their drinking water for 90 days. No chromosome damage was observed in any of the treatment group in bone marrow cells, but spleen cells showed damage in all treatment groups. In vitro exposure, human lymphocytes were exposed to either 1 $\mu\text{g}/\text{ml}$ linuron, 0.001 $\mu\text{g}/\text{ml}$ atrazine or simultaneously exposed to 0.5 $\mu\text{g}/\text{ml}$ linuron and 0.005 $\mu\text{g}/\text{ml}$ atrazine. No chromosomal damage was observed in lymphocytes exposed to linuron or atrazine alone. However, a significant increase in chromosome break frequency and aberrant cells was observed in lymphocytes simultaneously exposed to atrazine and linuron.

Atrazine was negative in vitro assays for SCEs in human lymphocytes and in the alkaline elution assay for DNA single strand breaks in rat hepatocytes, V79 cells and human lymphocytes (Dunkelberg *et al.*, 1994). In a recent study Chinese Hamster Ovary (CHO) cells were exposed to atrazine, simazine, cyanazine, and all possible combinations of these chemicals for 48 hrs at two concentrations for each sample: 1) the U.S. Environmental Protection Agency (EPA) MCL and 2) the highest contamination levels found in Illinois water supplies. The nuclei were then isolated and analyzed by flow cytometry. The effects of clastogenicity were measured by the coefficient of variation (CV) of the G1 peak. Flow cytometry is a sensitive method of measuring the whole cell clastogenicity. At both levels tested, atrazine, alone or mixed with cyanazine, showed whole cell clastogenicity. Atrazine also showed whole cell clastogenicity when mixed with simazine at the MCL. Simazine alone showed whole cell clastogenicity at the maximum level found in water (Taets, 1996). No synergy was observed when all three herbicides were combined (Taets and Rayburn, 1988).

The genotoxicity of the herbicides alachlor, atrazine, maleic hydrazide, paraquat and trifluralin was evaluated in the single-cell gel electrophoresis (SCGE) assay by using human peripheral blood lymphocytes (Ribas *et al.*, 1995). Lymphocyte cultures were treated with selected chemicals for 4 hours at 37 °C and checked for viability. The remaining cells ($3 \times 10^6/\text{ml}$) were used for microgel electrophoresis. All treatments were conducted with and without the presence of an external bioactivation source (S9 mix). The results indicated that

all the herbicides tested are able to give positive results by increasing the comet tail length. Alachlor and atrazine give similar results in treatments with and without S9; paraquat and trifluralin genotoxicity was higher when S9 mix was not used. On the other hand, maleic hydrazide genotoxicity was higher when S9 mix was used at normal pH (7.4). The authors suggested that genotoxicity depends largely on the pH of the solution, increasing as the pH decreases.

Ribas et al. (1998) also studied the genotoxicity of atrazine in cultured human peripheral blood lymphocytes. Sister-chromatid exchange (SCE), chromosomal aberration (CA), and micronuclei were scored as genetic end points in cultured cells treated with 5-100 ug/ml of atrazine. Lymphocyte cultures grown for SCE and MN assays were also pretreated for two hours with S-9 fraction. There was no increase in any of the genetic endpoints in the presence of atrazine as compared to controls.

The expression of P⁵³ protein was studied in peripheral blood lymphocytes of rats treated with 2.7 and 5.4 mg atrazine/kg-d, 5-d/week, for 6 and 12 months (Cantemir *et al.*, 1997). Wild type and mutant P⁵³ protein expression was measured by immunochemical techniques using a monoclonal antibody against a common epitope. Serum concentration of atrazine was also measured. The results indicate that the atrazine concentration in serum is proportional to the given dose after 6 and 12 months. The percentage of lymphocytes expressing P⁵³ protein was significantly increased in treated animals but the expression levels were not dose related. At the 2.7 mg/kg level, 19.8% of lymphocytes expressed intracytoplasmic P⁵³ after 6 months exposure and 32.8% after 12 months of atrazine exposure. At the 5.4 mg/kg level, 66.3% of lymphocytes expressed intracytoplasmic P⁵³ protein after six months and 73.8% after 12 months exposure; in controls about 60% of lymphocytes expressed intracytoplasmic P⁵³ protein at 6 and 12 months. The serum concentrations of atrazine in the controls, low and high dose groups were 0.12, 0.42 and 1.80 ug/mL, respectively. The lack of dose response and the presence of atrazine in the control rats diminish the significance of this study. The author suggested that the presence of the small quantity of atrazine in the serum of the control rats suggests that persisting atrazine in soil and water finds its way into the food used for rats.

Summary of Genetic Toxicity

From study data submitted by the registrant to DPR, atrazine was not found to be positive in assays designed to test for gene mutation, chromosomal aberration or DNA damage (DPR, 1998). From the open literature, Brusick (1994), using ICP/MS weight of evidence scheme for assessing multi-test mutagenicity studies, concluded that intrinsic mutagenicity or clastogenic activity for atrazine is quite low. In another series of tests reported by Cova (1996), atrazine was negative for 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 in vitro clastogenicity tests in Chinese hamster ovary cells, and for gene mutation and p⁵³ protein expression in human peripheral blood lymphocytes. Given the genotoxic potential of atrazine (albeit only at high doses), a genotoxic mode of action cannot be totally dismissed at this time. However, the weight of evidence suggests that a genotoxic mode of action alone may not have an important influence on the carcinogenic process at low atrazine doses.

Immunotoxicity

The effects of atrazine (formulation AAtrex, 85.5%) were examined on various parameters of immunologic responsiveness in C57Bl/6 female mice following a sublethal exposure to doses equivalent to 0.5 to 1.64 times the LD₅₀ (Fournier *et al.*, 1992). The animals were sacrificed 7-40 days after exposure and the following parameters were studied: organ weights, changes in spleen cell number and cell viability, frequency of L3T4-positive and Lyt-2-positive T-cells, mitogen response to lipopolysaccharide (LPS), phytohemagglutinin (PHA) and concanavalin A (Con-A), interleukin-2 (IL-2) production by splenic cells, primary humoral IgM response to sheep erythrocytes (SRBC), T-cell response to alloantigens in mixed lymphocyte reaction (MLR), and phagocytic activity of peritoneal macrophages. While there were transient and reversible changes observed in various parameters, normal humoral and cellular responses were restored at 14-40 days after the herbicide exposure. Overall, no effects could be attributed to the sublethal exposure to AAtrex.

Neurotoxicity

The effect of atrazine was studied on the cerebellar forelimb projection area in rats (Podda *et al.*, 1997). Rats acutely treated with 100 mg/kg atrazine had reduced Purkinje cell firing. Also, atrazine decreased the cerebellar potentials evoked by electrical stimulation of the ipsilateral radial nerve, affecting mostly the response to climbing fiber input. The authors suggest that motor disorders observed in atrazine treated animals might be due to cerebellar somatosensory cortex effects.

The effects of atrazine were studied in Fischer rats and their offspring. Two groups of 10 rats were gavaged with 120 mg/kg bw atrazine in paraffin oil once every 48 hours for six days. Two groups of 10 rats were used as controls and were given equivalent amounts of paraffin oil. By the end of the intubation period, the treated rats weighed less than controls. No effect was observed on the estrus cycle, gestation and delivery, litter size or pup survival. However, adult female offspring displayed increased activity in the activity cage and the male offspring avoided more electric shocks during the shuttle box acquisition trial compared to controls. In addition, the offspring of mothers treated with atrazine had shorter latency time during extinction trials than the control offspring (Peruzovic *et al.*, 1995). The results of this study, while suggesting that some neurological effect has occurred from atrazine treatment to the offspring of mothers treated with atrazine are questionable due to the small sample size.

