NATIONAL TOXICOLOGY PROGRAM

EXECUTIVE SUMMARY OF SAFETY AND TOXICITY INFORMATION

ATRAZINE

CAS Number 1912-24-9

February 21, 1992

Submitted to:

NATIONAL TOXICOLOGY PROGRAM

Submitted by:

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Draft Report

TABLE OF CONTENTS

OVERVIEW [1]

Nomination History: *Atrazine was nominated for toxicological and carcinogenic evaluation by the National Institute of Environmental Health Sciences (NIEHS) in November, 1991 with a high priority. The nomination was based on the following: evaluations by IARC indicated that there was a need for chronic animal studies to help establish risk for humans; atrazine is the active ingredient in an herbicide widely used on corn in the midwest, and is stable in the environment and found in ground water; the compound is known to cause hormonal changes in animals; the de-ethyl derivative of atrazine is described to cause gene mutation and mitotic recombination in Saccharomycete; and the compound was identified as one of the chemicals needing carcinogenesis studies to fill a gap in toxicity information by the "Farm Chemical Workgroup". Other reasons cited by the Farm Chemical Workgroup include the large annual usage of atrazine as an agricultural pesticide (up to 100 million pounds a year), the lack of adequate data in the areas of exposure assessment (especially to agricultural worker families), immunotoxic action (hypersensitivity and chemically induced immune injury), and the potential for nitrosation in the presence of nitrate, and the need for testing in an animal species that is susceptible to development of non-Hodgkin's lymphoma.*

Chemical and Physical Properties: *Atrazine is a solid in the form of white powder or crystals. The compound has a melting point of 171-174°C. Atrazine is moderately soluble in water and readily soluble in many organic solvents. Although atrazine does not react with water or other common materials, it may be hydrolyzed to its hydroxy derivative in strong acids and alkalis, and at higher temperatures in neutral media.*

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[1]The information contained in this Executive Summary of Safety and Toxicity Information (ESSTI) is based on data from current published literature. The summary represents information provided in selected sources and is not claimed to be exhaustive.

Production/Uses/Exposure: *Atrazine is used as a pre- and post-emergence herbicide, plant growth regulator, and weed-control agent primarily for corn, grain sorghum, and sugarcane. Atrazine, the second most widely used herbicide in the United States, is manufactured by Ciba-Geigy Corporation and the DuPont Company. SRI reports that approximately 80 million pounds of atrazine were produced annually during 1984-1988. Although atrazine is listed in the United States International Trade Commission's publication, Synthetic Organic Chemicals, no production data were available for the years 1985-1989. In addition, no production data were available form the public file of the EPA Toxic Substances Control Act (TSCA) Inventory. The amount of atrazine imported to the United States between July, 1990 and November, 1991 was reported to be 19,580,103 pounds.*

Atrazine may be released to the environment via effluents at manufacturing sites and at points of application where it is used as an herbicide. This herbicide has been detected in surface water, groundwater, rainwater, soil, and sediment samples collected throughout the United States, (primarily in agricultural areas). Atrazine is subject to breakdown in the environment by both biological (microbial degradation) and physio-chemical (photolysis, hydrolysis) mechanisms.

The major transformation products of microbial degradation include 2-chloro-4-ethyl-amino-s-triazine, 2-chloro-4-amino-6-isopropylamino-s-triazine, 2-hydroxy-4-ethylamino-6-isopropyl-amino-s-triazine, and 2-hydroxy-4-ethylamino-6-amino-s-triazine. Both hydrolysis and photolysis of atrazine yield hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropyl-amino-s-triazine) as the major transformation product. Hydrolysis of this herbicide is rapid in acidic and basic environments, but is very slow or nonexistent in neutral environments.

Consumer exposure may result from contaminated groundwater, drinking water, and food. Persons involved in the manufacture, formulation, or application of atrazine may potentially be occupationally exposed. Data from the National Occupational Exposure Survey (NOES) conducted by NIOSH during the years 1981-1983 estimated that 1,001 employees, including 123 female employees, were potentially exposed.

The OSHA PEL and ACGIH TLV for this compound is 5 mg/m ^{3}. The EPA has adopted a restricted use policy for atrazine and has established tolerances for this compound in, or on, raw agricultural commodities.

Toxicological Effects:

Human: *The following atrazine metabolites have been detected in the urine of humans with known occupational exposure: 2-chloro-4-amino-6-(ethylamino)-s-triazine and 2-chloro-4,6-diamino-s-triazine. Contact dermatitis was attributed to atrazine exposure in 1 case report. However, atrazine was not found to induce irritant or allergic reactions based on patch test results of agricultural workers. Results form an epidemiological study of farmers who were exposed to atrazine as well as other herbicides and insecticides, showed significant differences between high and low pesticide use groups with respect to hematocrit, hemoglobin, and prothrombin time. A significant correlation was also observed between the use of atrazine and 1-minute and 30-minute bilirubin values. In a case-referent study carried out to evaluate the specific role of triazine herbicides, including atrazine, in ovarian carcinogenesis, women exposed to triazine herbicides were found to have a 2- to 3-fold greater risk of epithelial ovarian cancer as compared to unexposed women. An increased risk of non-Hodgkin's lymphoma was associated with several classes of herbicides, including atrazine in a population-based case control study. No data on the prechronic, reproductive/teratogenic, or mutagenic effects of atrazine in humans were found.*

Animal: *The following metabolites have been detected in the urine of rats following oral atrazine administration: 2-chloro-4-amino-6-(ethylamino)-s-triazine; 2-chloro-4-amino-6-(ethylamino)-s-triazine, 2-chloro-4,6-diamino-s-triazine, 2-hydroxyatrazine, 2-hydroxy-4-amino-6-isopropylamino-s-triazine, and 2-hydroxy-4-amino-6-ethylamino-s-triazine. Atrazine was found to be mainly excreted in the urine, with 85-95% of the atrazine being excreted within 24 hours after dosing; approximately 19% of ^{14}C-atrazine was found to be excreted in the feces. After oral ^{14}C-atrazine administration in rats, radiolabel was detected at the highest concentrations in the liver, kidney, and lung. In another study, atrazine and its dealkylated metabolites were primarily detected in the liver, kidney, and brain of atrazine treated rats, with the highest concentrations being found in the kidney.*

The oral rat LD_{50} for atrazine has been found to range from 672-3080 mg/kg, a 1-hour LC_{50} of >0.71 mg/L, and a dermal rat LD_{50} of >2500 mg/kg have been reported. Six studies on the carcinogenic effects of dietary administration of atrazine in rats have been reported. In one study conducted by Ciba-Geigy, a significant increase in mammary gland tumors was observed in female Sprague-Dawley rats. In another study, significant increases in uterine adenocarcinomas and leukemia/lymphomas was observed in female Fisher 344 rats following oral administration; in male rats of this strain, significant increases in mammary tumors were observed. In the other 4 studies, no carcinogenic effects were found. When atrazine was administered interperitonaelly in male swiss mice, a significant increase in lymphomas was observed. Atrazine was not found to cause dose-related and statistically significant increases in carcinogenic effects in mice following dietary administration, and this herbicide was determined to be noncarcinogenic to beagle dogs by this exposure route. Nononcogenic effects observed in chronic studies included reduced weight gain (rats, mice, dogs), decreased heart and liver weight (dogs), liver necrosis (rats), and cardiopathy syndrome (dogs).

Atrazine was not found to cause reproductive effects in rats in 3-generation and 2-generation feeding studies. However, in the 2-generation reproduction study, a significant decrease in body weight, body weight gain, and food consumption were observed in males and females, and a significant increase in relative testes weight was observed in both generations of male rats as well as the F_0 and F_1 males. Atrazine was embryotoxic following subcutaneous administration in rats and was classified as being fetotoxic, but not teratogenic, following subcutaneous administration in mice. Developmental effects observed in rats and rabbits were primarily observed at doses that were maternally toxic. However, in one study, oral administration to pregnant rats on days 6-15 of gestation caused retarded skeletal development at a dose level that did not cause maternal toxicity. No data on the prechronic effects of atrazine were found in the literature.

Genetic Toxicology: *Numerous studies have been conducted on the genotoxicity of atrazine. Atrazine did not cause genotoxic effects in the majority of studies conducted in prokaryotic organisms. In addition, negative results were obtained in most lower eukaryotes, ;positive results were generally achieved only in the presence of plant activation. Atrazine induced sex-linked recessive and dominant lethals in Drosophila; and caused dominant lethal mutations and chromosomal aberrations in mouse bone marrow cells. Atrazine also induced forward mutations in host-mediated rat and mouse assays.*

Structure Activity Relationships: *Structurally related s-triazine herbicides, including simazine and propazine, have been found to cause carcinogenic effects in rats. These compounds are under review by the EPA for evidence of human carcinogenic potential. Simazine and cyanazine were genotoxic in Drosophila, and simazine was mutagenic in cultured human lymphocytes. Cyanazine caused reproductive and developmental effects in rats and is currently under review by the EPA for developmental effects.*

I. NOMINATION HISTORY AND REVIEW

A. Nomination History

1. Source: National Institute of Environmental Health Sciences (NIEHS) [NIEHS, 1991; NIEHS, 1992]

2. Date: November, 1991

3. Recommendations:

4. Priority:

5. Rationale/Remarks:

   - Need for chronic animal studies to help establish risk for humans.
   - Lack of adequate data in the areas of exposure assessment (especially to agricultural worker families), immunotoxic action (hypersensitivity and chemically induced immune injury), and the potential for nitrosation in the presence of nitrate.
   - Need for testing in an animal species that is susceptible to development of non-Hodgkin's lymphoma.
   - Known to cause hormonal changes in animals.
   - The de-ethyl derivative of atrazine is described to cause gene mutation and mitotic recombination in *Saccharomycete*.
   - Identified by the "Farm Chemical Workgroup" as one of the chemicals needing carcinogenesis studies to fill a gap in toxicity information.
   - Large annual usage as an agricultural pesticide (up to 100 million pounds a year).
   - Stable in the environment and found in ground water.

B. Chemical Evaluation Committee Review

1. Date of Review:

2. Recommendation:

3. Priority:

4. NTP Chemical Selection Principle(s):

5. Rationale/Remarks:

C. Board of Scientific Counselors Review

1. Date of Review:

2. Recommendations:

3. Priority:

4. Rationale/Remarks:

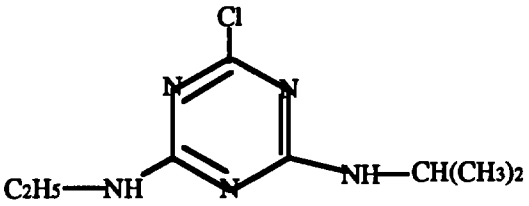
D. Executive Committee Review

1. Date of Review:

2. Decision:

II. CHEMICAL AND PHYSICAL DATA

A. Chemical Identifiers



ATRAZINE

CAS No. 1912-24-9
RTECS No. XY5600000

Molecular formula: $C_8H_{14}ClN_5$ Molecular weight: 215.68

B. Synonyms and Trade Names

Synonyms: 1,3,5-triazine-2,4-diamine, 6-chloro-N-ethyl-N'-(1-methylethyl)- (9CI); *s*-triazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-(8CI).

Trade Names: AAtrex®, Atranex®, Atratol®, Atranex®, Cekuzina-T®, ENT 28244®, Fenamin®, G-30027®, Gesaprim®, Maizina®, Primatol A®, Inakor®, Radazin®, Vectal®, Zeaphos®; Zeazin®

C. Chemical and Physical Properties

Description: While powder [Worthing, 1991] or crystals [Budavari, 1989].

Melting Point: 171-174°C [Budavari, 1989].

Boiling Point: Decomposes [USCG, 1985].

Specific Gravity/ Density: 1.2 @ 20°C (estimated {solid})[USCG, 1985]/ 1.187 g/cm^3 [Kearney and Kaufman, 1975].

Refractive Index: No data available

Solubility in Water: 70 ppm @ 25°C [Budavari, 1989]; 33 ppm @ 25°C [Meister, 1990].

Solubility in other Solvents: ether (12,000 ppm @ 25°C); chloroform (52,000 ppm @ 25°C), methanol (18,000 ppm @ 25°C) [Budavari, 1989]; n-pentane (360 ppm @ 25°C); ethyl acetate (28,000 ppm @ 25°C); dimethyl sulfoxide (183,000 ppm @ 25°C) [Meister, 1990].

Log Octanol/ Water Partition Coefficient: 2.63 [Banerjee and Baughman, 1991].

Reactive Chemical Hazards: No reaction with water or other common materials [USCG, 1985]. Hydrogen chloride and oxides of nitrogen may be formed upon decomposition [USCG, 1985; Sax and Lewis, 1989]. Atrazine is hydrolyzed to the hydroxy derivative in strong acids and alkalis, and, at higher temperatures, in neutral media [RSOC, 1987].

Flammability Hazards:

- Not flammable [USCG, 1985].
- Vapor Pressure: 3.0×10^{-7} mm Hg @ 20°C [Hayes, 1982].

III. PRODUCTION/USE

A. Production

1. Manufacturing Process

Atrazine is prepared by the reaction of cyanuric chloride with one equivalent of ethylamine followed by one equivalent of isopropylamine in the presence of an acid binding agent [Kearney and Kaufman, 1975]. This compound may also be prepared by the reaction of 2,6-dichloro-4-ethylamino-*s*-triazine with isopropylamine [SRI, 1992].

2. Producers and Importers

Producers:

U.S. Producers	Reference
Ciba-Geigy Corporation, Agricultural Division (AAtrex®) St. Gabriel, Louisiana	SRI, 1991a; USITC, 1990
DuPont Company Agricultural Products Department Axis, Alabama	SRI, 1991a; USITC, 1990

European Producers	Reference
Industria Prodotti Chimici SpA Navate Milanese, (Milano), Italy	SRI, 1991b
Oxon Italia SpA Pavia (Pavia), Italy	SRI, 1991b

Importers:

Importers	Reference
Alexander International New Orleans, Louisiana Kenner, Louisiana	Piers Imports, 1992
Ciba-Geigy Hawthorne, New York	Piers Imports, 1992
Fritz Savannah, Georgia Norfolk, Virginia New Orleans, Louisiana	Piers Imports, 1992
Heemsoth Kerner New York, New York	Piers Imports, 1992

John V. Carr & Son Norfolk, Virginia	Piers Imports, 1992
John Hearne Memphis, Tennessee	Piers Imports, 1992
Marman Tampa, Florida	Piers Imports, 1992
Micro Flo Sparks, Georgia	Piers Imports, 1992
Panalpina Memphis, Tennessee	Piers Imports, 1992
Schenkers International FWDRS Kenner, Louisiana	Piers Imports, 1992

3. Volume

Production Volume

Atrazine is listed in the United States International Trade Commission's (USITC) publication Synthetic Organic Chemicals, but no specific production data were available for the years 1985-1989 [USITC, 1986-1990][2]. The only year the USITC reported atrazine production data was in 1982, but industry sources maintain that the production level reported (68 million pounds) was vastly understated and that an output of 85-90 million pounds was a more realistic estimate. SRI reports that atrazine production dropped to about 60 million pounds in 1983 (as Ciba-Geigy closed a major plant for 6 months) before recovering to the 80 million pound level during 1984-1988. SRI noted that future production levels will depend directly on corn acreage and the use of direct applications or components in blends [SRI, 1992]. No production data were available from the public file of the EPA Toxic Substances Control Act (TSCA) Inventory [USEPA, 1992a].

[2]Production statistics for an individual chemical are given only when there are three or more producers, no one or two of which may be predominant. Moreover, even when there are three or more producers, statistics are not given if there is any possibility that the publications would violate the statutory provisions relating to unlawful disclosure of information accepted in confidence by the Commission. Data are reported by producers for only those items where the volume of production or sales or value of sales exceeds certain minimums. Those minimums for all sections are 5,000 pounds of production or sales, or $5,000 value of sales with the following exceptions: plastics and resin material—50,000 pounds or $50,000; pigments, medicinal chemicals, flavor and perfume materials, and rubber processing chemicals—1,000 pounds or $1,000.

Import Volume

The amount of atrazine imported to the United States by the importers listed above and 4 unspecified importers was 19,580,103 pounds between July, 1990 and November, 1991 [Piers Imports, 1992]. SRI reports that atrazine imports and exports essentially balance at 12-14 million pounds annually [SRI, 1992].

The net quantity of pesticides containing an unfused triazine ring (a class which includes atrazine) exported to the United States for the years 1989 and 1990 was reported in the U.S. Department of Commerce's publication U.S. Imports for Consumption and General Imports[3] to be 6,930, 790 pounds and 7,955, 886 pounds, respectively. Data on this pesticide class was not reported for the years 1987-1988 [USDC, 1988 -1991].

4. Technical Product Composition

Atrazine is available as 50 and 80% wettable powders and in a flowable formulation [Hayes, 1982]. Other formulations include suspension concentrates, granules, and water-dispersible granules [RSOC, 1987].

B. Use

Atrazine was introduced in 1958 by J.R. Geigy [Hayes, 1982]. This chemical is used as a pre- and post-emergence herbicide, plant growth regulator, and weed-control agent [Sax and Lewis, 1987] for corn, asparagus, forestry, grasslands, grass crops, pineapple, roses, sorghum, sugarcane [Worthing, 1991], vines, fruit orchards, citrus groves, bananas, pineapples, guavas, macadamia orchards, coffee, oil palms, and roses. This herbicide is also used non-selectively on non-crop areas, and may be used in combination with a wide variety of other herbicides [RSOC, 1987]. SRI reports that the majority (98%) of atrazine consumed is applied to corn acreage, grain sorghum and sugarcane, while secondary consumption of this compound includes industrial/commercial applications (2%) and home and garden use (2%) [SRI, 1992].

Atrazine is reported to be the second most widely used (by volume) pesticide in the United States (alochlor is the most heavily used [C&EN, 1987]). Approximately 75 million to 85 million pounds of atrazine are used each year in the United States, depending on corn price supports. Four states (Illinois, Iowa, Nebraska, and Indiana) account for 50% of atrazine use, but 10 additional states use one million pounds or more annually [Pesticide and Toxic Chemical News, 1991a].

The rate of atrazine application varies depending on its specific use. The equivalent of 2.24-4.48 kg/ha (2-4 lb/acre) are required for selective weed control for most situations. Higher rates are used for nonselective weed control while lower rates will effectively control cheatgrass and most of the weeds in chemical fallow or rangeland uses as well as many common annual broadleaf weed species [SRI, 1992].

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[3]Imports for consumption is a measure of the total volume of merchandise that has cleared through Customs, whether such merchandise enters consumption channels immediately, is withdrawn for consumption from warehouses under Customs custody, or is entered into U.S. Customs territory from Foreign Trade Zones.

IV. EXPOSURE/REGULATORY STATUS

A. Consumer Exposure

Consumers may be exposed to atrazine from a variety of sources including ground water, drinking water (see section IV.C, Environmental Occurrence) and contaminated food. The EPA reports that the estimated daily intake of atrazine from drinking water ranges from 0.0 to 0.45 µg/kg/day; however, these values do not account for variances in individual exposures or uncertainties in the assumptions used to estimate exposure levels. According to the EPA, data obtained on levels of atrazine in foods in the United States were insufficient for use in determining typical dietary intake levels. Tolerances have been set by the EPA for atrazine in or on raw agricultural commodities (see section IV.D, Regulatory Status). It is not expected, however, that individuals would be exposed to all the foods for which tolerances have been set, at their tolerance levels [USEPA, 1990].