Carcinogenicity

Rat-

Sprague-Dawley rats dietary study; Ciba-Geigy, 1986

Atrazine (95.8%) was administered in feed to groups of 70 male and 70 female Sprague-Dawley rats at dietary concentrations of 0, 10, 70, 500, or 1000 ppm for 24 months. Estimated doses were 0, 0.5, 3.5, 25, and 50 mg/kg-day, based on the default conversion

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factor of 0.05. Additional groups of 20 rats each received diets containing either 0 (control) or 1000 ppm atrazine and were sacrificed at either 12 or 13 months (after receiving control diets for one month). In males, survival was increased in a dose-related manner, but was significantly higher only in males receiving 1000 ppm as compared to controls. In contrast, the survival rate was decreased in females in a dose-related manner and was significantly lower in high-dose females as compared with controls. Mean body weights were significantly reduced in males and females at 500 and 1000 ppm except for males in the last 2 months of the study. The 24-month weight gains for males and females in the high dose groups were 76% and 64.5% of controls, respectively. While there was a substantial increase in weight gain in males and females in the recovery groups for month 13, it was still significantly lower in males as compared to controls. Organ to body weight ratios were increased in high-dose females for several organs, but these changes were due to the decreased body weights and were not considered biologically significant. Mammary fibroadenomas and adenocarcinomas were increased in females in a dose-related manner (see Table 3). The percentages of control females with these neoplasms in four recent studies conducted in the same laboratory were 40, 40, 48, and 51%.

Table 3. Mean life span and mammary tumor incidence in female Sprague-Dawley rats fed atrazine (Ciba-Geigy, 1986).

Dose (mg/kg-day)	0	0.5	3.5	25	50
Mean life span (days)	656	632	666	632	620
Incidence ¹	35/66 (53%)	39/64 (61%)	47/68 (69%)	47/65 (72%)	56/64* (88%)
Incidence ²	35/88 (40%)	40/69 (58%)	48/69 (70%)	48/70 (69%)	65/89 (73%)

¹Number of rats with mammary tumors/number of rats that were examined - based on CDFA's interim risk characterization document (1990). These values were used for fitting the LMS model.

²Number of rats with mammary tumors/number of rats that were examined based on Stevens *et al.*, (1994)

Other non-neoplastic changes at the high dose were: lower mean red cell counts, reduced hemoglobin and hematocrit at 6, 12, and 18 months; an increase in mean platelet count at 6 months in the 1000 ppm dose group; increased platelet counts at 6 and 12 months in females and lower serum triglycerides. Serum triglyceride levels were also significantly lower in the 12 month sacrifice group males. In the recovery group at 13 months the triglyceride level was similar to control males and those that were in the 1000 ppm dose group. In females, glucose levels were reduced in the high-dose group at 3, 6, and 12 months when compared with controls.

Acinar hyperplasia of the mammary gland and epithelial hyperplasia of the prostate were increased in males receiving 1000 ppm when compared to controls. In females receiving 500 and 1000 ppm, there was an increased myeloid hyperplasia in the bone marrow of both the femur and sternum. It was reported that the bone marrow changes, as well as an increase in extramedullary hematopoiesis in the spleen, were sequelae related to mammary fibroadenoma and adenocarcinomas (Table 2). The myeloid hyperplasia was characterized by a decrease in the number of fat cells in the marrow and an increase in the hematopoietic tissue, particularly cells of the granulocytic series. Muscle degeneration (femoral muscle) was found in both high-dose males and females. Retinal degeneration was increased in both males and females, the incidence being significantly higher in high dose females than in controls. In high dose females there was an increase in coagulative centrilobular necrosis in the liver. Based on the decreased body weight gain the NOEL for systemic toxicity for this study is 70 ppm (3.5 mg/kg-day) (Ciba-Geigy, 1986).

Fischer and Sprague-Dawley rats dietary study: Wetzel *et al.* (1994)

The dietary effects of atrazine (97%) on selected endocrine and tumor profiles were evaluated in 60 Fischer-344 or Sprague-Dawley rats/sex/group. Fischer rats were given 0, 10, 70, 200 or 400 ppm and Sprague-Dawley rats were given 0, 70 and 400 ppm dose levels for two years. The endocrine portion of this study is reviewed separately. There was no effect of atrazine on mammary tumor development in Fischer 344 rats. Sprague-Dawley rats fed 400 ppm atrazine had significantly higher mammary and pituitary tumors between week 0 -54. There was no increase in the numbers of mammary tumors at the end of the study suggesting an earlier onset, but not an increase incidence.

F344 rats dietary study: Pinter *et al.* 1990

In a third study carcinogenic effects of atrazine (98.9%) were observed in a life-long study in Fischer F344/LAT1 rats fed 0, 500 and 1000 ppm in diet (Pinter *et al.*, 1990). The number of rats in various dose groups was: Control, 56 males and 50 females; low dose, 55 males and 53 females; high dose, 53 males and 55 females. Because of decreased body weight and increased water consumption, the doses were lowered to 375 and 750 ppm in the low and high dose groups, respectively, after 8 weeks of treatment. No differences were observed in food consumption in treated and control groups for 104 weeks. Dose dependent reduced body weight gains were observed in treated animals, and the effects were more marked in females as compared to males. Survival was greater in treated males especially in the high dose group as compared to controls. No difference was observed in female survival in the various dose groups.

There were no significant differences in tumor frequency or latency in males among the experimental groups except for mammary gland tumors. The incidences and latency of benign mammary tumors were as follows: Control, 1/48 (11 weeks); low-dose, 1/51 (119 weeks); high-dose, 9/53 (121 weeks)($p < 0.05$ Peto's incidental tumor test). In females, the number of uterine adenocarcinomas occurred at an increased frequency in treated animals, with incidence and latency as follows: control, 6/45 (104 weeks); low-dose, 8/52 (110 weeks), and high-dose, 13/45 (108 weeks) ($p < 0.05$ Cochran-Armitage trend test). The incidence of uterine adenomatous polyps showed a negative trend as follows: control, 9/45;

low dose 9/52, and high dose, 3/45. The incidence of leukemia and lymphoma was increased in females in a dose-dependent manner (control, 12/44; low-dose, 16/52; high dose, 22/51) with a positive trend test ($p < 0.05$) for leukemia/lymphoma combined.

Chronic (12-24 month) dietary study: Morseth, 1998

Atrazine (97.1%) was fed in the diet to eighty intact CrI:CD®BR female rats per group at concentrations of 0, 25, 50, or 400 ppm. Twenty rats per group were designated for 1-year interim sacrifice. Parallel groups of ovariectomized rats were similarly treated. The primary purpose of this study was histopathology of reproductive tissues and pituitary. The NOEL for non-neoplastic effects was 70 ppm based on reduced body weights at the 400 ppm level in intact and ovariectomized females. There was a decreased time-to-tumor for mammary carcinoma in 400 ppm intact rats, but there were no tumors in ovariectomized rats (as reviewed by DPR, 1998).

3. Mouse:

Mice dietary; Ciba-Geigy, 1987

Atrazine (97.6%) was administered in the diet to 60 CD-1 mice/sex/dose group for 91 weeks at concentrations of 0, 10, 300, 1500 and 3000 ppm. The NOEL was 10 ppm based on reduced body weight gain at 300, 1500 and 3000 ppm in both sexes. Also, there was an increase in mortality in females at the 3000 ppm dose level. There was a reduction in hematocrit, hemoglobin and red blood cell counts at 1500 ppm in males and 3000 ppm in both sexes. A reduction in absolute brain weights in males and females and in absolute kidney weights in females was observed at the 3000 ppm level. There was an increased incidence of cardiac thrombi at the 1500 ppm level in females and at the 3000 ppm level in both sexes. No oncogenic effects were observed in this study (Ciba Geigy, 1987 as reviewed by DPR, 1996).

In vitro mesothelial cell differentiation; Dona *et al.*, 1991

The effects of atrazine were investigated on phenotypic differentiation of mesothelial cells treated with 5 μ M atrazine. Human peritoneal mesothelial cell cultures were characterized using a polyclonal anti-microthelial cell antibody. Differentiation of cells was based on the desmin, fast myosin and myoglobin markers of muscle differentiation. The myogenic phenotype was desmin -, fast myosin +, and myoglobin +. The authors hypothesized that differentiation of mesothelial cells to the myogenic phenotype in culture by atrazine involves the metabolism of atrazine to a possible DNA hypomethylating agent, such as 5-aza-chlorocytidine (Donna *et al.*, 1991).

Summary of Carcinogenicity studies

There is sufficient evidence of carcinogenicity in the female Sprague-Dawley rats for mammary fibroadenomas and adenocarcinomas. Positive results were also obtained in male F344 rats for benign mammary tumors ($P < 0.05$) and leukemia and lymphoma (positive trend test, $P < 0.05$). Atrazine was negative in mouse oncogenicity study. In vitro, atrazine was able to differentiate human peritoneal mesothelial cells to myogenic phenotype in culture. It is hypothesized that atrazine may be acting by metabolizing to a DNA hypomethylating agent.

Hormonal Effects

The ovarian hormones are known to play a role in the development of mammary tumors. Therefore, to understand the role and possible mechanism of ovarian hormones in atrazine induced mammary tumors a series of studies has recently been submitted in support of atrazine's registration (DPR, 1998). The following is a brief summary of these studies as well as related studies found in the open literature.

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 to determine various parameters such as estrous cycle, plasma hormone levels and tumor profiles. In Sprague-Dawley rats, tumor latency for mammary and pituitary gland tumors during the first year was shortened at the 400 ppm level. 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. Based on vaginal cytology, the 400 ppm females had an increased percentage of days in estrus during the first 18 months of the study. Estradiol levels in the 400 ppm dose group were elevated compared with other groups at 3 and 9 month (statistically significant only at 3 months). Data are variable, but are generally consistent with the hypothesis that elevated estrogenic stimulation early in the adult life of Sprague-Dawley rats influenced the elevated incidence of mammary tumors (or earlier onset of such tumors).

In Fisher-344 rats, reduced 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 suggest 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 but there was no concomitant increase in or earlier onset of mammary tumors in this dose group (Wetzel *et al.*, 1994). We believe that the data do not support the conclusion drawn by the authors. 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 atrazine develop as a result of endocrine-mediated effects (Stevens *et al.*, 1994). The authors compiled data from previously conducted carcinogenicity studies to substantiate this hypothesis. Atrazine and simazine and to a lesser extent propazine and terbutylazine have been shown to induce mammary tumors in female Sprague-Dawley rats. The 2-thiomethyl-s-triazines (ametryn, prometryn and terbutryn and 2- methoxy-s-triazines) indicated a 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 (estradiol, prolactin, progesterone, growth-stimulating, luteinizing, and follicle-stimulating hormones) in aged females (Stevens *et al.*, 1994).

The effects of atrazine, simazine and the common metabolite diaminochlorotriazine (DDA) 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 DDA for 1-3 days. On days 2 and 3, half of each group of rats received estradiol by injection. Dose related decreases in uterine wet weights were obtained in rats treated with estradiol and atrazine. No effect was observed on uterine wet weight with atrazine alone. For thymidine uptake studies, immature females were given 0, 1, 10, 50, 100, or 300 mg/kg-day atrazine, simazine or DDA for two days. On day 2 all animals received estradiol by injection. After 24 hours, all animals were killed and uterine slices were incubated with ³H-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 DDA. Each dose was followed by sc injection of estradiol. Parallel groups were treated with 0 or 300 mg/kg-day of atrazine, simazine or DDA without estradiol. Net progesterone receptor binding was reduced significantly in high dose animals in the atrazine and simazine treated groups subjected to estradiol treatment, and non-significantly in estradiol-pretreatment DDA rats. In the absence of estradiol treatment, lesser but nevertheless statistically significant reductions in progesterone receptor binding were observed. The authors conclude that triazine displayed very low antagonistic potency against estradiol function. It is postulated that atrazine may operate through cellular interactions unrelated to these hormone effects.

The effects of atrazine, simazine or DDA 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 concentration of 300 mg/kg-day of atrazine, simazine or DDA for 2 days reduced ER binding capacity by approximately 30%. This suggests that triazine competition against ER binding occurred to a much lesser degree than inhibition of estrogen-mediated responses. The authors suggest that atrazine binds weakly to the ER and other molecular interactions may play a part of 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.*, 1996). Atrazine was administered by gavage to females displaying regular 4-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 4-day ovarian cycle; but no distinct alteration in ovarian function (i.e., irregular cycles but not persistent estrus or diestrus) was observed. At the 150 mg/kg-day dose level, atrazine induced repetitive pseudopregnancies in females of both strains. At the 300 mg/kg-day dose level, 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. These data demonstrate that atrazine 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 28-31 days prior to ovariectomy and continued for an additional 10 days at 0, 2.5, 5, 40, or 200 mg/kg-day (Morseth, 1996a as reviewed by Cal/EPA, 1996). The rats were evaluated for variations in estrous cycle stages by vaginal smear analysis during weeks 2-4 of the treatment. Seven days after ovariectomy, rats were implanted with a silastic capsule designed to deliver estradiol levels ≥ 12 pg/mL. On the 10th day after ovariectomy, blood samples were taken at intervals (20-25 samples/interval) for assays of estradiol (to verify capsule implantation), LH and prolactin. Prior to ovariectomy, estrous cycling was disturbed, most remarkably by prolonged periods of diestrus at the 40 and at the 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 prolactin surge. The author suggests 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 ip for 3 days) suppressed the estrogen induced surge of luteinizing hormone (LH) and prolactin in ovariectomized rats (Cooper et al., 1996). However, the pituitaries of atrazine treated rats did release LH in response to gonadotropin-releasing hormone (GnRH). Using this model, 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 disrupt the CNS control of pituitary function.

Atrazine (97%) or DDA (97.4%) was administered to groups of 15 female Crl:CD®BR rats for at least 2 wk at doses of 100, 200, or 300 mg/kg-day (Morseth, 1990 as reviewed by Cal/EPA, 1996). Initially the high dose 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 corn starch suspension vehicle, and the other (a positive control for prolactin secretion) received an ip dose of metoclopramide 20 min before sacrifice. Rats were sacrificed at the time of first determination of diestrus stage after at least 14 daily treatments. Cycle stage was determined by vaginal cytology. Serum collected at sacrifice was assayed for prolactin, LH, FSH, progesterone, and estradiol. The majority of atrazine treated rats at doses of 200 to 300 mg/kg-day and DDA treated rats at doses 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. Also, there was a reduction in thymus weight in all groups. Coincident with these general toxicity signs, there were possible hormone level changes, particularly decreases of LH, progesterone, and estradiol in the 200 to 300 mg/kg-day DDA treated rats. In general, the high variability in hormone levels coupled with high general toxicity in groups with apparent hormone level changes makes this study of limited value for assessing intrinsic effects of these chemicals on hormone control (DPR, 1996).