B. Occupational Exposure

Personnel involved in the manufacture, formulation, or application of atrazine may potentially be exposed to this herbicide [Sittig, 1985].

Data from the National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) during the years 1981 to 1983, estimated that 1,001 employees, including 123 female employees, were potentially exposed to atrazine. Occupations that had over 50 employees potentially exposed included the following: mechanics and repairers (not specified); supervisors of production operations; miscellaneous plant and system operators; separating, filtering, and clarifying machine operators; miscellaneous machine operators, machine operators (not specified); and industrial truck and tractor equipment operators [NIOSH, 1992].

C. Environmental Occurrence/Fate

1. Occurrence

Atrazine is not known to occur naturally; however, the compound may be released into the environment via effluents at manufacturing sites and at points of application where it is used as an herbicide [Howard, 1991]. Residues of atrazine have been detected in surface water, groundwater, rainwater, soil, and sediment samples throughout the United States (especially in agricultural areas), and several studies and surveys have been conducted to quantify the incidence and level of atrazine contamination in the environment. For instance, the United States Environmental Protection Agency's (USEPA) "National Survey of Pesticides in Drinking Water Wells" revealed that atrazine was the second most frequently found pesticide in 540 community wells (from 50 states) and 752 rural domestic wells (from 38 states) sampled. The maximum concentrations of atrazine detected in the survey were 0.92 ppb in community water wells and 7.0 ppb in rural domestic wells [Pesticide and Toxic Chemical News, 1990a; Worthy, 1991]. The National Alochlor Well Water Survey detected atrazine in 12% of the 1,430 wells sampled from 26 states, and a database compiled by the USEPA showed that atrazine was found in 343 of 3,208 groundwater samples analyzed for pesticide residues. The database also showed that atrazine was found in the groundwater of 13 states at a maximum concentration of 700 µg/L [Pesticide and Toxic Chemical News, 1990b; USEPA, 1990; Ritter, 1990].

In addition to groundwater, atrazine has been widely detected in surface water and soil samples. The USEPA database showed that atrazine was present in 4,123 of 10,942 surface water samples analyzed, and was found in samples from 31 states at a maximum concentration of 2,300 µg/L [USEPA, 1990; Ritter, 1990]. A study conducted by the U.S. Geological Survey (USGS) detected atrazine residues in 98% of the 127 streams tested throughout 10 states; 71 of these sites were above the USEPA health advisory standard of 3 ppb [Klassen, 1990]. Another source reports that the USGS detected atrazine in most of the 146 water samples taken from the Mississippi, Missouri, and Ohio rivers in April-June of 1991 [Toxic Materials News, 1991]. In 1971, 152 (71.4%) of the soil samples taken from agricultural areas of 37 states tested positive for atrazine; the concentrations ranged from 0.01-16.73 ppm. A year later, atrazine was detected in a total of 134 (88.7%) of the samples at concentrations ranging from 0.01-0.77 ppm [Howard, 1991]. Table 1 below summarizes additional data on atrazine concentrations measured in the United States' groundwater, surface water, drinking water, and rain water.

Table 1: Concentrations of Atrazine in Water Samples from the United States

Sample Type	Location	# Samples collected/# positive	Concentration	Reference
River water	Mississippi River; 14 sites	NS/NS [a]	<1-17 µg/L	EC, 1990
Filtered river water	Ohio; Maumee River basin	NS/NS	0.3-4.8 mg/L	EC, 1990
Surface water	Kansas; 58 lake sites	NS/48	1.2-23.0 µg/L	EC, 1990
Surface water	Kansas; 19 drinking water supply sites	NS/NS	1.2-16.0 µg/L	EC, 1990
Surface water	Iowa rivers	NS/NS	0.50-42.0 µg/L	EC, 1990
Surface water	Iowa reservoirs	NS/NS	0.06-9.40 µg/L	EC, 1990
Surface water	Ohio streams	13/NS	0.1-23.2 µg/L	Howard, 1991
Surface water	Ohio; Sanduski River Basin	8/NS	0.1-45.7 µg/L	Howard, 1991
Surface water (dissolved fraction)	North Carolina; Cape Fear River Basin	107/~38 (35%)	mean of 56 µg/L	Howard, 1991
Surface water (particulate fraction)	North Carolina; Cape Fear River Basin	106/~23 (22%)	mean of 0.4 µg/L	Howard, 1991
Surface water	Pennsylvania; Susquehanna Drainage Basin	NS/NS	1.1-67.8 µg/L	USDI, 1989
Surface water	Maryland; Rhode River	NS/NS	0.003-3.3 µg/L	EC, 1990; USDI, 1989
Surface water	Maryland; Wye River	NS/NS	usually <3.0 µg/L	USDI, 1989
Surface water	Chesapeake Bay	NS/NS	max. of 0.3-1.1 µg/L	USDI, 1989
Runoff water	C hesapeake Bay	NS/NS	max. of 480 µg/L	USDI, 1989
Surface water	Chesapeake Bay tributaries	NS/NS	0.1-46.0 µg/L	USDI, 1989
Surface water	Chesapeake Bay; Choptank estuary	NS/NS	0.0-9.3µg/L	USDI, 1989
Rain water	Indiana	14/8	0.1->1.0 µg/L	EC, 1990
Rain water	Maryland	NS/NS	max. of 2.2 µg/L	USDI, 1989

Table 1: Concentrations of Atrazine in Water Samples from the United States (Continued)

Sample Type	Location	# Samples collected/# Positive	Concentration	Reference
Rain water	Ohio	24/14	<0.1->1.0 µg/L	EC, 1990
Rain water	West Virginia	20/11	<0.1-0.5 µg/L	EC, 1990
Rain water	New York	21/10	<0.1->1.0 µg/L	EC, 1990
Rain water	Maryland	68/NS	0.003-0.97 µg/L	EC, 1990
Drinking water	Colorado	NS/NS	mean of <1.8 µg/L	USDI, 1989
Drinking water	Tiffin, Ohio	NS/NS	mean of 3.3-16.4 µg/L	USDI, 1989
Ground water	Pennsylvania Mahantango Creek watershed	38/28	<0.003-1.110 µg/L	EC, 1990
Ground water	Nebraska; Platte River Valley (30 wells)	116/116	0.01-8.29 µg/L	EC, 1990
Ground water	Nebraska; Platte River valley (35 wells)	NS/64	<0.01-88.36 µg/L[b]	EC, 1990
Ground water	Nebraska; agricultural land	47/13	0.01-1.2 µg/L	EC, 1990
Ground water	Delaware; Appoquinimink watershed, wells	3/11	1-45 µg/L	Ritter, 1990
Ground water	Nebraska; agricultural areas	NS/NS	200-800 ng/L	Howard, 1991
Ground water	Central Nebraska (irrigation wells)	14/NS	0.06-3.12 µg/L	Howard, 1991
Ground water	Iowa; Big Springs watershed, wells	NS/NS	2.5-10.0 µg/L	Ritter, 1990
Ground water	Iowa; aquifers	NS/51%	max. of 3.0 µg/L	Ritter, 1990
Ground water	Iowa; areas around farm chemical supply dealers	NS/NS	max. of 65 µg/L	Ritter, 1990
Water pools/soil	Iowa; loading and rinse areas of farm chemical supply dealers	NS/NS	max. of 70,000 µg/L	Ritter, 1990

[a] NS = Not Specified

[b] The highest concentrations of atrazine were measured in bottom land wells in the principal aquifer and down gradient from irrigated cropland. Bottom land wells in the principal aquifer where "near pristine conditions" exist had atrazine concentrations ranging from 0.01-0.27 µg/L

2. Fate

Once introduced into the environment, atrazine is broken down by both biological (microbial degradation) and physico-chemical (photolysis, hydrolysis) mechanisms. Microbial degradation of atrazine proceeds mostly through dealkylation, and the major transformation products include 2-chloro-4-ethyl-amino-*s*-triazine, 2-chloro-4-amino-6-isopropylamino-*s*-triazine, 2-hydroxy-4-ethylamino-6-isopropyl-amino-*s*-triazine, and 2-hydroxy-4-ethylamino-6-amino-*s*-triazine. Decomposition studies show that ^{14}C ring-labeled atrazine evolved labeled CO_2 at slow rates, indicating that the s-triazine ring is fairly resistant to microbial degradation. For instance, seventy days after ^{14}C labeled atrazine was applied to a Webster soil at 10-20,000 ppm, <1% was recovered as CO_2. Also, only 0.49 and 0.76% of ^{14}C-labeled atrazine evolved as CO_2 from a soil/barley plant system treated with 1 and 6 ppm, respectively; 1.69% of 1 ppm radio-labeled atrazine evolved from a soil/maize system. For this reason, chemical degradation of atrazine may be more important environmentally than biodegradation, and much of what appears to biodegrade is actually bound to soil and sediments. Atrazine was also shown to be degradable by bacterium in a defined medium under anaerobic conditions; however only 0.59% of ring-labeled atrazine was mineralized to CO_2 [Howard, 1991].

Both hydrolysis and photolysis of atrazine yields hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropyl-amino-*s*-triazine) as the major transformation product. Hydrolysis of atrazine is rapid in acidic and basic environments, but is very slow or nonexistent in neutral environments. The hydrolysis half-lives of atrazine in aqueous buffered systems at 25°C and pHs of 1, 2, 3, 4, 11, 12, and 13 were 3.3, 14, 58, 240, 100, 12.5, and 1.5 days, respectively. In addition, the hydrolysis of atrazine is accelerated in the presence of humic materials, indicating this compound could be catalyzed. For example, the half-life of atrazine at 25°C and pH 4 was 244 days; however, after the addition of 2% humic acid, the half-life was 1.73 days. Also, at this same temperature, the addition of 5% fulvic acid resulted in half-lives of 34.8, 174, 398, and 742 days at pHs of 2.9, 4.5, 6.0, and 7.0, respectively. Photolysis of atrazine can occur in surface soils and water. Atrazine was not photolyzed, however, in methanol, ethanol, butanol, and water at wavelengths >300 nm. At wavelengths ≥ 290 nm, the photolysis half-life of atrazine (10 mg/L) in aqueous solution at 15°C was 25 hours; however, under identical conditions with an acetone sensitizer added, the half-life was decreased to 4.9 hours. Fulvic acid was found to increase the photostability of atrazine in aqueous solution, and the photolytic half-life of atrazine in fulvic acid solution increased with decreasing pH [Howard, 1991].

Microbial action, chemical hydrolysis, photodecomposition, and volatilization, all play a role in the degradation of atrazine in soil [USEPA, 1990]; however volatilization is not considered an environmentally important pathway (only 0.5-0.8% of radio-labeled atrazine was lost to volatilization from soils treated with 1-6 ppm) [Howard, 1991]. The persistance of atrazine in soils is variable, with half-lives ranging from 20-100 days in some soils and 330-385 days in others. For instance, atrazine is lost more rapidly from moist, warm soils that favor microbial activity than from dry, cold soils [USDI, 1989; USEPA, 1990]. This compound is also degraded

more quickly in soils with high organic and high clay content than in sandy mineral soils, in acidic as opposed to alkaline soils, at shallow soil depths than at deeper depths, and under conditions of increased ultraviolet irradiation [USDI, 1989]. In one study, phytotoxic residues of atrazine persisted for more than 1 year after application in loam to silt-loam soils, and were detected at maximum depths of 30-42 inches. In silty clay-loam and loam soils, phytotoxic residues persisted for 16 months after application and were detected at depths of 12-24 inches [USEPA, 1990]. In the summer of 1981, samples were collected from loamy soil that had been treated with radio-labeled atrazine in the spring of 1973. The soil still contained about 83% of the initial ^{14}C activity [Howard, 1991].

Atrazine is moderately to highly mobile in soils [USEPA, 1990], with Koc values ranging from 39-13,600 [Howard, 1991]. Atrazine can leach into the soil by rain or irrigation water; however the extent of the leaching is limited by the low water solubility of the compound and by its adsorption onto soil constituents. Run-off loss in soils ranges from 1.2-18% of the total quantity or atrazine applied, but is usually less than 3%. The downward movement of atrazine was measured in cornfield soils to a depth of 30 cm when applied at 1.7 kg/hectare to moist soils [USDI, 1989]. Field dissipation studies conducted by Ciba-Geigy, however, showed no leaching of atrazine and its metabolites below 6-12 inches of soil depth [USEPA, 1990].

In aqueous environments, chemical hydrolysis and biological dealkylation are the two primary pathways of atrazine degradation. Although photodegradation of this compound occurs, it was shown to be slow and was not expected to be a significant factor in the removal of atrazine from the water [EC, 1990]. The degradation of atrazine in water was found to be rapid; the half-life was reported to be 3.2 days in fresh water and between 3 and 30 days in estuarine environments (shorter half-lives were found at higher salinities). In sediments from estuaries, the atrazine half-life ranged from 15-35 days [USDI, 1989]. Adsorption of atrazine to suspended particulate material in the water column might also be a major factor in the removal of the compound from water [EC, 1990; USDI, 1989]. Colloidal organic matter from an estuary was found to have a higher adsorption capacity for atrazine than sediments from this environment, with an adsorption constant of 1850 compared to 78-213 for sediments [EC, 1990].

D. Regulatory Status

United States

Federal:

- The current OSHA permissible exposure limit (PEL) for atrazine is 5 mg/m^3 averaged over an 8-hour work shift [Office of the Federal Register, 1989].

- Atrazine is regulated by the EPA under the Safe Water Drinking Act. An MCLG of 0.003 mg/L and an MCL of 0.003 mg/L have been established for this pesticide [USEPA, 1992b].

- Atrazine is listed on the SARA Section 110 Priority List of CERCLA Hazardous Substances (ATSDR) [Roytech, 1990].

- The EPA has adopted a restricted use policy for atrazine under which only certified pesticide applicators, or those directly under their supervision, may purchase or spray this pesticide. (All products sold after September 1, 1990 must carry revised end-use product labels although EPA will not require relabeling of products already in the channels of trade {40 CFR 152.175} [Kassen, 1990; PTS Newsletter, 1992].[4]

- The EPA has established the following tolerances for atrazine in, or on, raw agricultural commodities {40 CFR 180.220 (a)}[Office of the Federal Register, 1991]:

  — cattle (fat, meat, and meat byproducts)--0.02 ppm {negligible residues}
  — corn, fodder (field, sweet, and pop)--15 ppm
  — corn, fresh (sweet kernels and cob with husk removed)--0.25 ppm
  — corn, grain--0.25 ppm
  — goats (fat, meat, and meat byproducts)--0.02 ppm {negligible residues}
  — guava--0.05 ppm
  — hogs (fat, meat, and meat byproducts)--0.02 ppm
  — horses (fat, meat, and meat products)--0.02 ppm {negligible residues}
  — macademia nuts--0.25 ppm
  — pineapples (fodder and forage)--0.25 ppm
  — poultry (fat, meat, and meat byproducts)--0.02 ppm {negligible residues}

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[4]EPA reports that this regulatory action was taken to reduce worker exposure and point-source ground water contamination [Chemical Marketing Reporter, 1990]. An EPA official recently reported that the EPA is currently considering banning atrazine as well as two other triazine herbicides (simazine and cyanazine) based on positive results of a Ciba-Geigy atrazine cancer study. The anticipated substitutes would reportedly be 2,4-D and dicamba [Pesticide and Toxic Chemical News, 1991b]. Another EPA official stated that Ciba-Geigy will be filing additional information on atrazine toxicity and environmental fate in 1992 [Pesticide and Toxic Chemical News, 1992], which would include toxicology studies intended to demonstrate that the carcinogenic effects observed in rats are a threshold effect resulting from a hormonal imbalance caused by atrazine [Pesticide and Toxic Chemical News, 1991a].

— rye grass, perennial--15 ppm
— sheep (fat, meat, and meat byproducts)--0.02 ppm {negligible residues}
— sorghum (fodder and forage)--15 ppm
— sorghum, grain--0.25 ppm
— sugarcane (fodder and forage)--0.25 ppm
— wheat, grain--0.25 ppm

- The EPA has established a tolerance for combined residues of atrazine and its metabolites (2-amino-4-chloro-6-ethylamino-*s*-triazine, 2-amino-4-chloro-6-isopropylamino-*s*-triazine, and 2-chloro-4,6-diamino-*s*-triazine) in or on the following raw agricultural commodities {40 CFR 180.220 (b)} Office of the Federal Register, 1991]:

  — grass, range--4 ppm
  — orchardgrass--15 ppm
  — orchardgrass, hay--15 ppm
  — proso millet (fodder, forage, and straw)--5 ppm
  — grain--0.25 ppm

State:

- Iowa (Department of Agriculture and Land Stewardship) has instituted an atrazine management plan to protect ground and surface waters. The plan is similar to the label changes approved by the EPA (see above), however it establishes a maximum use rate of 1.5 pounds of active ingredient per acre (compared to a federal maximum use rate of 3 pounds per acre) in certain regions of the state, primarily in northeastern and central Iowa [Ciba Geigy, 1991].

- Wisconsin has implemented a voluntary best management practices program which is similar to the Iowa initiative. In addition, atrazine use has been banned from approximately 2,000 acres of vulnerable farm lands in the lower Wisconsin River Valley [Ciba-Geigy, 1991].

Europe

- The sale and use of atrazine was banned in Italy in 1990. Italy plans to retest atrazine and eventually adopt a restricted use policy for this pesticide [Farmers Weekly, 1990].

- The agricultural use of atrazine was banned in Germany in 1991 [European Chemical News, 1991].

E. Exposure Recommendations

- The current ACGIH threshold limit value-time weighted average (TLV-TWA) for atrazine is 5 mg/m^3 averaged over an 8-hour work shift [ACGIH, 1991].

- NIOSH has not recommended an expousre limit (REL) for atrazine [NIOSH, 1990].

- The EPA has established an oral reference dose (RfD) of 5E-03 for atrazine based on a no observed effect level (NOEL) of 0.5 mg/kg/day [USEPA, 1992].

- The World Health Organization (WHO) has established a drinking water guideline for atrazine of 2 µg/L. The Organization noted that improvement of agricultural practices to reduce the use of atrazine should be encouraged and that the use of atrazine should be carefully controlled, particularly in ground water catchment areas [Kello, 1989].

V. TOXICOLOGICAL EFFECTS

The data summarized below on the toxic effects of atrazine were obtained from a comprehensive database search (see Appendix I) of the published literature. However, many of the toxicological investigations carried out on this compound were conducted by Ciba-Geigy (for submission to the EPA) and are not available in the published literature (with the exception of 2 studies described in section V.E.2, Reproductive and Teratogenic Effects). Ciba-Geigy studies conducted between 1971 and 1988 have been described in EPA publications including the Drinking Water Criteria Document for Atrazine and the Integrated Risk Information System (IRIS) database. These EPA documents were used as a source of the unpublished Ciba-Geigy data presented in this summary. In addition, although Ciba-Geigy was not able to provide copies of their studies on atrazine, they were able to supply a document entitled Atrazine Update: A Briefing Paper on Atrazine Groundwater Protection and Toxicological Risk Evaluation which was used to supplement the information contained in the EPA documents. According to the EPA, Ciba-Geigy will be filing additional toxicity information on atrazine with the agency in 1992.

Finally, several studies conducted by either Woodard Research Corporation, Hazleton Laboratories, Inc., or Industrial Bio-Test Laboratories were also not available in the published literature. Again, the EPA documents described above were used as sources of this unpublished data.

A. Chemical Disposition

1. Human Data

- ***inhalation, human***

Atrazine metabolism was studied in six men with known atrazine exposure who were engaged in the weeding of railway lines in Switzerland. Their duration of employment in this occupation was not reported. The concentration of atrazine in their breathing zone during an operation involving filling a tank wagon with atrazine was determined using personal sampling pumps. Sampling times varied from 7 to 240 minutes, depending on the specific task. Urine samples were collected from the men after the 8-hour workshift and analyzed for atrazine metabolites by gas chromatography. Pre-shift samples were also collected and analyzed 17-hours after the end of the workshift.

Equal amounts of the metabolites 2-chloro-4-amino-6-(ethylamino)-*s*-triazine and 2-chloro-4,6-diamino-*s*-triazine were detected in the urine; their concentrations were reported by the authors as the sum of the two metabolites. The sum of the two urinary metabolites ranged from 30-110 µmol/l for men exposed to atrazine breathing concentrations ranging from 1.1-4.1 µmol/m^3, respectively. The authors note that the rather high concentrations of 2-chloro-4-amino-6-(ethylamino)-*s*-triazine and 2-chloro-4,6-diamino-*s*-triazine may indicate that inhalation is not the primary route of atrazine

exposure among the 6 men, and that percutaneous absorption could be a more significant route. The two metabolites were not detected in the 17-hour samples. Ikonen *et al.* reported that practically no 2-chloro-4-(isopropylamino)-6-amino-*s*-triazine was detected in the urine (concentration not specified)[Ikonen, *et al.*, 1988].

2. Animal Data

In vivo and *in vitro* studies on the chemical disposition of atrazine in rodent species are described below. In addition, *in vitro* studies pertaining to the metabolism of atrazine in rodent and non-rodent species *(e.g.* sheep, goats, chickens, pigs) have briefly been described. Data concerning the *in vivo* chemical disposition of atrazine in non-rodent species are summarized at the end of this section in Table 7.

- ***oral, rat***

  As reported in an abstract by German authors concerning the metabolism of atrazine, Böhme and Bär isolated and identified 5 rat urinary metabolites of atrazine. These metabolites, all of which contained the 2-chloro group, were identified as the 2 mono-dealkylated metabolites of atrazine, the corresponding carboxylic acid derivatives, and the di-dealkylated derivative (See Figure 1) [Böhme and Bär, 1967] .

- ***oral, rat***

  To study the metabolism of atrazine, fifteen male 3-month-old Wistar rats were divided into 3 groups and given drinking water containing commercial atrazine at the following concentrations for 1 or 3 weeks: 0.1 g/l (0.45 mM), 0.2 g/l (0.9 mM), or 0.5 g/l (2.3 mM). Daily consumption of water was measured and the following ingested doses were calculated: 260, 556 and 1052 µmol/kg after 1 week and 760, 1641, and 3553 µmol/kg after 3 weeks for atrazine drinking water concentrations of 0.1 g/l (0.45 mM), 0.2 g/l (0.9 mM), and 0.5 g/l (2.3 mM), respectively. Twenty-four-hour urine samples were collected from metabolic cages, and atrazine metabolites were analyzed by gas chromatography.

  The only urinary atrazine metabolite detected was 2-chloro-4-amino-6-(ethylamino)-*s*-triazine. Its concentration was linearly related to the administered atrazine concentration at 1 and 3 weeks. At 1 week, this metabolite was detected at concentrations of 7 ± 5, 12 ± 10, and 46 ± 12 µmol/l for atrazine doses of 260, 556 and 1052 µmol/kg, respectively. At week 3, the urinary metabolite was detected at concentrations of 10 ± 4, 21 ± 14, and 53 ± 26 µmol/l for atrazine doses of 760, 1641, and 3553 µmol/kg, respectively. Animal weights were not affected, and no signs of clinical toxicity were observed. The authors concluded that atrazine metabolism is rapid in the rat and that the N-dealkylation metabolic pathway does not predominate [Ikonen, *et al.*, 1988].

- ***oral, rat***

To study the metabolism of atrazine, Bradway and Moseman dosed male Charles River rats (2 per dose group) by gavage with atrazine in peanut oil (1.0 ml suspensions) at concentrations of 0.005, 0.5, 5.0, or 50.0 mg/ml. The doses were repeated twice at 24-hour intervals. Six rats were maintained as controls. Urine was collected from all rats over 24-hour intervals. Following the last dose, urine was collected from animals over 48-hour intervals for 4 days. To analyze the atrazine metabolites, gas chromatography was performed on the silica gel eluates.

2-Chloro-4-amino-6-(isopropylamino)-*s*-triazine was the primary urinary metabolite identified. In rats administered 50 mg atrazine, this metabolite was detected at concentrations of 34.4 ppm (day 1), 32.0 ppm (day 2), and 156.0 ppm (day 3). In the 5 mg dose group, 2-chloro-4-amino-6-(isopropylamino)-*s*-triazine was detected at concentrations of 0.40 (day 1), 0.43 (day 2), and 0.94 ppm (day 3). A second metabolite, 2-chloro-4-amino-6-(ethylamino)-*s*-triazine, was detected in lesser amounts in urine of rats administered 50 mg/ml (9.3 ppm (day 1), 7.6 ppm (day 2), and 36.2 ppm (day 3)) and 5.0 mg/ml (0.28 ppm (day 1), 0.11 ppm (day 2), and 0.20 ppm (day 3)). Neither metabolite was detected in urine from rats in the low dose group. Only a small percentage of the administered atrazine was excreted as either metabolite. The percentage of administered atrazine excreted as 2-chloro-4-amino-6-(isopropylamino)-*s*-triazine and 2-chloro-4-amino-6-(ethylamino)-*s*-triazine was 3.67 and 0.27% for the 50.0 and 5.0 mg/ml dose groups, respectively.

The urine extracts all contained another peak which was not present in the urine of the control animals; it was identified as 2-chloro-4,6-diamino-*s*-triazine. To identify this metabolite, it was necessary to repeat the first study in order to obtain fresh urine. In the second study, 2 rats per dose group were gavaged with 0.005, .05, 0.5, and 5.0 mg atrazine in peanut oil. 2-Chloro-4,6-diamino-*s*-triazine was found to be excreted at detectable levels in rats exposed to 5.0 mg atrazine (15.9 ppm {day 1} and 28.8 ppm {day 3}) and 0.5 mg atrazine (0.43 ppm {day 1} and 0.858 ppm {day 3}). (No data from day 2 were obtained.) No detectable levels were found in the urine of rats in the 2 lower dose groups. The percentage of administered atrazine excreted as this third metabolite was 3.18% (day 1) and 31.9% (day 3) for the rats administered 5.0 mg atrazine, and 2.89% (day 1) and 4.29% (day 3) for the rats in the 0.5 mg dose group [Bradway and Moseman, 1982].

- ***oral, rat***

To study the distribution, excretion, and metabolism of atrazine, Bakke *et al.*, dosed rats by stomach tube with ethanolic acid solutions of ^{14}C-ring-labeled-atrazine. Fourteen rats of unspecified strain were administered 0.53 mg of ^{14}C-atrazine containing 3.54 µCi of ^{14}C. Two of these rats were housed in glass metabolism cages from which carbon dioxide was collected. Their urine and feces were also collected daily. At the end of 72-hours, the 2 rats were sacrificed and their total body radioactivity was determined. From the remaining 12 rats, urine and feces were collected daily and tissues were taken for analysis of radioactivity after sacrifice at 2, 4, or 8 days (4 rats sacrificed per time point). Eight additional rats were administered 5.5 mg of ^{14}C-atrazine containing 0.5 µCi of ^{14}C and their urine and feces were collected daily. Metabolites were isolated from urine and feces by gas chromatography.

Distribution: At sacrifice, 3 days after dosing, the carcasses from the rats administered 0.53 mg of ^{14}C-atrazine contained 15.8% of the administered dose. Analysis for ^{14}C-atrazine from various tissues revealed that the fat and muscle had lower residues than other tissues examined, with the liver, kidney, and lung containing higher residues. The liver, kidney, and digestive tract displayed the greatest decrease in residues with time (See Table 2).

Excretion: Rats administered 0.53 mg of ^{14}C-atrazine excreted the radioactivity mainly in the urine (65.5% in 72 hours). Eighty-five to 95% of the atrazine excreted in the urine appeared during the first 24-hours after dosing. The feces contained 20.3% of the administered dose, and less than 0.1% of the ^{14}C-atrazine appeared as ^{14}C-carbon dioxide in the expired air.

Metabolism: At least 19 radioactive compounds were detected by ion-exchange chromatography, gas chromatography, and mass spectrometry in the urine of animals administered 0.53 mg and 5.5 mg ^{14}C-atrazine. Four metabolites (constituting 47% of the urinary radioactivity) were identified: 2-hydroxyatrazine, 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine, 2-hydroxy-4-amino-6-ethylamino-*s*-triazine, and ammeline. Two other metabolites were characterized based on the mass spectra obtained from their silyl derivatives. Structures of the 4 primary metabolites and the 2 additional metabolites are presented in Figure 2.

In contrast to the results reported above by Böhme and Bär, 1967, none of the rat urinary metabolites isolated in this study by ion exchange chromatography contained the 2-chloro moiety. Bakke *et al.*, report that the atrazine metabolites identified, or characterized, represent N-dealkylation intermediates leading to ammeline and/or 2-chloro-4,6-diamino-*s*-triazine. Either, or both, of these compounds probably represent the terminal rat metabolites resulting from ingestion of 2-chlorotriazine herbicides. Bakke *et al.* state that the lack of ^{14}C-carbon dioxide in the expired air from rats given ring-labelled atrazine indicates that the triazine ring was not metabolized by the rat [Bakke *et al.*, 1972].

- ***oral, rat***

  The excretion and metabolism of ^{14}C-atrazine was studied by Ciba-Geigy (unpublished data, 1988) in rats of unspecified strain. Three groups of rats (5 males and 5 females/group) were administered a single oral dose of atrazine at a concentration of 1 mg/kg or 100 mg/kg, or were administered 1 mg/kg atrazine following a 14-day dosing period with nonlabeled atrazine at the same concentration (1 mg/kg/day). The elimination of radiolabel in urine and feces was monitored over a 7-day period.

  Excretion: In the 3 test groups, approximately 74% and 19% of the administered dose was eliminated in the urine and feces, respectively, within 7 days after administration of the ^{14}C-atrazine. No sex-, treatment-, or pretreatment-related differences were observed, and consistent urine:feces excretion ratios over the 1 - 100 mg/kg body weight dosage range suggested that absorption of the administered ^{14}C-atrazine was complete.

  Metabolism: The results of this study supported a metabolic pathway dominated by oxidative removal of the alkyl side chains, with 2-chloro-4,6-diamino-*s*-triazine being the major metabolite. The carbon-chlorine bond on the second carbon was stable to enzymatic hydrolysis but was subject to conjugation via the action of glutathione-S-transferase. Sulfur-containing metabolites were reportedly converted to 2-sulfhydryl-*s*-triazines, which in turn were subject to methylation followed by oxidation to the corresponding S-oxides. Oxidation of primary positions of the alkyl side chains to carboxyl function appeared to be a minor alternative metabolic route[USEPA, 1990].

- ***oral, rat***

  Timchalk *et al.*, studied the effect of tridiphane on the distribution, excretion, and metabolism of ^{14}C-atrazine.[5] Four cannulated male Fischer rats were administered, by gavage, a single oral dose of corn oil (vehicle) and approximately 2 hours later, were orally administered ^{14}C-atrazine at a target dose of 30 mg/kg. Samples of blood were collected via the indwelling jugular vein cannula at 4, 8, 10, 12, 24, 36, 48, and 72 hours post-dosing and analyzed for radioactivity. Released ^{14}C-carbon dioxide was trapped and also analyzed for radioactivity. All urine voided during successive 12-hour intervals through 24-hours post-dosing and at 24-hour intervals thereafter was analyzed. Tentative identification and quantification of metabolites in 0-24 hour urine samples was carried out by HPLC. Feces collected at 24-hour intervals were also analyzed for radioactivity. Rats were sacrificed 72-hours after dosing. Their skin was

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[5]Tridiphane is the active ingredient in TANDEM® herbicide. It is usually applied to weeds as a mixture with atrazine to enhance its herbicidal effect. In this study, no significant differences were found in the absorption, distribution, metabolism, and excretion between rats administered only ^{14}C-atrazine and those administered both tridiphane and ^{14}C-atrazine, and the authors concluded that tridiphane has no meaningful effect on the pharmacokinetics and/or metabolism of atrazine in the rat. In this summary, only the results for atrazine administration alone have been described.

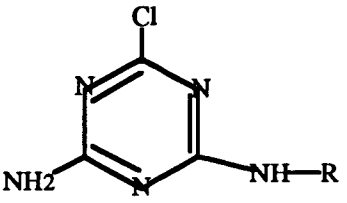
removed and weighed, and carcasses were homogenized and weighed. A representative sample of skin was removed from the back neck region of each rat and was oxidized directly. The ^{14}C-carbon dioxide released on oxidation was trapped and quantified. A one-compartment pharmacokinetic model was used to describe the time-course of radioactivity in the plasma of the rats.

Distribution: The distribution of recovered ^{14}C-atrazine is summarized in Table 3. The ^{14}C plasma time-course data exhibited a mono-exponential decrease in radioactivity and can be described by a one-compartment pharmacokinetic model (see Table 4). The authors noted that their data are similar to the results reported by Bakke *et al.*, concerning the distribution of atrazine.

Excretion: The urine was found to be the primary route of excretion accounting for approximately 66% of the administered dose. The 0-12 and 12-24-hour urine collection intervals accounted for approximately 37 and 20% of the administered dose, respectively; the 24-48 and 48-72-hour intervals represented approximately 7 and 1% of the radioactivity, respectively. The feces, on the other hand, accounted for only 19% of the administered dose. The greatest amount of radioactivity was detected in the feces during the 0-24-hour collection interval (14% of the administered dose). Timchalk *et al.*, note that these results also compare well with those reported by Bakke *et al.*

Metabolism: Metabolites were identified by HPLC in 0-24-hour urine extracts. The majority (67%) of the radioactivity eluted from the HPLC in 5.7 minutes and was identified as 2-chloro-4,6-diamino-1,3,5-triazine. A peak eluting at 15.3 minutes and accounting for 5% of the radioactivity was found to be only 10% 2-chloro-4-amino-6-methylethylamino-1,3,5-triazine; attempts to identify the remaining 90% of the radioactivity collected in this fraction were unsuccessful. The HPLC fraction eluting at 12.7 minutes (<1.0% of total urinary ^{14}C-atrazine) was determined to be 2-chloro-4-amino-6-ethylamino-1,3,5-triazine. Other metabolites detected included *s*-(2,4-diamino-1,3,5-triazin-6-yl)-mercapturic acid, which eluted at 6.7 minutes (9.0 % of total urinary ^{14}C-atrazine), and *s*-(2-amino-4-methylethylamino-1,3,5-triazin-6-yl)-mercapturic acid, which eluted at 14.2 minutes (13.2 % of total urinary ^{14}C-atrazine). Two remaining mercapturic acids in the HPLC fractions could not be identified [Timchalk *et al.*, 1990].

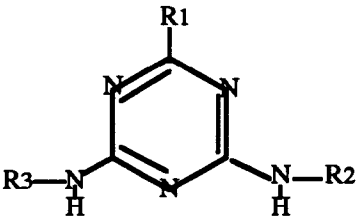
Figure 1. Atrazine Metabolites Isolated from Rat Urine



Metabolite	R
1	H
2	CH_2H_5
3	iso-C_3H_7
4	CH_2COOH
5	$CH(CH_3)COOH$

Reference: Böhme and Bär, 1967

Figure 2. Atrazine Metabolites Identified in Rat Urine



Metabolite	R1	R2	R3
2-hydroxyatrazine	HO	CH_3CH_2	$(CH_3)_2CH$
ammeline	CH_3O	CH_3CH_2	CH_3CH_2-CH-CH_3
2-hydroxy-4-amino-6-isopropylamino-*s*-triazine	HO	H	$(CH_3)_2CH$
2-hydroxy-4-amino-6-ethylamino-*s*-triazine	HO	CH_3CH_2	H
Proposed structure #1 (fraction 7)	HO	H	HOOC-CH
Proposed structure(s) #2 (fraction 12)	HO	CH_3CH_2	CH_3-CH-COOH
	HO	$HOOCCH_2$	$(CH_3)_2CH$

Reference: Bakke *et al.*, 1972

Table 2. Radioactivity in Rat Tissues After a Single Dose of ^{14}C-Labelled Atrazine

	Tissue Residues, ppm of Atrazine-^{14}C equivalent (freeze-dried tissue basis) [a]							
Time of Sacrifice (days)	Liver	Brain	Heart	Lung	Kidney	Digestive Tract	Omental Fat	Leg Muscle
2	3.5	1.6	2.5	3.0	4.0	5.8	0.5	0.6
4	2.6	1.4	1.6	2.3	2.3	0.9	0.1	0.5
8	1.7	1.1	1.4	2.0	1.7	0.9	0.1	0.5

[a] Average of tissues from four rats.

Reference: Bakke *et al.*, 1972

Table 3. Distribution of Radioactviity Recovered 72 Hours After Oral Administration of 30 mg ^{14}C-Atrazine/kg Body Weight to Male Fischer 344 Rats

	Percent of Administered ^{14}C-Atrazine [a]
Urine	65.83 ±1 .39
Feces	18.69 ± 1.76
Skin	1.48 ± 0.37
Carcass	3.95 ± 0.23
Cage wash	0.45 ± 0.21
Plasma [b]	0.08 ± 0.0
RBC [b]	1.24 ± 0.15
Total	91.72 ± 1.60

[a] Values represent the mean ± s.d. for 4 rats/group.
[b] Values represent the total radioactivity collected in these tissues throughout a 72-hour interval.

Table 4. Parameters For 1-Compartment Model That Best Describes the Time Course of ^{14}C-Atrazine in the Plasma of Male Fischer Rats

Parameters [a]	Units	^{14}C-Atrazine
Absorption (Ka)	h^{-1}	0.270
Half-life of absorption (t1/2)	h	2.6
Elimination (Ke)	h^{-1}	0.064
Half-life of elimination (t1/2)	h	10.8
Volume of distribution (V)	ml/kg	108.9
Area under plasma curve (AUC)	$\mu g\ ml^{-1} h^{-1}$	200.57

[a]The data sets were optimized by varying Ka, Ke, and V for each treatment group independently.