Estrogenic activities of atrazine and simazine 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 stably 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-atrazine, suggesting that chloro-s-atrazine does not interact with the ER (Balaguer *et al.*, 1996).

The effects of atrazine and simazine 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. Cell growth was measured in the estrogen dependent recombinant yeast strain PL3, which requires an estrogenic substance and functional ERs 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. Also, 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 the estrogenic and antiestrogenic effects elicited by these chemicals are not mediated by the ER (Connor *et al.*, 1996).

The effects of atrazine, atrazine deisopropyl, cyanazine, and simazine on estrogen receptor 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, atrazine 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 the atrazine displaced radiolabeled estradiol from recombinant hER. These results suggest that the ability of atrazine 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 prometryne were studied on the formation of the ER complex (Tezak *et al.*, 1992). For the *in vivo* studies, 21 days old rats were administered lindane at 3 or 6 mg/100g bw; atrazine at 3, 6, or 12 mg/100 g bw; prometryne at 12 mg/100 g bw for 7 days. Animals were killed after 28 days for *in vivo* and *in vitro* studies. Both *in vivo* and *in vitro*, atrazine significantly inhibited the formation of the estradiol ER complex in rat uterus cytosol. The inhibition was non-competitive; atrazine decreased the number of binding sites but not the affinity of the ER for estradiol.

The influence of s-triazine compounds (atrazine, prometryne and de-ethylatrazine) was studied *in vivo* and *in vitro* on testosterone metabolism and binding of 5 alpha-dihydrotestosterone to its receptor in the rat prostate (Kniewald *et al.*, 1994a). Both atrazine and prometryne reduced 5 alpha-dihydrotestosterone (5α -DHT) formation. Also, both significantly decreased the number of binding sites for 5α -DHT on the receptor molecule following *in vivo* or *in vitro* exposure, but the K_d 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.

The effects of s-triazine compounds were studied on the metabolism of testosterone by the rat prostate in vivo and in vitro (Kniewald *et al.*, 1997). For in vivo studies, Fischer rats were intubated daily with atrazine or de-ethylatrazine for 7 days and killed 24 hours after the last intubation. Prostate glands were removed for analysis. The metabolism of testosterone and the formation of the cytosolic 5 α -DHT receptor complex was assayed in vitro following incubation of prostate tissue slices with atrazine and/or prometryne. Testosterone was found to be converted by the prostate primarily into 5 α -DHT and to a lesser extent into androstanediol, androstenedione, and androstane-3 α , 17 β -diol. The formation of 5 α -DHT was decreased with the addition of atrazine and/or prometryne. The formation of the 5 α -DHT receptor complex in rat prostate cytosol was significantly decreased in vitro following exposure to either atrazine or de-ethylatrazine and in vivo following atrazine treatment. The authors concluded that the inhibition of steroidogenic enzyme activities and steroid hormone formation in the rat prostate by s-triazine compounds is noncompetitive and reversible.

The effects of atrazine on androgen converting enzymes and protein synthesis are studied in male porcine pituitary adenohypophysial gland (Kniewald *et al.*, 1994b). The pituitaries were removed from six-month-old pigs castrated at the age of three months. Fresh tissues were incubated with ¹⁴C-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 μ mol to 0.7 μ mol significantly inhibited 5 α -reductase, which converts testosterone to DHT (24 \pm 2 pg/mg vs. 32 \pm 3.1 pg/mg in control), and 17 β -hydroxysteroid dehydrogenase which converts testosterone to 5 α -androstane-3 α , 17 β -diol (A-diol) (66 \pm 5.4 pg/mg vs. 109 \pm 8.2 pg/mg in control). Atrazine also inhibited protein synthesis in pituitary cytosol. Electrophoresis analysis of cytosolic protein indicated that while other major bands remained unchanged the purified fraction of 28.2 kD was increased, and 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 on the thyroid gland was studied in female albino rats administered oral doses of atrazine at 0.2 LD₅₀ for periods of 6 and 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 thyroxine (T4). A dose-dependent decrease in serum T3 concentrations was observed in all the treatment groups (control: 0.57 nmol/L; atrazine for 6 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 epitheliomers. Also, there was 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 as a result of a decrease in T3 levels (Kornilovskaya *et al.*, 1996).

The ability of various xenobiotics including atrazine to inhibit the binding of [³H] physiological ligands (present at a concentration of 7 nM) to the androgen and estrogen receptors, to rat androgen-binding protein (ABP), and to human sex hormone-binding globulin (hSHBG) was examined. Atrazine caused a statistically significant inhibition of

specific binding of [³H]5 α -DHT to the androgen receptor. The binding of [³H]5 α -DHT to rat ABP was inhibited 40% by atrazine. There was no inhibitory effect of atrazine on the binding of 5 α -DHT to hSHBG (Danzo, 1997).

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-1/2-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-1/2-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 xenobiotics might increase the risk of breast cancer by altering the ratio of 16- α -OHE-1/2-OHE-1. Estradiol metabolite 2-hydroxyestrone (2-OHE-1) inhibits breast cell proliferation while 16- α -hydroxyestrone (16- α -OHE-1) enhances breast cell growth, increases unscheduled DNA synthesis, and oncogene and virus expression, and increases anchorage-independent growth.

Summary of Hormonal Effects

The effects of atrazine and other triazines were studied on estrus cycle, estrogen mediated responses, estrogen receptor binding, hormonal induction and metabolism. Overall the in vivo and in vitro study data suggest that atrazine disrupts the ovarian cycling and induces mammary tumors. It binds weakly to the estrogen receptor, alters a few estrogen mediated parameters and has no direct agonist or antagonist activity. In one study in Sprague-Dawley rats, LH and estradiol levels were reduced at 40 and 200 mg/kg dose levels by influencing the hypothalamus/pituitary control mechanism. The relevance of these observations to humans is not clear at this time.

Effects on estrous cycle

In Sprague-Dawley female rats, atrazine exposure at about 20 mg/kg-day dose level increased the percentage of days in estrous and increased incidence and earlier 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 nor earlier onset of mammary tumors in this dose group. In another study conducted in Long-Evans hooded (LE-hooded) and Sprague-Dawley rats, a 75 mg/kg dose level for 21 days disrupted the 4-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 the 150 mg/kg-day dose level, atrazine induced repetitive pseudopregnancies in females of both strains. At the 300 mg/kg-day dose level, 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 in rats 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 atrazine and other triazines 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. Also, thymidine incorporation in uterine slices was decreased at the 50, 100 and 300 mg/kg-day dose levels. 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. Also, 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 regulate luciferase reporter gene (17m5-G-Lucia) activity, a dose-dependent induction in luciferase activity was observed following treatment of the cells with 17 β -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

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 1 to 3 days inhibited basal cytosolic PR binding. In rat prostate, atrazine inhibited 5 alpha-dihydrotestosterone (5 α -DHT) formation and decreased the number of binding sites for 5 α -DHT on the receptor molecule following in vivo or in vitro exposure to atrazine, but the K_d value was not changed. Atrazine also inhibited the binding of tritiated physiological ligands, [³H]5 α -DHT and ABP (40%) to androgen receptors. There was no inhibitory effect of atrazine on the binding of 5 α -DHT to hSHBG in rat prostate.

Effects on hormone induction and metabolism

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 5 α -reductase in male porcine pituitary adenohypophysial, which converts testosterone to DHT and 17 β -hydroxysteroid dehydrogenase, which, in turn, converts testosterone to 5 α -androstane-3 α , 17 β -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 16- α -hydroxyestrone (16- α -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 1/10th of those found in DMBA-treated cells.

Toxicology of Metabolites

DDA, a major metabolite of atrazine in rats and humans, was fed to dogs at nominal dietary concentrations of 0, 5, 100, or 1500 ppm for 52 weeks, with some animals fed for only 13 weeks prior to a recovery period or interim sacrifice. In the high-dose group, the dietary levels of atrazine were changed three times, including periods of 750 and 0 ppm; five males and two females in the high dose group were euthanized in a moribund condition with signs related to cardiac dysfunction and anemia/hypoproteinemia. Body weight and feed consumption were reduced in this group, and electrocardiographic abnormalities were detected in most animals. Gross and microscopic pathology corroborated the clinical findings. The NOEL was 100 ppm, which is similar on a molar basis to the 150 ppm level in the atrazine dog study (Ciba-Geigy 1990, as reviewed by DPR, 1996).

Hydroxyatrazine (97.1%) was administered in the diet to 70 to 80 CD rats/sex/group at 0, 10, 25, 200, or 400 ppm. Sixty rats/sex/group were designated for a 2-yr study, other rats were sacrificed at 12 months. The NOEL was determined to be 25 ppm, based on progressive nephropathy in females, which was characterized by dilatation with crystalline deposits, particularly in collecting ducts and renal pelvis, and interstitial papillary fibrosis. High dose animals had an accelerated onset of severe progressive nephropathy, with associated widespread mineralization of tissues, secondary parathyroid hyperplasia, and associated changes in bone (fibrous osteodystrophy), heart (progressive cardiomyopathy), and testes (degeneration or atrophy). A sharp dose-response relationship was observed in this study. However these findings were limited to relatively high dose levels, and did not represent the active ingredient (Chow, 1995 as reviewed by DPR, 1996).

Hydroxyatrazine (97.1%) was administered orally for 13 weeks to four beagle dogs/sex at levels of 0, 15, 150, 1500, or 6000 ppm. Hematology, clinical chemistry, and urinalysis were performed prior to and post-treatment. Standard gross and microscopic pathology was performed at study termination. The primary finding was chronic nephropathy with associated crystalline casts in renal tubules and papillae. All dogs with 1500 and 6000 ppm dose levels were affected (Chau *et al.*, 1990 as reviewed by CDFA, 1996).

Developmental Toxicity

Hydroxyatrazine technical grade (97.1%) was administered by gavage to 26 rats (CrI:COBS CD (Sprague-Dawley) BR/group at 0, 5, 25, or 125 mg/kg-day in aqueous suspensions of cornstarch/Tween 80 on gestation days 6-15. The maternal NOEL was 25 mg/kg-day, based on enlarged, mottled kidneys in two females and slightly reduced food consumption at 125 mg/kg-day during gestation days 8-12. The developmental NOEL/NOAEL was 25 mg/kg-day based on slightly delayed ossification at several sites, a very slight decrement in fetal

weights, and a low incidence of pups with malformations in the abdominal wall (one gastroschisis and one umbilical hernia in separate high dose litters). The malformations appeared plausibly related to treatment, and constitute a possible adverse effect of hydroxyatrazine (Giknis, 1989 as reviewed by DPR, 1996).

Deisopropylatrazine (G 28279 technical, 97.4%) was orally administered to 25 Tif: RAI f (SPF) rats (hybrids of RII/I x RII/2) per group at 0, 5, 25, or 100 mg/kg-day on gestation days 6-15. The developmental NOEL/NOAEL was 5 mg/kg-day, based on fused sternebrae (#1 and #2)]. Ossification delays were common at 100 mg/kg-day. The maternal NOEL was 5 mg/kg-day, based on minor decrements in body weight and food consumption at the 25 mg/kg dose level (Marty, 1992 as reviewed by DPR, 1996).

De-ethylatrazine (G-30033 technical, 97.5%) was administered by gavage to 24 Tif: RAI f (SPF) rats/group at 0, 5, 25, or 100 mg/kg-day on gestation days 6-15. The maternal NOEL was 5 mg/kg-day, based on slightly reduced food consumption at the 25 mg/kg-day dose level. At the 100 mg/kg-day dose level, a more pronounced reduction in food consumption, a statistically significant bw decrement, and hunched posture were observed in one dam. The developmental NOEL was 25 mg/kg-day based on fused sternebrae (sternebrae #1 and #2 fused) (Marty, 1992 as reviewed by DPR, 1996).

DDA (at least 98.1%) was orally administered to 26 CrI:COBS CO (SD)BR rats/ per group at levels of 0, 2.5, 25, 75, or 150 mg/kg day on gestation days 6 through 15. The maternal NOEL was 25 mg/kg-day based on reduced food consumption and body weight gain. The developmental NOEL was 25 mg/kg-day, based on ossification delays in parietal, interparietal, and hyoid bones at ≥ 25 mg/kg-day. There were decrements in fetal body weights, and ossification delays in the skull, hindpaw, and ribs at dose level of 75 to 150 mg/kg-day. At 150 mg/kg-day there was also an increase in resorptions (Hummel et al, 1989 as reviewed by DPR, 1996).

Structure Activity Relationships (SAR)

S-triazine compounds are derived from symmetrical 1,3,5-triazine by substitution in position 2, 4 and 6. They are classified in to chloro-, 2- alkoxy, 2-alkylthio,-triazine depending on the substituents on position 2. A number of the pesticides of the 2-chloro group (i.e., atrazine, simazine, propazine, and cyanazine) are carcinogenic inducing adenoma and adenocarcinoma in mammary gland of rats. Also, an alkylthio-substituted herbicide, terbutryn is carcinogenic inducing mammary, thyroid and liver tumors in rats. However there is a 10-fold difference in the potency of chloro substituted and methoxy substituted herbicides suggesting that the nature of substitution may have a profound effect on the carcinogenic potency as well as target specificity. Of the five sulfonyleurea herbicides, only one was carcinogenic at the highest dose tested inducing mammary tumor similar to those induced by atrazine type s-triazine herbicides.

An overview of the genotoxicity data indicates a heterogeneous response by s-triazine pesticides. The positive data are mostly associated with chlorinated s-triazine compounds (atrazine, cyanazine, propazine and anilazine) although at high dose levels. For non-chloro s-triazine compounds there is no evidence of genotoxicity. Thus, the role of mutagenicity in the development of mammary tumors can not be dismissed at this time.

U.S. EPA's (1990) Office of Pesticide and Toxic Substances has conducted SAR analysis of s-triazine pesticides. The carcinogenicity of any given s-triazine compound depends upon the nature of the substituents at the 2-, 4- and 6 position and the presence of N-alkyl group. The relative activity follows the order: 2-chloro >2-alkylthio >2 alkoxy. The enhancing effect of a 2-chloro group may be related to its ability to serve as a leaving group thus generating an electrophilic arylating intermediate. Further the document states that the available data and the SAR analysis do not suggest that pesticides and pesticide residues containing an intact s-triazine moiety are similar in terms of carcinogenic potential.