Reference: Timchalk *et al.*, 1990

- ***oral, rat:***

Gojmerac and Kniewald studied atrazine metabolism and distribution at the subcellular level following oral administration in male Fischer rats. Ten rats per dose group were treated orally by gavage with 15 or 30 mg atrazine (6 and 12 mg/100 kg body weight, respectively) in 0.5 ml paraffin oil daily for 7 days. An unspecified number of control animals received the same volume of paraffin oil only. Rats were sacrificed 24-hours after receiving the last atrazine dose, and tissue samples (liver, kidney, brain) were collected and freeze-dried. Freeze dried tissues were pooled from each group, homogenized, and extracted with methanol. Dried extract was dissolved in chloroform and portions were transferred to an acidic alumina column. The column was eluted with chloroform (eluate 1) followed by methanol (eluate 2). Eluate 1 was concentrated and analyzed by gas chromatography-mass spectrometry; eluate 2 was concentrated and combined with an excess of diazomethane (used to methylate hydroxy analogues) prior to gas chromatography.

Metabolism: The following metabolites were detected from eluates 1 and 2: 2-chloro-4-amino-6-isopropylamino-*s*-triazine (deethyl atrazine), 2-chloro-4-ethylamino-6-amino-*s*-triazine (deisopropylatrazine); 2-methoxy-4-ethylamino-6-isopropylamino-*s*-triazine (hydroxy atrazine), 2-methoxy-4-amino-6-isopropylamino-*s*-triazine (hydroxy deethyl atrazine), and 2-methoxy-4,6-diamino-*s*-triazine (hydroxy diamino atrazine). (The latter three compounds were prepared by methylation of the corresponding hydroxy analogues with diazomethane.)

Distribution: The residues of atrazine and its five metabolites detected in the tissues of the liver, kidney, and brain are presented in Table 5. The authors note that the recoveries of hydroxyatrazine were low due to poor efficiency of the methylation (approximately 30-50%), and that since a considerable variation occurred in the recoveries of the hydroxy analogues, the data reported for the residues of atrazine hydroxy metabolites should only be regarded as qualitative.

According to Gojmerac and Kniewald, the data suggest that atrazine and the dealkylated metabolites of atrazine, are primarily present in the liver, kidney, and brain of atrazine-treated rats, with the highest concentrations being found in the kidney. They conclude that partial and total N-dealkylation of lateral alkylamino groups in positions 4 and 6 and hydroxylation in position 2 of the *s*-triazine ring is, based on their results, the established metabolic pathway for atrazine in the rat [Gojmerac and Kniewald, 1989].

Table 5. Residues of Atrazine and its Metabolites in the Male Rat Following Oral Administration

			Residues (ppm)[a]					
Atrazine (mg/ 100 g bw)	Tissue	Wet wt (g)	Atrazine	Deethyl atrazine	Deiso-propyl atrazine	Hydroxy atrazine	Hydroxy deethyl atrazine	Hydroxy diamino atrazine
6	liver	70.00	2.30	ND	4.70	0.22	ND	0.24
6	kidney	25.00	4.00	6.20	2.40	ND	ND	ND
6	brain	18.20	0.87	2.08	ND	ND	ND	ND
12	liver	70.30	3.10	ND	4.80	0.75	ND	0.40
12	kidney	24.50	7.50	9.80	5.10	ND	0.18	ND
12	brain	19.70	1.67	2.73	ND	ND	ND	ND

[a]Calculated on a wet weight basis

Reference: Gojmerac and Kniewald, 1989

- ***oral, rat***

The distribution of atrazine was studied by Ciba-Geigy (unpublished data, 1988) in a subacute exposure study. Six groups of two female Sprague-Dawley Charles River CD albino rats were dosed daily with ^{14}C-atrazine for 10 consecutive days at a concentration of 1, 3, 7, 10, 50, or 100 mg/kg. In all dose groups, the highest concentrations of radiolabel (0.6% or less of the administered dose) were found in the red blood cells and liver; other tissues contained less than 0.25% of the total dose. Both total mammary (pectoral and inguinal) concentrations and total tissue (mammary, pituitary, liver, ovary, brain, and kidney) concentrations declined exponentially following cessation of dosing. Tissue concentrations 3 hours following the tenth dose were linearly related to plasma concentrations obtained at the same time point in all dose groups. Tissue concentrations 72 hours after the tenth dose were also significantly lower (27% {P value not specified}) and linearly related to plasma concentrations. Comparison of tissue concentrations 3 hours and 72 hours after cessation of dosing indicated a longer residence time for radioactivity in tissues other than plasma. The whole-body half-life was approximately 1.5 days.

Based on plasma concentrations, the apparent volume of body distribution was 4.15 l/kg body weight. The apparent volume of body distribution for red blood cells (0.70 l/kg) supported the existence of extensive covalent binding to red blood cells with a half-life of 5-8 days, consistent with the lifespan of rat red blood cells (45-56 days). A steady-state red blood cell concentration was estimated to be achieved only after 30 days of daily dosing. It was concluded that atrazine and/or its metabolites appear to bind to red blood cells, but that accumulation in other tissues doses not appear to occur [USEPA, 1990].

- ***dermal, rat***

In a dermal absorption and distribution study performed by Ciba-Geigy (unpublished data, 1983a), an unspecified number of male and female Harlan Sprague-Dawley rats were treated dermally with ^{14}C-atrazine (specific activity of 17.2 µCi/mg). ^{14}C-atrazine (dissolved in tetrahydrofuran) was applied to a 1.5 cm^2 area of the shaved skin at a total volume of 15 to 20 µg/L, for a total administered dose of 0.25 mg/kg atrazine. To estimate tissue distribution, 4 males and 4 females were sacrificed at 2, 4, 8, 24, or 48 hours after compound administration.

Absorption: Approximately 70% of the applied dose was absorbed in 72 hours, resulting in a half-life absorption of approximately 41 hours for both sexes.

Distribution: The highest levels of radioactivity were detected in the liver and muscle at all time points examined, with the radioactivity reaching a peak of 2.1% in muscle and 0.5% in liver at the 8-hour time point [USEPA, 1990].

- ***dermal, rat***

Ciba-Geigy (unpublished data, 1983b) studied the excretion rate of ^{14}C-atrazine in an unspecified number of male and female Harlan Sprague-Dawley rats that were dermally administered atrazine (dissolved in tetrahydrofuran) at concentrations of 0.025, 0.25, 2.5, or 5.0 mg/kg. Urine and feces were collected from all animals at 24-hour intervals for 144 hours. At all dose levels tested, the majority of the radioactivity was excreted in the urine within 48 hours after compound administration, with excretion ranging from 37% at the lowest dose to 59% at the highest dose. Fecal excretion was approximately 15% at the lowest dose and between 20 and 21% at all other dose levels. Combined cumulative excretion in 144 hours for urine and feces was 52% at the lowest dose and 70 to 80% at all other doses [USEPA, 1990].

- ***dermal, rat***

The percutaneous absorption of ring-labelled ^{14}C-atrazine having a specific activity of 3.71 mCi/mM was studied in young (33 days old) and adult (82 days old) female Fischer 344 rats at the following 3 dose levels: 0.25 µmol/cm^2 (low), 0.536 µmol/cm^2 (medium), and 2.679 µmol/cm^2 (high). ^{14}C-atrazine solutions in acetone were applied to clipped mid-dorsal skin with treatment areas ranging from 2-3% of the body surface area for both young and adult rats. Following treatment, areas were protected

with a perforated plastic blister. Seventy-two hours following dermal application, treated animals were sacrificed, and skin, urine, feces, carcasses, and blisters were analyzed to determine compound absorption and recovery of radioactivity. Comparison of the skin penetration between young and adult rats was made at each dose level using 3 animals per group. Fractional absorption was calculated by dividing the radioactivity in the body and excreta by the total radioactivity recovered.

Atrazine exhibited a greater absorbtion in the young rats. In adult rats, penetration in the low, medium, and high dose groups was 7.7%, 4.5%, and 2.7%, respectively; in young rats, penetration in the three different dose groups was 9.6%, 6.7%, and 3.2%, respectively. The ratio of fractional penetration in young and adult rats was found to be statistically significant ($P \leq 0.05\%$) at the low and medium dose levels. The 72-hour dermal penetration for young rats was greater than that of the adults by 38% at the low dose level and by 49% at the medium dose level. The authors also noted that atrazine displayed decreasing absorption with increasing dose [Shah *et al.*, 1987; Hall *et al.*, 1988].

- ***in vitro, rat***

Dauterman and Muecke studied the *in vitro* metabolism of atrazine using liver subcellular fractions (10,800g supernatant, 100,000g supernatant, and microsomes) obtained from male RAI rats. The incubation mixture consisted of 800 mg of liver equivalents (wet weight), 0.42 µmoles (89.99 g) of ^{14}C-atrazine, 4.4 µmoles of nicotinamide-adenine dinucleotide phosphate (NADPH) or nicotinamide-adenine dinucleotide (NADH), or 8 µmoles of glutathione (GSH), and 480 µmoles of phosphate buffer in a final volume of 8 ml. The reaction was terminated after two hours by the addition of chloroform, and radioactivity was determined in each phase following centrifugation. Atrazine metabolites were analyzed by chromatography with known standards.

The following *in vitro* metabolites were identified: 2-chloro-4,6-bisamino-*s*-triazine; 2-chloro-4-amino-6-isopropylamino-*s*-triazine; and 2-chloro-4-amino-6-ethylamino-*s*-triazine. With the addition of NADPH, the dealkylation reaction proceeded at a faster rate than the conjugation reaction. With the 10,800g supernatant, the dealkylation reaction was more than 3 times faster than the conjugation reaction. The authors also noted that the endogenous NADPH and GSH were sufficient to dealkylate as well as conjugate a portion of the atrazine added. When NADPH was added to the 10,000g supernatant, only a trace of the atrazine conjugate (<0.1%), and no atrazine, was found. All of the metabolites were first formed by dealkylation and then a portion of them were conjugated with GSH. Dauterman and Muecke report that these findings indicate that the dealkylation reaction predominated over the conjugation reaction. They state that, based on the ratio of the metabolites observed, the dealkylation of the isopropyl group proceeds more readily than dealkylation of the ethyl group. Upon addition of NADPH to the 10,000g supernatant and the microsomal fraction, the

second alkyl group was also degraded (forming the metabolite 2-chloro-4,6-bisamino-*s*-triazine). In the presence of NADPH and GSH, all of the chlorotriazine metabolites of atrazine except 2-chloro-4,6-bisamino-*s*-triazine, formed GS-conjugates. The authors commented that there was no evidence that atrazine was dechlorinated enzymatically to form a hydroxytriazine [Dauterman and Muecke, 1974].

- ***in vitro, rat/mouse***

Adams *et al.* performed Phase I and Phase II *in vitro* studies on the metabolism of atrazine. For phase I investigations, 30-minute incubations contained 1.0 mg/l microsomes (male Sprague-Dawley rat or male ICR mouse), 50-100 µM ^{14}C-atrazine, an NADPH generating system, and potassium phosphate buffer. Heat-treated microsomes were used to assess non-enzymatic oxidations. Parent compound and metabolites were extracted into chloroform and analyzed by thin layer chromatography.

The primary atrazine metabolites in the SD rat and ICR mouse were the two dealkylation products produced by the loss of either the ethyl group (2-chloro-4-amino-6-(isopropylamino)-*s*-triazine) or the isopropyl group (2-chloro-4-amino-6-(ethylamino)-*s*-triazine). Using rat microsomes, the deisopropylated product was formed in greater quantity (3:1) than the deethylated product (as had been shown by previous investigators {Dauterman and Muecke, 1974; Bohme and Bar, 1967}). With mouse hepatic microsomes, however, the ration of deisopropylated to deethylated product was 1:1.

With both rat and mouse microsomes, 2-chloro-4,6-bisamino-*s*-triazine, a metabolite having neither an ethyl group nor an isopropyl group, was detected at low concentrations that could not be quantified accurately. No dechlorinated or ring cleavage products were detected with either rat or mouse preparations. In addition, no metabolites were found when the NADPH was omitted, suggesting that P-450, which requires NADPH as a cofactor, might be the metabolizing enzyme. Addition of NADH to the complete *in vitro* systems did not increase the rate of metabolism, suggesting that cytochrome b5, which requires NADH, does not contribute to the microsomal metabolism of atrazine.

The Phase I study was repeated using female Fischer rats and female Sprague-Dawley rats. As part of the phase I study, time course experiments using 5 mg of S-10 protein/ml over 30 minutes (5, 10, 15, 20, and 30-minute sampling times) were included. In addition, a Phase II study was conducted. For the Phase II study, a similar incubation system to the Phase 1 system was employed with the following changes: (1) the supernatant after 1000g centrifugation (S10) was used rather than microsomes; (2) the reaction mixture was supplemented with glutathione; (3) S-10 preparations were used at a higher concentration (~ 5.0 mg of protein/ml); and (4) the reaction was incubated for two hours.

Both strains produced the same atrazine metabolites, the deisopropyl product (2-chloro-4-amino-6-(isopropylamino)-*s*-triazine) and the deethyl product (2-chloro-4-amino-6-(ethylamino)-*s*-triazine). Time course experiments showed that both strains gave equivalent amounts of metabolites at all time points sampled. If rates of phase I metabolism were compared on the basis of microsomal P-450 content, Fischer and Sprague Dawley rats had roughly equivalent rates. However, if comparisons were made on the basis of liver weight or body weight, rates of metabolism were higher in the Fischer strain (The apparent Km was 27.5 μM for Sprague-Dawley and 27.8 μM for Fischer rat hepatic microsomes).

Conjugation rates for atrazine and its metabolites with glutathione (phase II metabolism) were the same in both strains of rats ($P \geq 0.05$), normalized for S-10 content. Increasing the levels of glutathione and/or NADPH in the reaction mixture did not increase the phase II reaction rate. The predominant phase II conjugation product was with atrazine rather than with the dealkylation products.

The authors concluded that Phase I metabolism of atrazine is P-450 mediated and that phase II metabolism proceeds via glutathione conjugation of atrazine, and, to a lesser extent, via the glutathione conjugation of the monodealkylated products. Subsequent induction studies performed with phenobarbital and 3-methylcholanthrene (known inducers of specific isoenzymes of P-450 having substrate affinities), in Sprague Dawley and Fischer rats suggest that metabolism can by catalyzed by several different isoenzymes of P-450 and that the reactions are qualitatively and quantitatively similar [Adams *et al.*, 1990].

- ***in vitro, rat/mouse/rabbit/sheep/goat/chicken/pig***

As part of the investigation described above, Adams *et al.*, studied species-related differences in Phase I *in vitro* atrazine metabolism. A series of incubations were carried out using ^{14}C-atrazine with hepatic microsomal preparations from various species of animals using the experimental conditions described above. Results, based on quantities of metabolites produced in 30 minutes, are presented in Table 6. From the data, Adams *et al.*, concluded that there are considerable differences in the rates of metabolism, both within and among species, but when the difficulty of detecting low levels of metabolites (*e.g.*,3%) is taken into consideration, there are striking qualitative similarities in the types of metabolites produced. This suggests a common pathway for the metabolism of atrazine in these species [Adams *et al.*, 1990].

- ***in vitro, chicken/goose/pig/sheep***

Several *in vitro* investigations by Foster and Khan concerning atrazine metabolism (not described in full in this summary), showed that the soluble fraction (105,000g) from chicken liver homogenate contains a heat-labile glutathione-dependent enzyme(s), which metabolizes atrazine in *in vitro* incubations. This was accomplished by conjugation with glutathione and subsequent hydrolysis with partial N-dealkylation to the hydroxy and dealkylated analogues. The major metabolic pathway was shown to proceed via enzymatic hydrolysis [Foster *et al.*, 1979 as described in

Foster *et al.*, 1980]. Similar studies by these authors using enzyme preparations from goose, pig, and sheep liver homogenates indicated that *in vitro* metabolism of a mixture of atrazine and simazine (2-chloro-4,6-bis(ethyl-amino)-*s*-triazine) proceeded via partial N-dealkylation accompanied by hydrolysis. However, hydrolysis to the corresponding hydroxy analogues appeared to be slower than partial N-dealkylation [Kahn *et al.*, 1979]. To ascertain whether, in the *in vitro* metabolism of atrazine by the enzyme preparation, the formation of 2-hydroxy partially N-dealkylated metabolites occurs by the hydrolysis of the respective 2-chloro analogues, or by the partial N-dealkylation of hydroxyatrazine, Foster *et al.*, incubated deethylatrazine (2-chloro-4-amino-6-(isopropylamino)-*s*-triazine) and deisopropylatrazine (2-chloro-4-(ethylamino)-6-amino-*s*-triazine) with the soluble fraction from goose liver homogenates. The incubation resulted in the formation of the corresponding hydroxy analogues. No dealkylation of hydroxyatrazine (2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine) occurred when it was incubated with the enzyme preparation. The authors report that these data suggest that, in the metabolism of atrazine by the soluble fraction from liver homogenate, the formation of 2-hydroxy partially N-dealkylated metabolites occurs by the hydrolysis of the respective 2-chloro analogues rather than by partial N-dealkylation of hydroxyatrazine [Foster *et al.*, 1980].

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Table 7. Chemical Disposition of Atrazine in Pigs, Cows, and Hens

Species/Strain/No.	Atrazine Administration	Experimental Design	Excretion	Metabolism	Distribution (Tissue Atrazine Levels)	Reference
Mini-pig/Pittman Moore/ NS (35-50 lbs)	gavage: 0.1 g in ethanol	urine samples collected, extracted, GC analysis	Atrazine and metabolites identified in urine:within 24 h	principal metabolite: deethyl atrazine	---	Erickson *et a* 1979
Cow/NS/NS	oral: case report-accidental consumption of AAtrex 80w (76% atrazine)	liver and kidney samples extracted, GC analysis	---	---	liver: 75-79 ppm kidney: 53-67 ppm	Jowett *et al.*, 1986
Hens/Single Comb White Leghorn /6	oral: dietary administration-100 ppm for 7 days in standard laying ration [a]	urine and eggs collected, extracted, GC analysis	Atrazine and metabolites identified in urine within 24 h; urine residues detected up to day 4 after chickens returned to non-fortified ration.	deethylated atrazine[b], hydroxyatrazine,[c] possibly deethylated hydroxy atrazine[d] (urine); route: partial N-dealkylation and hydrolysis	No residues of atrazine or metabolites detected in egg samples	Foster and Khan, 1976
Hens/Single Comb White Leghorn/6	oral: dietary administration- 100 ppm for 7 days in a standard laying ration [a]	tissues extracted, GC analysis	---	deethylated atrazine[b], hydroxyatrazine,[c] deethylated hydroxy atrazine[d] (tissues); route: partial N-dealkylation and hydrolysis	Atrazine: fat, leg muscle; hydroxyatrazine, deethylated hydroxy atrazine: liver,[e] kidney, gizzard, leg muscles; deethylated atrazine: gizzard, intestine, leg/breast muscle, fat[f]	Kahn and Foster, 1976

[a] followed by non-fortified ration for 7 days
[b] deethylatrazine = (2-chloro-4-amino-6-isopropyl-amino-s-triazine)
[c] hydroxyatrazine = (2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine)
[d] deethylated hydroxyatrazine = (2-hydroxy-4-amino-6-isopropylamino-s-triazine)
[e] liver had highest concentration of hydroxyatrazine (16.2 ppm) and deethyl hydroxyatrazine.
[f] relatively high concentration of unchanged atrazine found in fat (38.8 ppm)

B. Acute/Subacute

1. Human Data

- ***dermal, human***

A case report concerning herbicide-induced contact dermatitis has been reported by Schlicher and Beat, 1972. A 40-year-old farmer with a history of dermatitis induced by Ramrod® (2-chloro-N-isopropyl acetanilide), developed acute contact dermatitis of his hands and forearms after applying atrazine in the morning and cleaning clogged nozzles on the applicator without the use of protective equipment. In addition, the subject applied another herbicide, Bladex® (2-(4-chloro-6-ethylamino-*s*-triazine-2-ylamino)-2-methylpropionitrile) for 3 hours in the afternoon. After spraying with atrazine and Bladex®, blisters began forming on his hands and forearms. The condition became so severe that he sought medical assistance and upon examination, his hands were painful, swollen, red, and blistered. Hemorrhagic bullae were seen between the fingers. There were no other significant physical findings. Nine hours later his hands were more ecchymotic, more vesicles were present, and the condition had become incapacitating. He was treated with codeine, prednisone, and tetracycline. Within 4 days the pain was gone, and the swelling was slightly reduced. Ninety percent recovery of his hands and forearms was achieved in 28 days. Several months later, a patch test was done which included a 1:1000 dilution of a commercial atrazine formulation. Within 48 hours, the reaction was strongly positive with clusters of tiny vesicles on an erythematous base. The authors comment that it would be illogical to label as "atypical" what appears to be the only reported case of atrazine-induced dermatitis. They report that there is no doubt that the dermatitis was caused by atrazine; however, the fact that the farmer had a prior history of vesicular dermatitis in response to Ramrod®, and that one of these instances involved a granular formation, suggests a very unusual susceptibility to amides, and even in the absence of any testing, indicates that ancillary materials in the atrazine spray did not contribute significantly to the dermatitis [Schlicher and Beat, 1972].