Special Studies

A 26-week toxicity study was done with pesticide and fertilizer mixtures representative of groundwater contamination found in California and Iowa (NTP, 1993). The mixtures were administered in drinking water to F344/N rats and B6C3F1 mice of each sex at concentrations ranging from 0.1X to 100X, where 1X represented the median concentration of the individual chemical found in studies of ground water contamination in ppb. The California mixture was composed of aldicarb (0.3), atrazine (0.05), 1,2-dibromo-3-chloropropane (0.001), 1,2-dichloropropane (0.45), ethylene dibromide (0.09), simazine (0.03), and ammonium nitrate (1.0). The Iowa mixture contained alachlor (0.09), atrazine (0.05), cyanazine (0.04), metolachlor (0.04), metribuzin (0.06), and ammonium nitrate (1.0). Parameters evaluated were: histopathology, clinical pathology, neurobehavior/neuropathology, reproductive system effects, and genetic toxicity as measured by the frequency of micronuclei in peripheral blood of mice and evaluating micronuclei and sister chromatid exchanges in splenocytes from female mice and male rats. The report also described additional studies with these mixtures which included teratology studies with Sprague-Dawley rats and continuous breeding studies with CD-1 Swiss mice. While there were some marginal variations of toxicity parameters with increasing dose, they were not considered to be of any biological significance. Therefore, no potential toxicity was associated with the consumption of mixtures of pesticides and fertilizers representative of groundwater contamination in agricultural areas of Iowa and California in subchronic toxicity studies in rats and mice.

The N-nitrosation of atrazine was studied in vitro in human gastric juices following incubation of atrazine with various concentrations of NaNO₂ (0.5, 1.5, and 3 mM) (Cova *et al.*, 1996). The yield of N-nitroso compound increased with the highest nitrite concentration. With 3 mM NaNO₂ atrazine nitrosation reached a maximum conversion of 23% after 1.5 hours. The nitroso derivatives were unstable, and after 6-12 hour of incubation they revert to the parent compound atrazine. The authors suggested that if the amount of nitrate is high in drinking water it might significantly raise the risk of N-nitrosoamine formation.

The effects of atrazine on hepatic drug metabolizing enzymes related to age and sex was studied in rats (Ugazio *et al.*, 1991). Atrazine (commercial product with 45% atrazine) was fed to 12 Wistar rats/dose group at concentrations of 0 or 450 ppm for 60 days before mating. The same concentration was fed to the F₁ generation from day 21 to 100. Hexobarbital sleeping time and hepatic xenobiotic metabolism in vitro were determined in F₁ rats at weaning and at 45 days of age. Atrazine administration significantly shortened the sleeping time of rats at weaning. This effect continued after weaning, but statistically significant differences were observed only in males. At weaning, F₁ male showed a

significant increase in both cytochrome P-450 and b-5 activities. Also there was a sharp increase in both GSH and GST activity. No effects were observed for the tested parameters in post weanling animals. These results suggest that atrazine affects hepatic metabolism in an age- and sex-dependent manner and that the effects are transferred to weanlings either via the placental and/or the mammary route. This is further supported by an increase in the phenobarbital-inducible families of cytochrome P-450 enzymes following atrazine exposure (Ugazio *et al.*, 1993).

Toxicological Effects in Humans

Epidemiological studies

A relationship between exposure to atrazine and increased cancer risk was evaluated in various counties of Kentucky (Kettles *et al.*, 1997). Exposure to atrazine was assessed from data on the use of contaminated water, corn crop production and pesticide use. Based on the exposure data, counties were classified into low, medium or high exposure groups. Data on breast cancer rates by counties were obtained from the state cancer registry. Odds ratios of 1.14 ($P < 0.0001$) and 1.2 ($P < 0.0001$) were obtained for the mid- and high-dose exposure groups, respectively, suggesting an association between atrazine exposure and breast cancer. This study is suggestive of, but cannot establish a causal relationship between triazine exposure and breast cancer, due to the lack of direct atrazine measurements in the exposed population.

Loosli (1995) reviewed the existing studies on the health effects of atrazine exposure, including studies of a manufacturing cohort, rural populations and case reports of acute exposures of humans and domestic animals. A few case control studies with chronic exposure are of special interest. For example, 66 cases of ovarian tumors diagnosed during a 6.5 year period in a city hospital were analyzed with the history of atrazine exposure. The reported relative risk (RR) for ovarian cancer associated with atrazine exposure was 4.4 (95% CI 1.9-16.1) and for women under 55 years of age the RR rose to 9.9 (CI, 3.0- 28.2). A similarly high RR ratio was reported in another study by the same author for primary malignant epithelial ovary tumors with an odds ratio of 2.7 (90% CI 1.0-6.9). Other studies reported were of short duration and did not show any association with atrazine exposure. Loosli (1995) concluded that studies of chronically exposed workers in chemical plants have not revealed an increased incidence of benign or malignant disease attributable to atrazine. Some case-control studies showed a slight increase of non-Hodgkin's lymphoma (NHL) incidence, while others were negative. Loosli (1995) concluded that the weighted evidence does not support a causal association between malignant tumors and atrazine in farming populations. Two studies conducted in a rural population suggested an increase of ovarian tumors in exposed women. Neither the statistical analysis nor the exposure data from these studies was satisfactory, and supporting evidence is not provided by other studies.

In a retrospective study of workers involved for the past ten years in production and formulation of chlorotriazine herbicides (atrazine, simazine, propazine and terbuthylazine), there was no association of any disease with occupational exposure (Catenacci *et al.*, 1997).

A recent report reviewed the available epidemiological studies relating triazine herbicide to various types of cancer, non-Hodgkin 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 was to be the causal factor involved (Sathiakumar and Delkzel, 1997).

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic effects

The most significant and sensitive end point for noncarcinogenic effects is cardiomyopathy (myocardial atrial degeneration) observed in the one-year dog study. Clinical signs associated with cardiac toxicity were: ascites, cachexia, labored/shallow breathing, and abnormal EKG (irregular heart beat and increased heart rate, decreased P-II values, atrial premature complex, and atrial fibrillation). These effects were first observed as early as 17 weeks into the study. At 150 and 1,000 ppm, females experienced increased heart weights; electrocardiographic alterations in the heart and gross detectable pathology in both sexes. The reported NOEL for this study is 0.48 mg/kg (DPR, 1996). Initially U.S. EPA identified a NOEL of 0.5 mg/kg for the same dog study, but the registrant submitted additional data that resulted in a NOEL change from 0.5 to 5.0 mg/kg. Cal/EPA re-evaluated the study, but the NOEL was not changed (DPR, 1998). The other end point of concern is reduced body weight observed in the chronic rat study, with a NOEL of 3.5 mg/kg-day.

Carcinogenic Effects

Possible Mode of Action

U.S. EPA classified atrazine as a group C (possible human) carcinogen based on an increased incidence of benign and malignant mammary gland tumors in female Sprague-Dawley rats (Ciba-Geigy, 1987). Increases in benign mammary gland tumors were also observed in another study in male F344 rats. Epidemiological evidence suggesting an association of cancer with atrazine is equivocal. A recent analysis suggests that the epidemiological studies reported to date do not have sufficient statistical power to detect an association with cancer. The exact mechanism of tumor induction by atrazine is not known. Recently, the registrant has submitted a myriad of studies that suggest that hormones play a role in the induction of mammary tumors in Sprague-Dawley rats. It has been hypothesized that atrazine administration accelerates the age related endocrine changes in Sprague-Dawley rats leading to earlier onset or increased incidence of mammary tumors. Atrazine is positive in a number of mutagenicity studies. It has been suggested that atrazine is metabolized at sites other than the liver to genotoxic compounds. Also, atrazine may be converted to nitrosamine in the gastrointestinal tract in the presence of nitrate, which is commonly found in drinking water. Recent studies suggest that atrazine increases the ratio

of estradiol metabolites 16- α -OHE-1/2- α -OHE-1. 16- α -Hydroxyestrone can react directly with DNA, enhances breast cell growth, and increases oncogene and virus expression (Service, 1998; McDougal and Safe, 1998).