- ***dermal, human***

A pesticide/herbicide series of 36 substances, including atrazine, were patch tested in patients from Italy admitted for dermatitis and non-allergic skin disorders. Atrazine (1% in petrolatum) was tested on 149 agricultural workers, 34 ex-agricultural workers, and 109 subjects employed in "other" occupations. Patch tests were performed on the upper back and evaluated after 48 and 72 hours. Atrazine was not found to induce irritant or allergic reactions in any of the subjects tested [Lisi *et al.* 1987].

2. Animal Data

Acute LD_{50} data for atrazine are summarized in Table 8. Effects observed during rat oral acute lethality studies included central nervous system lesions excitation followed by depression with reduced respiration rate, motor incoordination, clonic and sometimes tonic spasms, and hypothermia. In addition to these effects, bloody discharge from the mouth, nose and throat were observed during an intraperitoneal LD_{50} determination in rats [Gzhetotskii, 1971]. Excitation and subsequent depression were also noted in mice following oral administration [Bashmurin, 1974]. Other studies concerning the acute toxicity of atrazine are described below.

- ***oral, rat***

  As described in an abstract by Romanian authors, the acute toxicity of atrazine was investigated in an unspecified strain of rats by intragastric administration of a single dose of 0.6 or 3 g/kg atrazine.[6] The rats developed pruritus within 10 minutes and lassitude, increased salivation, and enhanced respiration rates at 30 minutes. Six percent of the animals died within 6 hours of compound administration. Animals that survived after 6 hours developed cyanosis and dyspnea 24 hours after administration, which resulted in death in 25% of the remaining rats. Necropsy of the rats that died after 6 hours revealed lung edema with extensive hemorrhagic foci, cardiac dilation, and macroscopic hemorrhages in the liver and spleen. Most of the administered atrazine was found in the stomach and small intestine. Rats that died during the second day after treatment showed distinct signs of hemorrhagic bronchopneumonia. Histological examination revealed leukocytic infiltration in various stages of pneumonia accompanied by hemorrhage in all the parenchymal organs, with the kidneys presenting dystrophic changes of the tubular mucosa. Among surviving rats that were sacrificed 24 hours post-treatment, cerebral edema was observed. Histochemical alterations of the liver of rats sacrificed at an unspecified time point(s) included the occurrence of alkaline phosphatase in the peripheral portion of the lobes and its gradual disappearance from the hepatocellular nuclei, the gradual disappearance of acid phosphatase from the cellular protoplasm and subsequent appearance in the cell nuclei. The authors also report that 3 weeks of subacute adminstration at the same dose levels caused pathomorphological features characteristic of subacute hepatitis and irritation of the bronchial mucosa [Molnar, 1971].

[6]It could not be determined from the abstract if the effects observed following atrazine adminstration were associated only with the highest dose level tested, or with both test concentrations.

- ***oral, rat***

  Because the kidney had been shown by other investigators to be the primary elimination route in rats, Maria *et al.*, studied the effects of atrazine on rat renal function. Male albino rats were divided into 4 experimental groups (n=10/group) and treated with atrazine at concentrations of 100, 200, 400, or 600 mg/kg body weight. Doses were administered daily with gum arabic by oral intubation for a period of 14 days. Control animals received gum arabic only. Urine was collected daily for analysis of sodium, potassium, and chloride levels as well as levels of creatinine and albumin.

  The elimination of sodium, potassium, and chloride was significantly (P value not specified) increased in rats in a dose-dependent manner. This increase in the elimination of electrolytes was attributed to an alteration in the resorption mechanism that occurs in the renal tubules. A significant decrease (P value not specified) in creatinine clearance was observed among atrazine exposed rats (with the exception of the low dose group), compared to controls; this decrease may be attributed to a decrease in the glomular filtration rate. A significant increase (P value not specified) in urine protein levels was also observed in atrazine-exposed rats. These results suggest that lesions are produced on the glomerular membrane causing either an increase in the permeability of the plasma protein or a subsequent decrease in the resorption in renal tubules. Based on these results, the authors conclude that exposure to atrazine may produce alterations in renal function [Maria *et al.*, 1986].

- ***oral, rat***

  In another study by Maria *et al.*, the hepatotoxicity of atrazine was investigated by studying clinical parameters related to hepatic function, and by examining liver slices using electron microscopy. Groups of 10 male adult Wistar albino rats were dosed by gavage with 100, 200, or 400 mg/kg atrazine in gum arabic for 14 days, or 600 mg/kg atrazine for 7 days. Ten control rats received gum arabic only. Following dosing, animals were bled for the determination of serum total lipids, glucose, alanine aminotransferase (ALT), and alkaline phosphatase (SAP). In addition, body weights changes were recorded and livers were removed, weighed, and examined grossly and microscopically.

  Decreases in body weight were observed in all atrazine-treated rats in the 14-day (dose-related) and 7-day (not dose-related) treatment groups. Relative liver weights were significantly ($P<0.005$) increased in the 400 and the 600 mg/kg dose group compared to controls. Atrazine administration also resulted in a dose-dependent decrease in serum glucose concentration in the 100 mg/kg (not significant), 200, 400, and 600 mg/kg dose groups ($P<0.001$). A dose-related significant increase in the level of total serum lipids was observed among rats administered 100 mg/kg ($P<0.01$) and 200, 400, or 600 mg/kg ($P < 0.001$). In addition, a dose-related significant ($P<0.001$)

increase in SAP activity was noted at all dose levels tested. The increase in ALT was less pronounced, but was also determined to be significant at all dose levels tested (100 mg/kg {P<0.025}, 200 mg/kg {P<0.002}, 400 mg/kg {P<0.001}, and 600 mg/kg {P<0.001).

No significant histological changes were observed in the hepatocytes of the rats in the 100 mg/kg group. However, among rats in all other dose groups, electron microscopy revealed hepatocytic proliferation and degeneration of smooth endoplasmic reticulum, lipid accumulation, and alteration of bile canaliculi proportional to dose and duration of treatment. The authors concluded that these results indicate that subacute exposure to atrazine may produce alterations in liver morphology and function, and that more information is necessary to elucidate the mechanism of atrazine toxicity in the liver [Maria *et al.*, 1987].

oral, rabbit

The acute toxicity of Gesaprim® herbicide (containing 80% atrazine as the active ingredient) was studied by Salem *et al.* in Balady rabbits. To determine the LD_{50}, six groups of 10 rabbits of unspecified sex were dosed by gavage with 0, 0.25, 0.5, 1.0, 2.0, or 4.0 grams/kg of Gesaprim®. The mortality rate was recorded after 24 hours and signs of toxicity were noted. In addition, ten rabbits of unspecified sex were dosed by gavage with 3.32 gm/kg of Gesaprim (LD_{50}). Five untreated rabbits served as the control. For hematological analysis, blood samples were taken from the ear vein of the rabbits 12, 24, 48, and 72 hours post-dosing. All rabbits were sacrificed 72-hours after dosing for histopathological examination.

Indications of toxicity observed during the determination of the LD_{50} included conjunctivitis, excessive salivation, sneezing and coughing, general muscular weakness with tremors and trembling, incoordination and weakness of the hind quarters which ended in collapse and inability to rise. Enteritis occurred in the form of bloat and watery diarrhea. Finally, the rabbits became comatose, respiration became weak, and death occurred. Results obtained from the hematological studies revealed a gradual and significant (P<0.05) increase in the percentage of hemoglobin at 48 and 72 hours. In addition, the erythrocyte sedimentation rate gradually increased (not significant) at 24, 48, and 72 hours while the erythrocyte count decreased at 48 (not significant) and 72 (P<0.01) hours. The leucocyte count exhibited a gradual increase at 12, 24, 48, and 72 hours which was significant (P<0.01) only at the latter time point. Atrazine was found to cause a significant increase in serum glutamic-oxoalacetic transaminase at 48 hours (P<0.01) and in serum glutamic-pyruvic transaminase at 72 hours (P<0.01). The blood glucose level showed a significant (P<0.01) increase after 48 hours and total protein level was significantly (P<0.05) increased after 72 hours.

Gross pathological observations included slight congestion of the liver and hemorrhagic kidneys. The lungs were also edematous and had hemorrhagic patches. The intestines were observed to be edematous with excess exudate in their lumen. The spleen was generally congested, slightly enlarged, and showed some petechial

hemorrhages. Histopathological observation revealed hydropic degeneration of the liver, hemorrhagic patches and hyaline casts in the kidneys. Lung edema and bronchitis were also noted. Edema in the intestine and mucous in the intestinal lumen were found, and the spleen displayed dilated blood vessels and some hemorrhagic spots [Salem *et al.*, 1985].

Table 8. Acute LD_{50} and Skin/Eye Irritation Data for Atrazine

Route	Species/Strain	Number per group/Sex	LD50 /LC50 (time)	Reference
oral	rat/NS	NS [b]	1869-3080 mg/kg	Worthing, 1991
oral	rat/Sherman	4/male	737 mg/kg	Gaines and Linder, 1986
oral	rat/Sherman	4/female	672 mg/kg	Gaines and Linder, 1986
oral	rat/Sherman, weanling	4/male	2310 mg/kg	Gaines and Linder, 1986
oral	rat/NS	NS	1400 mg/kg	Gzhetotskii *et al.*, 1977
oral	rat/albino	NS	3000 mg/kg	Bashmurin, 1974
oral	mouse/albino	NS	1750 mg/kg	Bashmurin, 1974
oral	mouse/NS	NS	3000 mg/kg	Ceiba Geigy, 1988 as cited in USEPA, 1990
oral	rabbit/NS	NS	750 mg/kg	RSOC, 1987
oral	rabbit/Balady	10/NS	3320 mg/kg [a]	Salem *et al.*, 1985
oral	hamster/Syrian	NS/male & female	1000 mg/kg	Cabral *et al.*, 1979
inh.[c]	rat/NS	NS	>0.71 mg/L (1h)	Worthing, 1991
inh.	rat/NS	NS	>4.9 mg/L (NS)	Clayton and Clayton, 1981
ip.[d]	rat/NS	NS	125 mg/kg	Gzhetotskii *et al.*, 1977
dermal	rat/NS	NS	>3100	Worthing, 1991
dermal	rat/Sherman	4/male	>2500 mg/kg	Gaines and Linder, 1986
dermal	rat/Sherman	4/female	>2500 mg/kg	Gaines and Linder, 1986
dermal	rabbit/NS	NS	7500 mg/kg	RSOC, 1987
dermal	rabbit/NS	NS	38 mg (mild irritation)	Ciba-Geigy, 1977 as cited in RTECS, 1991
eye	rabbit/NS	NS	6320 ug (severe irritation)	Ciba-Geigy, 1977 as cited in RTECS, 1991

[a] Based on intubation with Gesaprim® herbicide which contains 80% atrazine.
[b] NR = Not reported
[c] inh. = inhalation
[d] ip. = intraperitoneal

C. Prechronic

1. Human Data/Case reports

No data were found.

2. Animal Data

No data were found.

D. Chronic/Carcinogenicity

Atrazine was previously listed as a Class C carcinogen (possible human carcinogen) by the EPA. This compound is is currently being re-evaluated for evidence of human carcinogenic potential by an Environmental Protection Agency (EPA) inter-office Agency work group [USEPA, 1992b]. Additional data concerning the carcinogenicity of this compound is scheduled to be submitted to the EPA by Ciba-Geigy in 1992.

1. Epidemiological studies

- Long *et al.*, published a preliminary report from a cohort study which was launched in Johnson County, Iowa in 1966. The study had 3 objectives: (1) to obtain data on the kinds and amounts of pesticides used; (2) to collect data on the plant and animal crops treated and information on the environment in which pesticides were used; and (3) to ascertain the effects of pesticides on human beings. A random sample of 155 farmers was studied with respect to the use of pesticides and farming habits. From this group, 33 subjects were selected for the in-depth epidemiological study. Fifteen of the 33 subjects were selected because they had the highest pesticide use (high use group), while 18 of the farmers had the lowest usage (low use group). A wide range of insecticides (chlorinated hydrocarbon, organophosphorus, and carbamate) and herbicides (halogenated, triazine, acetanilide, and amide) were used by the 33 test subjects. Of the 12 herbicides, atrazine was the 8th most commonly used among the high use group and the 5th most commonly used among the low use group. Each subject received a comprehensive medical examination once per year which included an occupational and past medical history and a clinical laboratory evaluation.

  Statistically significant differences were found between the high and low pesticide use groups with respect to hematocrit ($P<0.01$) hemoglobin ($P<0.01$), and prothrombin time ($P<0.05$) values. In addition, a significant ($P<0.01$) correlation was observed between the use of atrazine among farmers in the high-dose group and 1-minute and 30-minute bilirubin values. Concerning this latter finding, the authors note that the number of subjects involved was small (1-minute values {n=7} and 30-minute values {n=8}) and that testing with large numbers may alter these results. In addition, they recognized that a number of confounding factors were present which raise questions about the significance of the study results [Long *et al.*, 1969].

- A case-referent study was conducted by Donna *et al.* to evaluate the specific role of triazine herbicides, including atrazine, in ovarian carcinogenesis. The study was confined to 143 of the 190 municipalities forming the Alessandria Province in Piedmont, Italy where corn, on which triazine herbicides are used, is the primarily crop grown. The analysis was carried out on 65 women with histologically confirmed primary malignant epithelial tumors of the ovary and 126 referents selected from voter registration lists. The cases and referents completed a reproductive factors questionnaire, a general medical survey, and a lifelong occupational history. Questions were also asked regarding other non-occupational instances of herbicide exposure. Concerning exposure to triazine herbicides, 3 categories were defined: definitely exposed (prepared/used triazine herbicides or worked in corn cultivation), possibly exposed (possible personal/occupational exposure, but herbicide types could not be identified, or denied personal use, but worked in corn cultivation after the use of triazine herbicides become extensive), and unexposed (occupational/domestic exposure could reasonably be ruled out).

  The risk of ovarian cancer for the three categories of exposure was adjusted for age and reproductive factors that affect the incidence of ovarian cancer (number of live births, use of oral contraceptives). A significant risk ratio (RR) estimate of 2.7 (90% confidence interval (CI) 1.0-6.9) was found for those subjects definitely exposed to triazines. Those possibly exposed had a lower risk (RR 1.8, 90% CI 0.9-3.5). Among both the definitely exposed and the possibly exposed subjects, the risks were related to the duration of the exposure. The logistic estimate of the regression coefficient for the definitely exposed was 1.7 (P=0.05) and for the possibly exposed it was 1.4 (P=0.08).

  Donna *et al.*, concluded that women with previous exposure to unspecified triazine herbicides showed a two- to three-fold increased risk of epithelial ovarian cancer as compared to unexposed women. They commented that these findings were consistent with those of their previous study (Donna *et al*, 1984 {not described in this summary}) in which a possible role in ovarian carcinogenesis was suggested for triazine herbicides [Donna *et al.*, 1989].

- To investigate the possible association between non-Hodgkin's lymphoma (NHL) and exposure to agricultural chemicals, a population-based case-control study was conducted in eastern Nebraska in 1985. Telephone interviews were conducted with 201 men from 66 counties having histologically confirmed NHL and with 725 controls. An increased risk of NHL was associated with several classes of insecticides, and several herbicides, including atrazine (OR 1.4, 95% CI 0.8, 2.2). The risk associated with atrazine use increased with duration with odds ratios of 0.9, 0.8, 2.0 and 2.0 for use durations of 1-5, 6-15, 16-20, and 21+ years, respectively. Weisenburger reports that further analysis of these data as well as data on other agricultural and non-agricultural factors evaluated in this study are forthcoming [Weisenburger *et al.*, 1990a].

2. Animal Data

Studies concerning both the carcinogenic and noncarcinogenic effects of atrazine are described below. Data on the potential carcinogenicity of this compund are summarized at the end of this section in Table 11.

- ***oral, rat***

As described in a study performed by Hazleton Laboratories, Inc. (unpublished data, 1961), atrazine was fed to groups of 30 male and female rats of unspecified strain for 2 years at dietary levels of 0, 1, 10, or 100 ppm (0, 0.05, 0.50, or 5.0 mg/kg/day). After 65 weeks, the 1 ppm dose was increased to 1000 ppm (50 mg/kg/day) for the remainder of the study. Five males and five females from each group were randomly sacrificed after 26 and 52 weeks. At necropsy, the following organs were collected for examination: brain, pituitary, thyroid, heart, lung, liver, kidney, adrenal, spleen, stomach, pancreas, small and large intestine, bladder, testes or ovary, bone marrow, skeletal muscle, and peripheral nerve. Blood hematocrits and leukocyte counts were determined, and urinalysis for sugar, protein, bile pigments, and pH were performed. No treatment-related effects on pathology, hematology, or urinalysis were found among rats sacrificed at 26 weeks, 52 weeks, or 2 years, or in animals that died and were necropsied during the study. In addition, atrazine had no effect on the general appearance or behavior of the rats. A transient roughness of the coat and piloerection were observed in some animals at the 10 and 100 ppm dose levels after 20 weeks, but not after 52 weeks of treatment. Body weight gains, food consumption, and survival were similar in all groups up till 18 months, but from 18-24 months, there was high mortality resulting from infections (not related to atrazine) in all groups, including controls. Microscopic examination of both males and females revealed no differences in tumor development or total number of tumors between treated animals sacrificed at 52 or 104 weeks or animals that died during the study and control animals [USEPA, 1990].[7]

- ***oral, rat***

A chronic study (specific duration not reported) on the effects of atrazine on rats (unspecified number and strain) administered this compound at doses of 140 or 280 mg/kg has been described in an abstract by Russian authors. Adverse effects noted included hypercholesterolemia; reduced peroxidase and carbonic anhydrase activities; reduced hemoglobin levels and monocyte counts; and increased lymphocyte counts. Reduced body weight gain was also observed. No other data were reported [Gzhetotskii *et al.*, 1977]

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[7]The EPA reported that this study cannot be considered adequate as very few animals of either sex remained alive at 104 weeks, probably due to the infection noted by the laboratory. In addition, EPA stated that the paucity of animals examined, the poor viability due to the infection rate of those on test, and the failure of the lab to analyze feed for the active ingredient further limit the applicability of this study [USEPA, 1990].

- ***oral, rat***

  The chronic toxicity of atrazine was studied by Suschetet *et. al.*, in male and female Sprague-Dawley rats. Sixteen rats/sex/group received 100 or 500 ppm atrazine in their diet for 183 days. Sixteen untreated males and 16 untreated female controls were employed. Body weight and food intake were recorded throughout the study. Complete histopathologic examinations were carried out on 5 males and 5 females from each group.

  Males and females in the high dose group had significantly ($P<0.001$) reduced food intake during days 6-27, 75-95, and 162-183. Males in the high dose group also exhibited decreases in weight gain during days 6-27 ($P<0.001$), 75-96 ($P<0.001$), and 162-183 (not significant). Similar decreases in weight gain were observed among females during days 6-27 ($P<0.05$), 75-96 ($P<0.01$), and 162-183 (not significant). The authors were not able to determine whether the diminished food intake was a result of atrazine-induced toxicity or taste aversion, and accordingly, the significance of the observed body weight changes is unclear. No histological lesions were observed in the atrazine-treated animals [Suschetet, *et al.*, 1974].

- ***oral, rat***

  A 2-year chronic feeding oncogenicity study was conducted by Ciba-Geigy (unpublished report, 1986) using 37- to 38-day-old Sprague-Dawley rats. Technical atrazine (98.8% a.i.) was administered in the diet at dose levels of 0, 10, 70, 500, or 1000 ppm (0, 0.5, 3.5., 25.0, or 50.0 mg/kg/day.) Twenty rats/sex/group were used to evaluate chronic toxicity (*i.e.*, hematology, serum chemistry, and urinalysis) and 50/rats/sex/group were used for the oncogenicity study. Two additional groups of 10 rats/sex were placed on the control or high-dose diet (1000 ppm) for 12-month or 13-month interim sacrifices. (The 1000 ppm group sacrificed at 13 months was placed on a control diet for 1 month prior to sacrifice.) The total number of animals/sex in this study was 90 in each of the control and high-dose groups, and 70 in the 10, 70, and 500 ppm groups. Histopathology was performed on all animals.

  At the mid- and high-dose levels, significant (P values not reported) decreases were observed in mean body weight gain for males and females. In addition, increased bone marrow myeloid hyperplasia was observed in females at an unspecified dose level. Survival was decreased in high-dose females, but increased in high-dose males. Red cell parameters (hemoglobin, hematocrit, and red cell count) were decreased in high-dose females, but not in males. The serum glucose level was decreased in high-dose females at 3, 6, and 12 months, and serum triglyceride levels tended to be decreased in high-dose males throughout the study. There were also decreases in organ-to-body weight ratios in the high-dose animals which were most likely the result of body weight decreases. Hyperplastic changes in high-dose males (mammary gland, bladder, prostrate) and females (myeloid tissue of bone marrow and transitional epithelium of the kidney) were of questionable toxicologic importance. An increase in retinal degeneration and in centrilobular necrosis of the liver in high-dose females and an increase in degeneration of the rectus femoris muscle in high-dose males and

females compared to controls were also observed. Based on decreased body weight gain, the lowest observed adverse effect level (LOAEL) for nononcogenic effects in both sexes was reported to be 25.0 mg/kg/day (500 ppm) and the no observed adverse effect level (NOAEL) was concluded to be 3.5 mg/kg/day (70 ppm).

Oncogenic effects were noted at doses equal to, or greater than, the NOAEL. In females, atrazine was associated with a statistically significant ($P<0.05$) increase in mammary gland fibroadenomas (1000 ppm), mammary gland adenocarcinomas, including two carcinomas at the highest dose tested (70, 500, and 1000 ppm), and in the total number of animals bearing mammory gland tumors (1000 ppm) (See Tables 9 and 10[8]). Each of these increases was associated with a statistically significant and dose-related trend and was reportedly outside of the high end of the historical control range. In addition, 12-month interim data indicated that there was evidence for decreased latency for mammary gland adenocarcinomas. A statistically significant (P value not reported) increase in testicular interstitial cell tumors was seen in male rats in the high dose group; however, this increase was reported to be within the historical control range. In both males and females, the highest dose tested exceeded the maximum tolerated dose (MTD) based on body weight gain decrement in males and increased mortality, liver necrosis, and bone marrow myeloid hyperplasia in females. The MTD was reached in males and females at 500 ppm (25 mg/kg/day) [USEPA, 1990].[9]

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[8]Table 9 presents the incidence of mammary tumors as reported by Ciba-Geigy (1986). This table was updated by the EPA in 1988 when, as a result of minor errors in the number of animals examined, the tumors were further analyzed using the Peto Prevalence method. The updated analysis is presented in Table 10.

[9]Ciba-Geigy believes that the mammary gland tumor response observed during this study is restricted to one strain and sex of rats, specifically the Sprague-Dawley female rat, which is highly prone to developing spontaneous mammary tumors. Ciba-Geigy also asserts that the response is caused by a perturbation of an already unstable endocrine system. Therefore, the response may have a threshold dose below which no tumor formation, other than that which occurs spontaneously, will be observed [Ciba-Geigy, 1991].

Table 9. Mammary Tumors in Female Rats Fed Atrazine for Two Years

	Dose level (ppm)				
	0	10	70	500	1000
12-month sacrifice/deaths and moribund sacrifices at 0 to 13 months					
No. tissues examined	12	5	1	5	15
Adenocarcinoma	0	1	1	0	3
Fibroadenoma	0	0	1	1	1
13-month sacrifice	10	---	---	---	10
No. tissues examined	0	---	---	---	5
Adenocarcinoma	0	---	---	---	2
Fibroadenoma					
terminal sacrifice/deaths and moribund sacrifices at 13 to 24 months					
No. tissues examined	66	64	68	65	65
No. rats-adenocarcinoma and carcinosarcoma	15	15	26	27	37
No. of adenocarcinomas	17	22	42	48	64
No. rats-fibroadenoma	29	29	35	38	42
No. of fibroadenomas	37	46	48	81	69
Mammary tumor-bearing rats	35	39	47	47	56
all animals in study					
No. of tissues	88	69	69	70	89
Carcinomas	15	16	27	27	45
Adenomas and fibroadenomas	29	29	36	39	46
All tumors	35	40	48	48	65
p values[a]					
Carcinomas:					
Cox-Tarone			0.0454	0.0071	<0.00005
Gehan Breslow			0.0290	0.0016	<0.00005
Adenomas and fibroadenomas:					
Cox-Tarone				0.0685	0.0004
All tumors:					
Cox -Tarone				0.0071	<0.00005
Gehan-Breslow				0.0050	<0.00005

[a]Life-table analysis, pairwise comparison.

Reference: Ciba-Geigy (unpublished, 1986) as cited in USEPA, 1990

Table 10. Female Rat Mammary Tumor Rates* and Peto's Prevalence Test Results

		Dose level (ppm)				
		0	10	70	500	1000
Benign		20/88	25/65	21/69	21/68[a]	20/89[b]
	%	(23)	(37)	(30)	(31)	(22)
	P	0.446	0.110	0.373	0.373	0.468
Malignant		15/88	16/67[c]	27/69	27/68	45/89[d]
	%	(17)[e]	(24)	(39)[f]	(40)[f]	(51)[e]
	P	0.000[e]	0.390	0.024[f]	0.019[f]	0.000[e]
Benign and Malignant Combined		35/88	40/67	48/69	48/68	65/89
	%	(40)[e]	(60)	(70)[f]	(71)[f]	(73)[e]
	P	0.000[e]	0.111	0.017[f]	0.015[f]	0.000[e]

*Number of tumor bearing animals/number of animals at risk. (Excluding animals that died before the observation of the first tumor or animals not examined.)

[a]First adenoma occurred at 81 weeks.
[b]First fibroadenoma occurred at 45 weeks.
[c]First adenocarcinoma occurred at 34 weeks.
[d]First carcinoma occurred at 69 weeks.
[e]P<0.05
[f]

Note: Significance of trend denoted at control. Significance of pairwise comparison with control denoted at dose level.

Reference: USEPA, 1990

- *oral, rat*

Pintér *et al.*, studied the effects of atrazine in a long-term carcinogenicity bioassay using Fischer 344/LATI rats of both sexes. Technical grade atrazine was administered at dietary concentrations of 0, 500, and 1000 ppm for 126 weeks. The following number of rats were used: 56 (control group), 55 (500 ppm), and 53 (1000 ppm) for males; and 50 (control), 53 (500 ppm), and 55 (1000 ppm) for females. After 8 weeks of treatment, concentrations were lowered to 750 ppm in the high dose group and 375 ppm in the low dose groups because of signs of toxicity at 1000 ppm (decreased body weight gain and increased water consumption). Body weight and water/food consumption were measured twice per week for 4 months, once per week for 8 months, and every 4 weeks after 4 months. Four surviving moribund males were killed at week 126, and 6 severely ill females were sacrificed at week 123. A complete macroscopic necropsy was performed on all animals and the Zymbal gland and sternum were microscopically examined.

For both males and females, food and water consumption of treated groups were similar to that of the control group. However, atrazine treatment caused a dose-dependent depression of body weight gain in both sexes (P values not reported). There was no difference in the survival rate of treated females compared to controls, and the males in the treated groups lived significantly (P value not reported) longer than controls. Non-neoplastic lesions occurred at background levels and were not related to atrazine treatment. The number of males and females with malignant tumors showed a statistically significant ($P < 0.01$), and dose-related increase compared to controls, as described below.

Concerning neoplastic changes in males, a significantly increased ($P < 0.05$) incidence of mammary tumors was observed in the high dose group. The incidences and latencies of benign mammary tumors for each group were as follows: controls (1/48; 111 weeks), low-dose (1/51; 119 weeks), and high dose (9/53; 121.3 weeks). All but one of the mammary tumors were benign fibromas, fibroadenomas, or adenomas. The adenomas were of the highly cellular type and were seen only among the high-dose group. The only mammary adenocarcinoma was observed in the high dose group. A dose-dependent increased incidence of combined leukemias/lymphomas was also observed in males; however, the increase was not significant. Other types of tumors seen in males appeared in a random fashion and corresponded to the background levels for Fischer rats.

In females, uterine adenocarcinomas were observed at a dose-related, and significantly increased (P>0.05) incidence with the following incidences and latencies: control (6/45; 104 weeks), low dose (8/52; 110 weeks), and high dose (13/45; 108). The number of uterine adenomatous polyps, however, was inversely related to the administered atrazine dose. There was no significant difference in the latency of either tumor type. Regarding the hematopoietic system of females, the numbers of combined leukemias/lymphomas were significantly increased (P<0.05) in a dose-related fashion (control (12/44), low dose (16/52), and high dose (22/51). No statistical difference was observed in the latency of these tumors and the incidences and latencies of other tumors did not differ significantly from controls among females.

The authors concluded that the high number of benign mammary gland tumors in males, the malignant uterine tumors and the significantly increased incidence of leukemias/lymphomas in females accompanied by the overall dose-related increase in malignant tumors in both sexes, can be regarded as suggestive evidence for the tumorigenic activity of atrazine [Pintér, *et al.*, 1990].

- ***oral, rat***

As reported in an abstract from the Proceedings of the American Association for Cancer Research, Weisenburger *et al.*, studied the carcinogenicity of atrazine and N-nitrosoatrazine compounds (see section V.I.) in female Wistar rats. Rats (n=50) were administered 500 ppm atrazine in their feed for a planned duration of 104 weeks. A group of 50 untreated control rats was included in the study. Due to excessive, unspecified toxicity, the doses of atrazine were decreased over time to unspecified levels for 67 weeks. No significant increase in tumors were found in the atrazine-treated group. No other data were reported [Weisenburger *et al.*, 1990b].

- ***oral, mice***

Innes *et al.*, investigated the tumorigenicity of atrazine in male and female F_1 hybrid mice (C57BL/6 x C3H/Anf and C57BL/6 x AKR). Atrazine was administered by gavage at a concentration of 21.5 mg/kg/day (determined to be the maximum tolerated dose) in 0.5% gelatin beginning when the mice were 7 days-old and continuing until the mice were 4 weeks of age. After the mice were weaned at 4 weeks, the chemicals were mixed directly in the diet which was provided *ad libitum* to 18 mice/sex/strain for 18 months at a concentration of 82 ppm. An untreated control group (18 mice/sex/strain), a vehicle control group (18 mice/sex/strain), and a positive control group (24 mice/sex/strain) administered ethyl carbamate were employed. Following the 18 month treatment period, an external examination and an examination of thoracic and abdominal cavities (which included histologic examination of major organs and of all grossly visible lesions), were performed. In addition, blood smears were made from mice prior to sacrifice, and were examined only if there was evidence of splenomegaly or lymphadenopathy. The incidence of hepatomas, pulmonary tumors, lymphomas, and total tumors in the atrazine-treated mice was not significantly different from the negative controls [Innes *et al.*, 1969].

- ***oral, mice***

  As part of the study by Weisenberger *et al.* described above, 50 female Swiss mice were administered 1500 ppm atrazine in their feed for a planned duration of 96 weeks. This dose was found to be excessively toxic, and the concentration of atrazine in their diet was reduced to unspecified levels for 67 weeks. No significant increase in tumor formation was observed compared to the untreated control. No other data were reported [Weisenburger *et al.*, 1990b].

- ***oral, mice***

  In a carcinogenicity study conducted by Industrial Bio-Test Laboratories (unpublished data, 1981), Swiss white mice (60 males and 60 females per dose group) were given atrazine in their feed at doses of 0, 10, 300, or 1000 ppm (0, 1.5, 45, or 150 mg/kg). Males and females were treated for 21 and 22 months, respectively. Males and females in the 1000 ppm dose group had decreased body weights and females in this group also exhibited decreased survival. No gross pathology was found in any of the groups, and there was no indication of any inflammatory, degenerative, proliferative, or neoplastic lesions associated with atrazine administration [USEPA, 1990].[10]

- ***oral, mice***

  In a 91-week oral feeding/oncogenicity study conducted by Ciba-Geigy (unpublished data, 1987c), atrazine (97% a.i.) was fed to 5-week-old CD-1 male and female mice. Sixty mice/sex/group received atrazine in their diet at concentrations of 10, 300, 1500, and 3000 ppm (actual mean daily intakes of 1.4, 38.4, 194.0, and 385.7 mg/kg/day for males, and 1.6, 47.9, 246.9, and 482.7 mg/kg/day for females). The dose-related effects in males and females consuming diets containing 1500 or 3000 ppm atrazine included a 23.5% and 11.0% decrease in the mean body weight gain at 91 weeks in males and females, respectively and decreases in erythrocyte count, hematocrit, and hemoglobin concentrations. In addition, an increase in the incidence of cardiac thrombi was observed in females in the 1500 and 3000 ppm dose groups. Amyloidosis and cardiac thrombi contributed to the deaths of mice who did not survive to terminal sacrifice. Based on these results concerning nononcogenic effects, the lowest observed adverse effect level (LOAEL) was determined to be 1500 ppm (194.0 mg/kg/day {M}, 246.9 mg/kg/day {F}) and the no observed adverse effect level (NOAEL) for systemic toxicity was reported to be 300 ppm (38.4 mg/kg/day {M}, 47.9 mg/kg/day {F}).

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[10]The data submitted by Industrial Bio-test Laboratories have been evaluated by the EPA and a number of deficiencies were found. For instance, there were no diet preparation records to verify that the animals received the test material in the diet during the first 6 months of the study; and analysis of dietary concentrations and stability of atrazine in the diet indicated that, for the first year of the study, the animals probably received lower dose levels than those required by the protocol. In addition, clinical observations and mortalities recorded were limited mainly to collective group notations for the first 16 months of the study [USEPA, 1990].

Atrazine was not found to be oncogenic in this strain of mice, and no statistically significant and dose-related increases in the incidences of neoplasms (mammary adenocarcinomas, adrenal adenomas, pulmonary adenomas and malignant lymphomas) were noted. However, among male mice in the 10 ppm dose group, a statistically significant (P value not specified) increase in the incidence of hepatocellular adenomas was observed. No statistically significant increase in the incidence of this type of tumor was observed in groups of mice fed higher levels of atrazine and this effect was determined not to be dose-related. In addition, 2 male mice fed 10 ppm atrazine had fibrosarcomas, and 1 male mouse in the 1500 ppm exposure group developed a hemangiosarcoma. Palpable masses (mammary adenocarcinomas) were observed in 3 female mice (1 mouse in the control group and 2 mice in the 3000 ppm group). One female in the 10 ppm exposure group developed a fibroma, and one female in the 300 ppm group developed malignant lymphoma. [USEPA, 1990; USEPA, 1991].

- ***oral, dogs***

In a study by Woodard Research Corporation (unpublished data, 1964), atrazine (80W formulation) was fed to groups of four male and four female beagle dogs for 105 weeks at dietary levels of 0, 15, 150, or 1500 ppm (0, 0.35, 3.5, or 35 mg/kg/day). Survival rates, body weight gain, food intake, behavior, appearance, hematology, urinalysis, organ weights, and histology were determined. In the 15 ppm dose group, no indications of toxicity were observed, but male and female dogs in the 150 ppm dose group had decreased food intake and females exhibited increased heart and liver weights. Among dogs that received 1500 ppm atrazine, there was a decrease in food intake and body weight gain, an increase in adrenal weight, a decrease in hematocrit, and occasional tremors or stiffness in the rear limbs. There were no histological changes in the 30 tissues examined. Based on changes in the organ weights observed at 150 ppm, the no observed adverse effect level (NOAEL) for chronic atrazine toxicity was determined to be 15 ppm (0.35 mg/kg/day) [USEPA, 1990].

- ***oral, dogs***

In an unpublished study conducted by Ciba-Geigy (1987b), the chronic toxicity of technical grade atrazine (97% a.i.) was evaluated in dogs. Male and female 5-month-old beagle dogs were fed atrazine for 1 year at dietary levels of 0, 15, 150, and 1000 ppm. (The atrazine-fortified diets corresponded to actual average intakes of 0.48, 4.97, or 33.65/33.8 {male/female} mg/kg/day.) Six animals/sex/group were assigned to the control and high-dose groups, and four animals/sex/group were assigned to the low- and mid-dose groups. One mid-dose male, one high-dose male, and one high-dose female were sacrificed in a moribund state during the study period. The death of the high-dose female was reported to be compound-related, and the deaths of the two males, especially the mid-dose male, appeared to be related to spontaneous dog pathology rather than to treatment.

Decreased body weight gains and food consumption were noted among males and females in the high dose group. Statistically significant ($P<0.05$) reductions in erythroid parameters (red cell count, hemoglobin, and hematocrit) in high dose males were noted throughout the study as were mild increases in platelet counts in both sexes. Slight decreases in total protein and albumin ($P<0.05$) were noted in high-dose males as well as decreased calcium and chloride in males and increased sodium and glucose in females. Decreased absolute heart weight and increased relative liver weight were noted in high-dose females and males, respectively.