U.S. EPA follows a general procedure in deriving MCLGs for group C carcinogens in water. Either an RfD approach is used (as with a noncarcinogen) but an additional UF of 1 to 10 (usually 10) is applied to account for the limited evidence of carcinogenicity, or a quantitative method (potency and low dose extrapolation) is used and the MCLG is set in the 10^{-5} to 10^{-6} cancer risk range. According to the 1996 draft proposed guidelines for carcinogenic risk assessment (U.S. EPA, 1996), 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, the default is to use a linear extrapolation.

For the non-linear approach either a NOEL or a LED₁₀ value can be used with an appropriate UF. For the linear approach, either q_1^* value or a carcinogenic slope factor (CSF) as has been suggested in the new cancer guidelines can be used to determine PHG levels. Since the mechanism of action of atrazine is unknown, OEHHA has adopted the default option of linearity for this chemical and has used cancer slope factor (CSF) and not the q_1^* , as has been done traditionally, for this PHG determination. Estimation of Cancer Potency and LED₁₀

The most relevant data for estimating the cancer potency are based on increased incidences of mammary tumors in Sprague-Dawley rats (Ciba-Geigy, 1986) which showed a dose related increase in mammary tumors. The multistage model is fit to the carcinogenicity dose response data and the 95% 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 their 1996 draft guidelines, was also calculated by linear extrapolation below the LED₁₀ dose. LED₁₀ is the lower confidence limit on a dose associated with 10% extra risk. As a result of slight differences in survival rate among the groups and a shorter than lifetime study (104 weeks), we averaged survival rate and used U.S. EPA's correction to adjust for the duration of the study. Based on these parameters, the ED₁₀ was calculated using the linearized multistage model and (body weight)^{3/4} interspecies scaling. A good fit criterion of $p > 0.05$ was adopted for the Chi-square test. Also, a graphical presentation of observed and predicted tumor response derived from Tox-Risk in the observed range showed a good fit with the LMS model. The LED₁₀ value and other potency estimates are given in Table 3. The earlier quantitative estimate by U.S. EPA using the linearized multistage model is $2.2 \times 10^{-1} (\text{mg/kg-day})^{-1}$. OEHHA's cancer potency estimate (q_1^*) and CSF are 0.26 and 2.3 $(\text{mg/kg-day})^{-1}$, respectively. The LED₁₀ value is $4.4 \times 10^{-1} (\text{mg/kg-day})$. The potency estimates and the LED₁₀ value were calculated using Tox-Risk (version 3) software (K.S. Crump Division, Clement International Corp., Ruston, LA).

Table 3. LED₁₀ and potency estimates for atrazine based on rat mammary tumors in Sprague-Dawley rats (Ciba-Geigy, 1986).

Parameters	q ₁ *(mg/kg-d) ⁻¹	Chi-square	P	K	MLE ₁₀ ¹ (mg/kg-d)	LED ₁₀ ¹ (mg/kg-d)	CSF (mg/kg-d) ⁻¹	US EPA q ₁ *(mg/kg-d) ⁻¹
Values	0.256	2.80	2.80	4	0.84	0.44	0.23	0.22

¹MLE and LED are given as dietary concentration on a 100% food basis in Tox-Risk. They were converted to mg/kg-day assuming 1.5 kg diet/day for 70-kg human body weight.

CALCULATION OF PHG

Noncarcinogenic Effects

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

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

where,

- NOAEL/LOAEL = No-observed-adverse-effect level or lowest-observed-adverse-effect level
- BW = Adult body weight (a default of 70 kg for male, 60 kg for female, or 10 kg for a child).
- RSC = Relative source contribution (defaults of 20%, 40%, or 80%)
- UF = Uncertainty factor (to account for uncertainty in the scientific analysis).
- L/day = Daily water consumption rate: 2 L/day for 60-70 kg adult, 1 L/day for 10 kg child; higher values of liter equivalents (Leq/day) are used for volatile organic compounds to account for inhalation and dermal exposure through showering, flushing of toilets, and other household uses of tap water.

This calculation is based on the assumption of a 70-kg adult person consuming two liters of water per day, with an RSC of atrazine from water of 20%.

Therefore,

For atrazine, the NOAEL is 0.48 mg/kg-day for myocardiopathy derived from the one year dog study. A cumulative uncertainty factor of 100 is used to account for inter- (10), and intra-species (10) variation.

$$C = \frac{0.48 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{100 \times 2 \text{ L/day}} = 0.034 \text{ mg/L} = 30 \text{ ppb}$$

Carcinogenic Effects

The following general equation can be used to calculate the public health-protective concentration (C) for atrazine in drinking water (in mg/L):

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

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% confidence limit on the cancer potency slope calculated by the LMS model, and CSF is a potency derived from the lower 95% confidence limit on the 10% tumor dose (LED₁₀). $CSF = 10\% / LED_{10}$. Both potency estimates are converted to human equivalent [in $(\text{mg/kg-day})^{-1}$] using $BW^{3/4}$ scaling.
- L/day = Daily volume of water consumed by an adult (a default of 2 L/day).

For atrazine, q_1^* , CSF and the LED₁₀ values of 0.26 and 0.231 $(\text{mg/kg-day})^{-1}$ and 0.44 mg/kg-day were calculated from the mammary tumor data in rats from the two-year dietary carcinogenicity study of Ciba-Geigy (1986). The potency estimates q_1^* , as used traditionally by U.S. EPA, and CSF, as suggested in the 1996 draft proposed cancer guidelines, are used for comparative purposes. An RSC is not included in the calculations for carcinogenic health protective concentrations. It is assumed that the use of the low dose extrapolation is adequately health protective without additional source contribution.

$$\underline{C \text{ using } q_1^*} \quad \frac{1 \times 10^{-6} \times 70 \text{ kg}}{0.26 (\text{mg/kg-day})^{-1} \times 2 \text{ L/day}} = \mathbf{0.00013 \text{ mg/L} = 0.1 \text{ ppb}}$$

$$\underline{C \text{ using CSF}} \quad \frac{1 \times 10^{-6} \times 70 \text{ kg}}{0.23(\text{mg/kg-day})^{-1} \times 2 \text{ L/day}} = \mathbf{0.00015 \text{ mg/L} = 0.15 \text{ ppb}}$$

The LED₁₀ value can be considered as a LOAEL for mammary tumors in rats. The health protective atrazine concentration then can be calculated as for a noncarcinogenic end point using UF and RSC. We used an uncertainty factor of 3 for interspecies extrapolation since LED₁₀ incorporates an interspecies adjustment; a factor of 10 for intraspecies; a factor of 10 for adjusting from LOAEL to NOAEL, and another factor of 10 for carcinogenic effect, for a total UF of 3,000. Therefore,

$$C \text{ using LED}_{10} \quad \frac{\text{LED}_{10} \times \text{BW} \times \text{RSC}}{\text{UF} \times \text{W}} = \mathbf{0.0010 \text{ mg/L} = 1.0 \text{ ppb}}$$

$$\frac{0.44 \times 70 \times 0.2}{3,000 \times 2\text{L/day}}$$

The estimated health protective atrazine concentrations in drinking water calculated using various approaches are summarized in Table 4. There is a 30 fold difference in the calculated values using q₁* based on the cancer endpoint versus using the NOAEL based on the non-cancer endpoint, cardiomyopathy in dogs. There is a 10-fold difference between the linear and non-linear approach using cancer as the endpoint. Values calculated using cancer as the endpoint are lower than the values calculated for non-cancer endpoints. When cancer is used as the endpoint, the values calculated using the linear approach are lower than that using the non-linear approach. Therefore, the PHG is based on the more health protective concentration calculated using the cancer endpoints with a linear approach. OEHHA has chosen to use the linear approach using the CSF method for this chemical because the value obtained is less model-dependent. In the absence of a clear mechanism of action for mammary tumors in rats and their relevance to humans, a linear default is appropriate to use for atrazine. OEHHA considers a concentration of 0.15 ppb for atrazine based on CSF calculated from the mammary tumor data in Sprague-Dawley rats (Ciba-Geigy, 1996) as the most appropriate value.