The most significant effect of atrazine in this study was cardiopathy syndrome, reflected as discrete myocardial degeneration in the high-dose animals of both sexes. Clinical signs associated with cardiac pathology such as ascites, cachexia, labored/shallow breathing, and abnormal EKG were observed in the group as early as 17 weeks into the study. Gross pathology revealed severe dilation of the right atrium and occasionally the left atrium. These findings were also noted histopathologically as degenerative atrial myocardium (atrophy and myelosis). In the mid-dose group, two males appeared to be affected. Gross necropsy in one male revealed a moderate degree of dilation of the right atrium and minimal dilation of the left atrium, plus a pale lesion of epicardium of the left ventricle. However, no histopathological findings were noted. In the second mid-dose male, a red right atrium "thickened with edema" was observed. Although this finding is consistent with "polyarteritis nodosa," this animal's death occurred before the end of the study and was considered by laboratory investigators to be unrelated to atrazine administration. The mid-dose females in this study also exhibited a decrease in P-II waves at day 175 of the study. These P-II levels were within the normal range, but they reflected a trend toward the development of cardiac pathology that was noted at the higher dose. Based on this data, a no observed adverse effect level (NOAEL) of 0.5 mg/kg/day and a lowest observed adverse effect level (LOAEL) of 5 mg/kg/day have been established [USEPA, 1990].

In order to evaluate the relationship between the lesions observed at the high dose and the less serious and less frequent effects seen at the mid dose, EPA requested that Ciba-Geigy carry out a subchronic study in dogs. In response to this request, Ciba-Geigy presented detailed analysis of the cardiac data outlined above and requested that the EPA reconsider the need for another subchronic study. After reviewing the additional data that was submitted, EPA reported that the original LOAEL was based on decreased amplitude of the P-II wave at only 1 time point in females, atrial dilation, and one case of polyarteritis nodosa. At the high dose, the first two effects occurred at a much higher frequency and magnitude and were accompanied by severe histopathologic cardiac lesions. Detailed analysis of the individual animal data indicated that the effects observed at the mid-dose were not likely atrazine-related. The apparent significant decrease in P-II amplitude of only 0.1 mV was observed in only 1 mid-dose female at only one time point (day 171); corresponding histopathological lesions were not observed at this dose level. EPA further commented that the amplitude change was very small and could readily have occurred as a result of slight positional change during EKG process. Changes of 0.1 mV were

present in other groups including controls at other time points. In addition, EPA stated that the polyarteritis lesion that occurred in 1 male dog was evaluated and found not to be of the type produced by chemical toxicity. Atrial dilation seen only at necropsy in the males could not be attributed to atrazine exposure due to the lack of supporting pathology data. Based on the supplemental data, EPA changed the NOAEL for this study from 0.5 to 5.0 mg/kg/day. In addition, the LOAEL was changed form 5 mg to 34 mg/kg/day based on death, cachexia, ascites, decreased body weight and body weight gain, decreased food consumption, EKG changes, and cardiac lesions [USEPA, 1990].

- ***intraperitoneal/subcutaneous, mice***

A preliminary carcinogenicity study on the effects of the herbicide Fogard S® containing both atrazine (25%) and simazine (37.5%) was reported by Donna *et al.* A solution of the herbicide containing 2 ppm atrazine and 200 ppm simazine in 0.9% saline was used for dosing animals in each of the following two dose groups (n=25/group): group 1 mice were given 13 subcutaneous injections of 0.25 mL (containing 0.0065 mg of atrazine and simazine) of the Fogard S® solution at 3-day intervals; group 2 mice were given 13 intraperitoneal injections with the same solution of Fogard S® as described for group 1. In addition, 2 groups of 50 control animals were given either 13 subcutaneous injections of 0.25 ml of saline solution at 3-day intervals or 13 identical intraperitoneal saline injections. Dosing continued for 7 months during which time one animal from each group was sacrificed at 15-day intervals. Animals that survived the treatment period were sacrificed, and all animals underwent complete necropsy.

Malignant lymphomas were observed in 3 animals (12.5%; P= 0.00113) from group 1 that died spontaneously. In addition, 1 sacrificed animal from this group (4.2%) had peritoneal mesothelioma. Malignant lymphomas were also observed in 2 animals (10%; P=0.02661) from group 2 that died spontaneously. No animals from the control group had either macroscopic or microscopic abnormalities.

The authors report that these results "hint" at the possibility of a carcinogenic risk associated with Fogard S® in mice which may be a result of atrazine and/or simazine. To test this hypotheses, Donna *et al.*, described their plans to conduct a carcinogenicity study on atrazine in mice (described below)[Donna *et al.*, 1986].

intraperitoneal, mice

As a continuation of their work described above, Donna *et al.*, studied the carcinogenicity of atrazine in male Swiss albino mice treated by intraperitoneal administration. Thirty 4-week old mice received 13 injections of a 0.25 ml solution of 2 ppm atrazine in saline every third day for 13 months (total administered atrazine dose of 0.26 mg/kg body weight). A control group (n=50) was given 0.25 ml of saline solution, by ip administration according to the same dosing schedule. A second control group underwent no treatment in order to assess the incidence of spontaneous tumors. The overall observation period for the three groups was extended to 375 days. Necropsy was performed on animals found dead and on animals that were sacrificed at the end of the observation period.

A statistically significant ($P<0.001$) increase in the number of lymphomas in treated groups compared to control groups was observed. Four of the atrazine treated animals developed plasma cell type lymphoma, and two other treated mice had histiocytic type lymphoma. In the control group administered saline, no tumors were found and in the untreated control, 1 spontaneous histiocytic lymphoma developed. Upon microscopic determination lymphomas were observed to involve mesenteric, lumbar, periaortic, and mediastinal lymph nodes as well as the spleen, liver, and lungs. The authors concluded that their experimental study provides only presumptive evidence of the potential oncogenicity of atrazine [Donna *et al.*, 1986].

Table 11: The Carcinogenic Effects of Atrazine Exposure in Animals

Route	Species/Strain	Sex/# per dose group	Dose/Duration[a]	Carcinogenic Effects[b]	Reference
Oral	Rats/NS [c]	M and F/ 5 per sex	1-100 ppm for 26 or 52 weeks	None	Hazelton Laboratories Inc., 1961,as reported in USEPA, 1990
Oral	Rats/NS	M and F/ 5 per sex	1-100 ppm for 65 weeks, the 1000 ppm for 39 weeks	None	
Oral	Rats/Sprague-Dawley	M and F/ 16 per sex [d]	100 or 500 ppm for for 183 days	None	Sushetet *et al.*, 1974
Oral	Rats/Sprague-Dawley	M and F/50 per sex at 10-500 ppm, 70 per sex at 1000 ppm	10-1000 ppm for 12 or 24 months	Females: significant increase in mammary gland FA (1000 ppm) and AC (70, 500, and 1000 ppm), and in # of tumor-bearing rats (1000 ppm) Males: significant increase in testicular interstitial cell tumors [e]	Ciba-Geigy, 1986 as reported in USEPA, 1990
Oral	Rats/Fisher 344/ LATI	M and F/ 50-56	500 or 1000 ppm for 8 weeks, then 375 or 750 for 118 weeks [f]	Females: significant and dose related increase in uterine AC, and leukemias/lymphomas Males: significant increase in mammary tumors (Fb, FA, Ad), and 1 AC in high dose group	Pintér et al., 1990
Oral	Rats/Wistar	F/50	<500 ppm(exact dose NS) for 67 weeks	None	Weisenburger *et al.*, 1990b
Oral	Mice/Swiss	F/50	<1500 ppm (exact dose NS) for 67 weeks	None	Weisenburger *et al.*, 1990b
Oral	Mice/Swiss	M and F/60 per sex	10-1000 ppm for 21 (males) or 22 (females) months	None	Industrial Bio-Test Laboratories, 1981 as reported in USEPA, 1990

Table 11: The Carcinogenic Effects of Atrazine Exposure in Animals (Continued)

Route	Species/Strain	Sex/# per dose group	Dose/Duration	Carcinogenic Effects	Reference
Oral	Mice/2 hybrid species	M and F/ 18 per sex	21.5 mg/kg/day on days 7-28, then 82 ppm for 18 months	None	Inness *et al.*, 1969
Oral	Mice/CD-1	M and F/ 60 per sex	10-3000 ppm for 91 weeks	Females: mammary AC in 2 mice (3000 ppm); Fb in one mouse (10 ppm); malignant lymphoma in one mouse (300 ppm) [results not significant] Males: significant increase in hepatocellular Ad (10 ppm); FS in 2 mice (10 ppm); HS in one mouse (1500 ppm) [results not dose-related]	Ceiba-Geigy, 1987c as reported in USEPA, 1990
Oral	Dogs/Beagle	M and F/ 4 per sex	15-1500 ppm for 105 weeks	None	Woodard Research Corporation, 1964 as reported in USEPA, 1990
Oral	Dogs/Beagle	M and F/ 4-6 per sex	15-1000 ppm for 1 year	None	Ciba-Geigy, 1987b as reported in USEPA, 1990
Intraperi-toneal	Mice/Swiss albino	M/30	0.25 ml of a 2 ppm solution every 3 days for 13 months	significant increase in lymphomas (plasma cell type and histiocytic type)	Donna *et al.*, 1986

[a] Doses reported as ppm refer to dietary levels of atrazine.

[b] FA = Fibroadenoma; AC = Adenocarcinoma; Fb = Fibroma; Ad = adenoma; FS = Fibrosarcoma; HS = Hemangiosarcoma

[c] NS = not specified

[d] Complete histopathologic examinations were only carried out on 5 males and 5 females per dose group.

[e] This statistically significant increase was reported to be within the historic control range.

[f] The dose was lowered after 8 weeks due to signs of toxicity seen at 1000 ppm

E. Reproductive Effects and Teratogenicity

1. Human Data

No data were found.

2. Animal Data

Reproductive Effects

- ***oral, rats***

A three-generation study on the effects of dietary administration of atrazine was conducted by Woodard Research Corporation (unpublished data, 1966). Groups of 10 male and 20 female rats of unspecified strain were administered 0, 50, or 100 ppm atrazine in their diet (0, 2.5, and 5 mg/kg/day). The animals initially received only half of these concentrations which were increased to the specified levels after 3 weeks. After 74 days of dosing at the full dose level, rats within each group were paired for mating over a 10-day period. Following birth of the litters, pups were weighed and examined, and the numbers of births and stillbirths were recorded. At weaning, the pups were weighed and examined and the number of survivors and the mean litter weights were noted. The pups were then sacrificed and necropsied. Approximately 13 days after the first weaning, the females in each group were remated with different males in the same group. The protocol employed following the first mating was repeated with the pups from the second mating. After the second weaning, the parents (F_0 generation) were sacrificed, and the weanlings (F_{1b} generation) were used to form another three dose groups.

The entire series of tests was repeated following the dosing of the F_{1b} generation with 0, 50, or 100 ppm atrazine for 105 days prior to breeding and thereafter. The F_{2b} generation was, in turn, fed atrazine for 75 days prior to breeding and thereafter. The protocol was repeated until weaning of the F_{3b} generation at which point the study was terminated. No adverse effects on reproduction related to atrazine administration were observed during the course of the 3 generation study. In addition, atrazine had no effect on any of the following parameters: mean parental body weight, survival, appearance, behavior, number of litters/group, number of live births, mean pup weights at birth and weaning, and pup survival at weaning. In addition, there were no histological changes in the weanlings and no atrazine-related effects on fetal resorption found. No malformations were observed in the atrazine treated group, and weanling organ weights were similar in F_{3b} controls and atrazine treated animals. A no observed adverse effect level (NOAEL) of 100 ppm was identified in this study. However, it was noted that the evaluation of this study is difficult because only two relatively low doses that were without observable toxicity were used. In addition, the dietary concentrations of atrazine were altered during an important maturation period of the neonates (F_0 parental generation) [USEPA, 1990].

- ***oral, rats***

  In a 2-generation reproduction study conducted by Ciba-Geigy (1987a), 120 rats/sex were randomly distributed into 4 treatment groups and fed technical grade atrazine (97% a.i.) at 0, 10, 50, or 500 ppm (0, 0.5, 2.5, or 25 mg/kg/day). Atrazine exposure began when male rats were 47 days-old and females were 48 days-old. They were maintained on these diets for 10 weeks prior to mating. Males and females were housed together in a 1:1 ratio and allowed 3 weeks for mating; following evidence of mating, the rats were separated. One litter was produced in each generation. After weaning, 30 males and 30 females from the first generation were selected to be the second parental generation. The remaining male parental animals were sacrificed on days 133 to 134 of the study. Animals selected for the second generation were exposed to test diets for 12 weeks prior to mating. Mating was conducted in the same manner as described above for the first generation. Parental males were sacrificed on day 138 of the study and parental females were sacrificed on days 138, 139, and 152 after litter weaning.

  Concerning parental toxicity, a statistically significant ($P<0.05$) decrease in body weight, body weight gain, and food consumption were observed among males and females in the 500 ppm dose group. In addition, a statistically significant (P value not reported) increase in relative testes weight was observed in both generations of male rats. There were no gross or macroscopic findings in any of the reproductive organs of the F_0, F_1, or F_2 generations although relative testes weights were increased in the 500 ppm F_0 and F_1 males as a result of reduced terminal body weights. Based on these results, the no observed effect level (NOEL) for parental toxicity was determined to be 50 ppm. A NOEL for reproductive toxicity of 10 ppm was based on statistically significantly lower F_2 generation pup weights at postnatal day 21 at 50 and 500 ppm (no P values reported). It was concluded that atrazine does not cause any impairment in reproductive performance in rats fed a maximum tolerated concentration of 500 ppm for two consecutive generations [Giknis *et al.*, 1988; USEPA, 1990; USEPA, 1992b].

- ***oral, rats***

  The effect of atrazine on reproductive parameters in an unspecified strain of rats was studied by Peters and Cook. Sperm-positive dams were divided into 8 groups of 4 rats each and were fed atrazine in their feed from day 1 of pregnancy throughout gestation at levels of 0, 50, 100, 200, 300, 400, 500, or 1000 ppm. The number of pups/litter and the weaning pup weight were recorded.

  Atrazine levels ranging from 0-1000 ppm did not affect the number of pups/litter or the weaning pup weight. No other data were reported [Peters and Cook, 1973].

- ***oral, rats/rabbits***

  In a study carried out be Infuna *et al.*, 1988 to evaluate the developmental effects of atrazine in rats and rabbits (described below), atrazine was not found to affect any of the reproductive parameters evaluated in rats following oral administration on gestational days 6-15. In rabbits, oral administration on days 7-19 of gestation caused a statistically significant (P value not reported) treatment-related increase in reproductive effects only in the high dose group (75 mg/kg). These effects included an increase in resorptions, a decrease in the number of viable fetuses, and an increase in the percentage of postimplantation losses. The effects were reported to be associated with the severe maternal toxicty observed in this dose group [Infurna *et al.*, 1988].

- ***subcutaneous, rats***

  The effect of atrazine on reproductive parameters in rats of unspecified strain was studied by Peters and Cook in a series of 4 experiments. For each experiment, atrazine was dissolved in 1ml dimethyl sulfoxide (controls were injected with 1mL of this vehicle). For experiments 1, 2, and 3, atrazine was administered on days 3, 6, and 9 of gestation. In experiment 4, atrazine was administered on days 3, 6, *or* 9 to determine the critical stage of gestation at which this chemical affects organogenesis. In experiment 1, 4 groups of 4 rats each were injected subcutaneously with 0, 50, 100, or 200 mg/kg atrazine. For experiment 2, 3 groups of 4 rats each were injected with atrazine at concentrations of 0, 800, or 2000 mg/kg[11] . Three groups of rats (5-7 rats per group) were used for experiment 3. These rats were injected with 0, 1000, or 2000 mg/kg atrazine. For experiment 4, 6 groups of 3 rats were each injected with atrazine at a concentration of 0, 1000, or 2000 mg/kg. In experiments 1 and 4, the number of pups/litter was determined; for experiments 2 and 3, both the number of pups/litter and the number of resorption sites/uterus were recorded.

  The number of pups/litter did not differ from the control at any dose level tested in experiment 1. However, injection of 800 or 2000 mg/kg atrazine in experiment 2 was found to cause a decrease (P value not reported) in the number of pups/litter (control {10 pups/litter}, 800 mg/kg {8 (3 rats) and 0 (1 rat) pups/litter}, and 2000 mg/kg {0 pups/litter) and an increase in the number of resorption sites/uterus (control {0 sites/uterus}, 800 mg/kg {0 (3 rats) and 11 (1 rat) sites/uterus}, and 2000 {9 sites/uterus). Based on these results, the authors classified atrazine as embryotoxic at these 2 dose levels. In experiment 3, a similar decrease in the number of pups/litter and an increase in the number of resorption sites was seen at dose levels of 1000 and 2000 mg/kg atrazine, and this chemical was again concluded to be embryotoxic. In experiment 4, 1000 mg/kg atrazine administered on day 6 of gestation reduced the number of pups/litter by 50%. The other dosing schemes in this experiment did not affect this reproductive parameter. Peters and Cook concluded that day 6 is the critical

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[11]Because atrazine at a concentration of 2000 mg/kg could not be dissolved in 1 ml dimethyl sulfoxide, the animals were injected with a 1 ml suspension of atrazine in dimethyl sulfoxide.

stage of organogenesis and that, since the higher concentration of atrazine (2000 mg/kg) did not affect the number of pups/litter on day 6, it may be that atrazine injected as a suspension is not readily absorbed into the blood [Peters and Cook, 1973].

Developmental Effects

- ***oral, rats***

In a three-generation study on the reproductive effects of dietary administration of atrazine in rats which was conducted by Woodard Research Corporation (described above [USEPA, 1990]), no histologic changes were noted in the weanlings and no external malformations were observed in the atrazine treated group [USEPA, 1990].

- ***oral, rats***

In a study performed by Ciba-Giegy (unpublished data, 1971), atrazine was administered by gavage to pregnant rats of unspecified strain and number at concentrations of 0, 100, 500, or 1000 mg/kg on days 6-15 of gestation. An increase in the number of prenatal deaths, a decrease in the mean weight of the fetuses, as well as retarded skeletal development was observed at doses of 500 and 1000 mg/kg. Anasarca was noted in 5/119 fetuses at the 1000 mg/kg dose only. At 1000 mg/kg, maternal mortality (23%) and various unspecified toxic effects were also observed. A no observed adverse effect level (NOAEL) of 100 mg/kg was determined based on the results of this study [USEPA, 1990; USEPA, 1991].

- ***oral, rats***

A study was carried out by Ciba-Geigy (unpublished data, 1984a) to evaluate the developmental effects of atrazine on Charles River rats. Twenty-seven rats per dose group were administered atrazine (96.7% purity) by gavage on days 6-15 of gestation at dose levels of 0, 10, 70, or 700 mg/kg/day. Excessive maternal mortality (21/27) was observed among rats in the 700 mg/kg/day dose group, and symptoms included reduced food consumption, reduced weight gain, salivation, ptosis, swollen abdomen, oral/nasal discharge, and bloody vulva. Maternal toxicity (reduced food consumption, reduced body weight, and reduced weight gain) also occurred at 70 mg/kg/day. No maternal toxicity was observed in the 10 mg/kg/day or control groups.