Table 4. Health protective concentrations of atrazine in drinking water based on various approaches.

Endpoints	Approach	Concentration (ppb)
Noncancer	NOAEL	30
Cancer	LED ₁₀	1.0 ^b
	q ₁ [*]	0.1 ^c
	CSF	0.15^d

^adetermined using NOAEL, (no-observed adverse-effect level)

^bdetermined using LED₁₀, 95% (lower confidence limit on a dose associated with 10% extra risk)

^cdetermined using q₁^{*} (carcinogenic potency determined by the linearized multistage model)

^ddetermined using CSF (cancer slope factor calculated from the LED₁₀ (0.1/LED₁₀))

RISK CHARACTERIZATION

Atrazine is readily absorbed from the gastrointestinal tract (80%). It is metabolized by oxidative dealkylation to mono- and then di-dealkylated products before being conjugated with glutathione or mercapturic acid. The toxicity potentials of dealkylated products are similar to that of the parent compound. U.S. EPA has classified atrazine as a group C carcinogen. Atrazine, as well as other triazines, has consistently produced mammary tumors (adenocarcinoma, fibroadenoma) at least in one species (rat) and one sex (female). Because of the high background incidence of mammary tumors in the test strain, it is not always possible to discern a clear dose response relationship at various doses. Atrazine was also positive for benign mammary tumors in males at the high dose and was positive in a trend test for combined leukemia and lymphoma in females in F344 rats. It is a genotoxicant. It has been suggested that atrazine is metabolized at sites other than the liver to genotoxic compounds. Also, atrazine may be converted to nitrosamine in the gastrointestinal tract in the presence of nitrate, which is commonly found in drinking water. Recent studies suggest that atrazine increases the ratio of estradiol metabolites 16- α -OHE-1 to 2- α -OHE-1. 16- α -Hydroxyestrone can react directly with DNA, enhances breast cell growth, and increases oncogene and virus expression (Service, 1998; McDougal and Safe, 1998).

Recently, it has been suggested that hormonal effects are involved in the development of atrazine induced mammary tumors. The role of ovarian hormone in the development and progression of mammary tumors is well documented (Bernstein and Press, 1998), but the actual mechanism of action is not known. Atrazine and related triazines disrupt ovarian function and have consistently produced increased mammary tumors or decreased time to tumor development in Sprague-Dawley rats. Atrazine decreases the LH surge, progesterone and estradiol levels, at least at the high doses of ≥ 400 ppm. This may disrupt the normal signal for ovulation and leads to prolonged estrus. Increased periods of constant estrus may result in an endocrine environment that supports the development of mammary tumors.

Atrazine is not directly estrogenic. It does not bind to the estrogen receptors and does not affect estrogen-mediated parameters such as uterine wet weight, uterine thymidine incorporation, MCF-7 cell proliferation or uterine progesterone receptor assay. An effect of atrazine on endocrine control at the hypothalamus/pituitary level has been hypothesized. Also, a dose-dependent decrease in serum T3 concentration was observed. Hypothalamus/pituitary control influence is also suggested by the reduced T3 levels observed in rats. Alternatively, a combination of aforementioned factors may play a role in the development of mammary tumors following atrazine exposure.

In developing the PHG, we have used a polynomial equation from the LMS model for fitting the mammary tumor incidence data from Sprague-Dawley rats in the observed range and to determine the 95% lower bound LED₁₀. From the LED₁₀, a model-free linear low dose extrapolation was made. Graphical representation of the observed and expected response suggested a good fit. In the absence of specific data on scaling between rats and humans, i.e., relative concentrations or activities of the active material at the target site, it was converted to human equivalent based on body weight to the 3/4 power scaling. This is an uncertainty in the calculation of atrazine's PHG.

This PHG is based on the parent atrazine compound alone. However, atrazine and its metabolites such as deethylatrazine and deisopropylatrazine have been detected in ground and surface waters. These two metabolites are toxicologically similar to atrazine. Therefore, the PHG value for atrazine may underestimate the possible risk to humans.

This PHG is based on exposure to atrazine in drinking water. Because of atrazine's physical and chemical properties, an insignificant amount of exposure is expected from inhalation and dermal exposure from bathing and showering. An estimated dermal absorption of 20% based on rat studies wherein high concentrations of atrazine in acetone or tetrahydrofuran were used is not appropriate in estimating dermal exposure to atrazine from bathing and showering. Contact time for the material in water is variable, but less than in the rat studies.

This atrazine PHG of 0.15 ppb is based on a cancer risk level of 10⁻⁶. The concentrations in water corresponding to cancer risk levels of 10⁻⁵ and 10⁻⁴ are 1.5 and 15 ppb, respectively. These values may be an over or underestimation depending on the assumptions used in body weight scaling and low dose extrapolation methods. Given the lack of scientific data in support of the value used for one or the other parameter, a stochastic analysis was not done because the parameters are not well defined and the methodology has not been finalized.

OTHER REGULATORY STANDARDS

The U.S. EPA MCL for atrazine is 3 µg/L (3 ppb). This MCL was calculated based on the RfD of 0.005 mg/kg-day derived from a NOEL of 0.5 mg/kg-day for decreased body weight of pups in a multi-generation rat reproduction study. An additional uncertainty factor of 10 was used to account for possible human carcinogenic effects. U.S. EPA's reference dose has since been changed to 0.035 mg/kg-day based on decreased body weight from a chronic rat study with a NOEL of 3.5 mg/kg-day. Recently, U.S. EPA used the linearized multi-stage model to extrapolate tumor responses observed at the high dose to predict tumor response at low doses. This model assumes that there is no threshold for carcinogenic

effects. Based on the model, U.S. EPA's cancer potency estimate (q_1^*) is 2.2×10^{-1} (mg/kg-day)⁻¹. The MCL of 3 ppb is associated with an estimated cancer risk level within the 10^{-5} range for drinking water (assuming a person consumes 2 liters of water per day containing atrazine at 3 µg/L over a 70-year lifetime) (U.S. EPA, 1994).

The current California MCL is 3 ppb for atrazine in water, based on a no-effect level of 0.35 mg/kg per day from a one-year dog study, with an uncertainty factor of 1,000 (DHS, 1989). In deriving the proposed MCL, OEHHA did not estimate oncogenic risks, but incorporated an extra 10-fold uncertainty factor to account for the possibility of an oncogenic effect. The MCL assumes that drinking water will be the source of 20% of the total atrazine dose.

Health Canada proposed an interim maximum acceptable concentration (IMAC) of 5 ppb based on an NOAEL of 0.5 mg/kg-day from a two-generation rat reproduction study, similar to U.S. EPA's initial NOAEL.

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

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