Fetal weights were significantly (P value not specified) reduced at 700 mg/kg/day. At 70 mg/kg/day, there were statistically significant (P value not specified) increases in both fetal and litter incidences for skeletal variations indicating delayed ossification. Variations included incomplete ossification of the skull, metacarpals, metacarpals bipartite, and phalanx.

This study established a no observed adverse effect level (NOAEL) of 10 mg/kg/day and a lowest observed adverse effect level (LOAEL) of 70 mg/kg for maternal toxicity, and the NOAEL and LOAEL for developmental toxicity was determined to be 10 mg/kg/day and 70 mg/kg/day, respectively [USEPA, 1990; USEPA, 1991].

- *oral, rats*

Technical grade atrazine was evaluated by Infurna *et al.*, 1988 for its embryotoxic, fetotoxic, and teratogenic potential in rats and rabbits (rabbit study described below). Twenty-seven sperm-positive female Charles River rats per group were administered atrazine (suspended in 3% aqueous corn starch) at daily doses of 0, 10, 70, or 700 mg/kg/day by gavage on gestational days 6-15. The volume administered (10 ml/kg/day) was adjusted for changes in body weight on gestational days 6, 10, and 14. Rats were observed for gross changes in appearance or behavior and feed consumption and body weight were monitored throughout gestation. Necropsy was performed on gestational day 20. The ovaries were examined, corpora lutea were counted, uteri and their contents were weighed, and live fetuses and intrauterine resorptions were counted. In addition, the liver weights were recorded for all full-term pregnant animals.

The rat fetuses were weighed, sexed, and examined for visceral, and with the exception of the high dose group skeletal abnormalities. Approximately one-third of the rat fetuses from each litter were fixed in Bouin's solution and then examined for soft-tissue abnormalities.[12]

Treatment-related mortality (21/27 dams died prior to the scheduled necropsy) and treatment-related clinical effects were observed only in high-dose dams. Effects included significantly ($P<0.01$) increased incidences of salivation, ptosis, bloody vulvas, and swollen abdomens. Significant (P value not reported) treatment-related reductions in feed consumption and body weight gains were also observed in the high dose group. Treatment-related effects at the intermediate dose level were restricted to small, but statistically significant, reductions in feed consumption (days 6 and 7) and a significant (P value not reported), decrease in body weight gain (days 6-10). No significant treatment-related adverse effects were observed among low-dose dams.

The mean body weight of fetuses obtained from the surviving high-dose dams was significantly ($P<0.01$) lower than control values. This effect was considered to result from severe maternal toxicity at the high dose level and was not observed at the intermediate or low dose level. No treatment related effects were noted for any of the reproductive parameters identified.

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[12]Due to the high incidence of maternal toxicity observed in the rat study, the number of litters (and fetuses) was greatly reduced in the high dose group. Fetal effects as indicated by the severely reduced fetal weight were also apparent in the surviving members of the high-dose group. Therefore, the rat fetuses in the high-dose group were not evaluated for skeletal abnormalities.

There were no treatment-related effects or visceral abnormalities (variations or malformations). In addition, there were no treatment-related increases in the incidence of any skeletal malformations. However, the incidence of minor peripheral skeletal variations was significantly increased in fetuses from the intermediate dose group. These variations included the incomplete ossification of the skull ($P<0.01$), hyoid bone ($P<0.05$), teeth ($P<0.01$), forepaw metacarpals ($P<0.05$), and hindpaw distal phalanges ($P<0.05$). In addition, a significantly increased incidence ($P<0.05$) of bipartite forepaw metacarpals was observed in the intermediate dose group.

The authors concluded that atrazine produced a dose-related pattern of maternal toxicity in rats and that effects on the fetus were observed only at dose levels that were maternally toxic. Reproductive parameters were not affected at any dose level tested and atrazine was not teratogenic at maternally toxic dose levels in either species [Infurna *et al.*, 1988].

- ***oral, rat***

Ciba-Geigy evaluated the embryotoxic, fetotoxic, and teratogenic potential of atrazine in an unspecified species of mated rats. Rats (n=26) were administered atrazine suspended in 3% cornstarch with 0.5% Tween 80 by gavage at doses of 5, 25, or 100 mg/kg on days 6-15 of gestation. An unspecified number of control animals were also employed.

During compound adminstration, high-dose maternal feed consumption, body weight, and body weight gain, were significantly (P value not specified) lower than control values. The authors report that these effects were, for the most part, reversible upon cessation of dosing. Subsequent examination of the fetuses for skeletal abnormalities revealed significantly (P value not specified) greater incidences of incomplete ossification of the hyoid, interparietal, occipital, and parietal bones in the high dose group. Because these effects were observed only at a dose that was determined to cause maternal toxicity, they were considered to be secondary effects and not indicative of direct fetotoxicity. Under the conditions of this study, the no observed effect level (NOEL) was 25 mg/kg and the maximum tolerated dose (MTD) was 100 mg/kg. The authors concluded that there was no evidence of embryotoxicty or teratogenicity in this study [Yau *et al.*, 1989].

- ***oral, rabbits***

Ciba-Geigy (unpublished data, 1984b) investigated the developmental toxicity of atrazine (96.3% purity) on groups of 19 New Zealand White rabbits which were administered this compound at dose levels of 0, 1, 5, or 75 mg/kg/day on days 7 through 19 of gestation. Maternal toxicity was observed at doses of 5 mg/kg/day (decreased body weight gain and food consumption) and 75 mg/kg/day (blood on vulva or in cage, decreased food consumption, abnormal stools, and decreased body weight and body weight gain). Does in the 75 mg/kg/day group did not recover from symptoms of toxicity during the period after dosing. No maternal effects were observed at 1 mg/kg/day or in the control group.

A significant (P value not specified) increase in the number of resorptions was observed in the 75 mg/kg/day group. At this dose level, reduced fetal weights, and delays in ossification of appendicular skeletal elements were also observed. No indications of developmental toxicity were noted at the other dose levels tested. No compound-related malformations were found.

Based on these results, a no observed adverse effect level (NOAEL) of 1 mg/kg/day and a lowest observed adverse effect level (LOAEL) of 5 mg/kg/day were established for maternal toxicity. The NOAEL and LOAEL for developmental toxicity were reported to be 5 mg/kg/day and 75 mg/kg/day, respectively [USEPA, 1990; USEPA, 1991].

- ***oral, rabbits***

As part of the teratology study by Infurna *et al*, 1988 (rat data described above), groups of 19 artificially inseminated New Zealand White does were administered atrazine by gavage at doses of 0, 1, 5, or 75 mg/kg/day in 3% aqueous corn starch on gestational days 7-19. The volume of administration (5 ml/kg/day) was adjusted for changes in body weight on days 7 and 14. The experimental protocol used in the rat study described above was employed with the following exceptions: does were necropsied on gestational day 29 and *all* rabbit fetuses were weighed, sexed, and examined for visceral and skeletal abnormalities. In addition, rabbit fetuses were examined for soft-tissue (visceral) abnormalities according to a modification of the Staples technique (Staples, 1974). Following the visceral examination, the skeletal elements of all fetuses were evaluated.

There were no treatment-related maternal deaths. Clinical signs related to treatment were observed only in the high dose group and included an increased incidence of stool variations and bloody vulvas. There were no treatment-related gross necropsy findings. The absolute liver weight of the high-dose animals was significantly (P value not reported) reduced; however, this finding was secondary to the changes in body weight as there were no significant differences in liver weight as a percentage of body weight. Significant (P value not reported) treatment-related changes (significant reductions followed by significant increases) in feed consumption were also noted in the high dose group which resulted in significant fluctuations in body weight gain. Slight, but significant (P value not reported), treatment-related reductions in feed consumption and body weight gain were recorded for the intermediate dose group, but not the low dose group.

Concerning the reproductive parameters evaluated, no treatment-related effects were observed at the low or intermediate dose levels. Statistically significant (P value not specified) treatment-related effects were observed only in the high dose group and included an increase in resorptions, a decrease in the number of viable fetuses, and an increased percentage of postimplantation loss. These findings in the high dose group were associated with the severe maternal toxicity observed in this dose group.

Although there were no significant differences in fetal weights at the low and intermediate dose levels, significant reductions in fetal weights were observed in the high dose group. No treatment-related gross (external) fetal malformations, or skeletal or visceral malformations were found, and no significant treatment-related differences in teh incidence or fetal visceral variations were noted. In addition, there were no treatment-related increases in the incidence of fetal skeletal variations in the low or intermediate dose groups. However, high-dose fetuses exhibited a statistically significant ($P<0.05$) increase in the incidence of nonossification of the forepaw metacarpals and middle phalanges, the patellae, and the hindpaw talus and middle phalanges.

The authors comment that atrazine induced maternal toxicity in a dose-related manner and that reproductive parameters and fetal effects were observed only at doses that were clearly maternally toxic. In addition, atrazine was not teratogenic at maternally toxic doses in either species [Infurna *et al.*, 1988].

- ***oral, sheep***

Binns and Johnson administered atrazine (15 or 30 mg/kg) by stomach tube to 2 groups of 6 pregnant ewes of unspecified strain. Administration continued throughout gestation as well as 30-days postpartem. All animals in the 30 mg/kg dose group died during gestation (on days 36-60). In addition, 1 ewe in this dose group had failed to conceive, 3 ewes had embryonic deaths, and 2 were carrying fetuses that appeared normal. In the group of ewes fed 15 mg/kg, all animals gave birth to full-term, normal live lambs that did not display clinical indications of toxicity during the 30-day postpartum observation period. Atrazine was not found to cause congenital malformation of the limbs at either dose level [Binns and Johnson, 1970].

- ***subcutaneous, mice***

A study was contracted by the National Cancer Institute and carried out by Bionetics Research Laboratories in 1968 to study the teratogenicity of selected pesticides, including atrazine. Atrazine was subcutaneously administered to an unspecified number of pregnant female C3H, C57BL6 {days 1-14 of gestation}, and AKR {days 1-15 of gestation}mice at dose levels of 46.6 mg/kg in dimethyl sulfoxide. Control mice for each strain receiving only dimethyl sulfoxide were employed. After being weighed, C3H and BL6 mice were sacrificed on day 18 of gestation while AKR mice were sacrificed on day 19. Weight gain and liver weight were evaluated to assess maternal toxicity.

To evaluate fetotoxicity and teratogenicty, the following parameters were assessed: fetal weight, crown-rump length and placental weight as well as litter number, total number of fetuses, implantations per litter, liver fetuses per litter, and fetal mortality. After gross inspection for abnormalities, 2/3 to 3/4 of each litter (16 {CH3}, 60{BL6}, and 78 {AKR} fetuses from 6, 13, and 15 litters, respectively) were selected at random for necropsy. The remainder of each litter was stained with Alizarin Red S to detect skeletal anomalies.

Maternal liver weight was increased in all strains of mice and maternal weight gain was reduced in the BL6 and AKR mice. However, in the C3H and AKR strains, there was a significant increase in fetal mortality. This increase in fetal mortality was not observed in the BL6 mice. In addition, an increase in amniotic fluid per fetus and a decrease in fetal weight was observed in the AKR litters. The incidence of abnormal fetuses was within the normal range in all groups. Because atrazine appeared to affect fetal growth and mortality, and not fetal development, this compound was categorized by the authors as fetotoxic, but probably not teratogenic [NCI, 1968].

F. Genetic Toxicology

1. Human Data

No data were found

2. Prokaryotic and Eukaryotic Data

The genotoxic effects of atrazine in prokaryotic organisms is summarized below in Table 12. *In vitro* eukaryotic data is summarized in Table 13, *in vivo* data for both prokaryotic and eukaryotic test systems are presented in Table 14, and Table 15 summarizes the genotoxicty of atrazine in plants.

Table 12: Genotoxicity of Atrazine in Prokaryotic Organisms

Test organism (strain)	Genetic endpoint	Dose	Test Conditions[a]	Response[b]	Reference
Salmonella typhimurium (8 unreported strains)	reverse gene mutations	NR[c]	- metabolic activation	negative	Anderson *et al.*, 1972
S. typhimurium (TA1530, TA1531, TA1532, TA1534, his G4-6)	reverse gene mutations	NR	NR	negative	Seiler, 1973
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	reverse gene mutations	5-5000 ug/plate	+/- rat liver S9 activation	negative	Simmon *et al.*, 1977
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	reverse gene mutations	10-810 ug/0.1 ml	+/- rat liver S9 activation	negative	Arni and Muller, 1978, as reported in USEPA, 1990
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	reverse gene mutation	50-10,000 ug/plate	+/1 rat liver S9 activation	negative	Sutou *et al.*, 1978, as reported in USEPA, 1990
S. typhimurium (TA98, TA100, TA1535, TA1538)	reverse gene mutations	0-100 ug/plate; 0-5 mg/plate	+/- rat liver[d] S9 activation	negative	Lusby *et al.*, 1979
S. typhimurium (NR)	reverse gene mutations	NR	+ plant activation	negative	Loprieno *et al.*, 1980; Adler, 1980
S. typhimurium (TA98, TA100)	reverse gene mutations	<= 5 umol/plate	+ rat liver[e] S9 activation	negative	Bartsch *et al.*, 1980
S. typhimurium (TA100 , TM-677)	reverse (TA100) and forward (TM-677) gene mutations	extracts of corn exposed to atrazine (concentration NR)	+ plant activation	negative	Bakshi, 1981
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	reverse gene mutations	up to full strength[f]	+/- rat liver S9 activation	negative	Eisenbeis *et al.*, 1981
S. typhimurium (NR)	reverse gene mutations	NR	- metabolic activation	positive	Gopalan and Njagi, 1981
S. typhimurium (TA98, TA100, TA1537)	reverse gene mutations	NR	+/- rat liver S9 activation	negative	Ishidate *et al.*, 1981

Table 12: Genotoxicity of Atrazine in Prokaryotic Organisms (Continued)

Test organism (strain)	Genetic endpoint	Dose	Test Conditions[a]	Response[b]	Reference
S. typhimurium (TA100)	reverse gene mutations	water soluble extracts of *Zea mays* (B37) exposed to 100 ppm[g]	+ plant activation	weak positive (2.5% significance level	Plewa *et al.*, 1984
S. typhimurium (TA98)	reverse gene mutations	extracts of *Zea mays* (B37) exposed to 1.66×10^{-5}M metolachlor/1.37×10^{-4}M atrazine[h]	+ plant activation	positive ($P<0.05$)	Weisenburger *et al.*, 1987
S. typhimurium (NR)	reverse gene mutations	NR	+ hamster liver S9 activation	negative	Kappas, 1988
S. typhimurium (TA97a, TA98, TA100, TA1535, TA1537, TA1538)	reverse gene mutations	1-1000 ug/plate	+/- rat liver S9 activation	negative	Zeiger *et al.*, 1988
S. typhimurium (TA98, TA100, TA1535, TA1537)	reverse gene mutations	10-10,000 ug/plate	+/- rat or hamster liver S9 activation	negative	Butler and Hoagland, 1989
S. typhimurium (TA97, TA98, TA100, TA1535, TA1537, TA1538)	reverse gene mutations	0.01-10.00 umol/plate	- metabolic activation	negative	Franekic *et al.*, 1989
S. typhimurium (TA98, TA100)	reverse gene mutations	NR	NR	negative	Xu and Schurr, 1990; Von der Hude *et al.*, 1988
Escherica coli (PQ37)	SOS induction	NR	+/- rat liver S9 activation	negative	Sutou *et al.*, 1979, as reported in USEPA, 1990
E. coli (WP2 hcr)	reverse gene mutation	50-5,000 ug/plate	+/- rat liver S9 activation	negative	Anderson *et al.*, 1972
T4 bacteriophage (NR)	induction of rII mutants	20 ug	- metabolic activation	negative	Anderson *et al.*, 1972
T4 bacteriophage (AP72 and N17)	reverse gene mutations	100 ug	- metabolic activation	negative	Means *et al.*, 1983; 1988

Table 12: Genotoxicity of Atrazine in Prokaryotic Organisms (Continued)

Test organism (strain)	Genetic endpoint	Dose	Test Conditions[a]	Response[b]	Reference
Bacillus subtilis (H17 and M45)	Rec assay	NR	- metabolic activation	negative	Shirasu *et al.*, 1976
B. subtilis (H17 and M45)	Rec assay	extracts of *Zea mays* (B37) exposed to 100 ppm	plant activation	negative	Means *et al.*, 1988
B. subtilis (H17 and M45)	DNA damage/ repair	100-10,000 ug/well	- metabolic activation	negative	Sutou *et al.*, 1979, as reported in USEPA, 1990

[a] Liver S9 fractions are from Aroclor-induced animals unless otherwise noted; + = with; - = without
[b] P values and levels of significance are included in the table when they are reported in the study
[c] NR = Not reported
[d] Liver S9 fractions were obtained from rats induced with Aroclor, phenobarbitol, 3-methylcholanthrene, atrazine, and Aatrex® (commercial form of atrazine)
[e] Liver S9 fractions were obtained from rats induced with Aroclor and 3-methylcholanthrene
[f] Atrazine was also tested for genotoxicity in combination with other herbicides (Banvel®, Bladex®, Lasso®, Paraquat®, Princep ®, Prowl®, Ramrod®, Sutan®); the response to these combinations was also negative
[g] Pellet material from extracts of atrazine treated plants was not found to be mutagenic in this assay
[h] Highest positive dose

Table 13 : Genotoxicity of Atrazine in Eukaryotic Systems (*In Vitro*)

Test Organism (strain)	Genetic endpoint	Dose	Test Conditions[a]	Response[b]	Reference
Saccharomyces cerevisiae (D4)	mitotic gene conversion	1000 ppm[c]	- metabolic activation	negative	Siebert and Lemperle, 1974
S. cerevisiae (D4)	mitotic gene conversion	extracts of *Zea mays* (W22, W23) kernels exposed to 5 and 20 ppm	+ plant activation	positive (2.5 and 4 x controls, respectively)	Gentile and Plewa, 1976
S. cerevisiae (D4)	mitotic gene conversion	extracts of *Zea mays* (W22, W23) leaves exposed to 5 and 25 ppm	+ plant activation	positive (18 and 30 x controls, respectively)	Gentile and Plewa, 1976
S. cerevisiae (D4)	mitotic gene conversion	extracts of *Zea mays* (H51, B37) exposed to 10-25 ppm	+ plant activation	positive and dose related	Plewa and Gentile, 1976a
S. cerevisiae (D4)	mitotic gene conversion	extracts of *Zea mays* (B37) exposed to 100 ppm	+ plant activation	weak positive	Means *et al.*, 1988
S. cerevisiae (D4)	mitotic gene conversion	1000 ppm	- metabolic activation	negative	Gentile *et al.*, 1977
S. cerevisiae (D4)	mitotic gene conversion	2.5-10.0 mM	+ plant activation (potato microsomes)	negative	Loprieno *et al.*, 1980; Adler, 1980
S. cerevisiae (D4)	mitotic gene conversion	1000 and 4000 ppm[d]	+/- mouse liver S9 activation	negative	Bertoldi *et al.*, 1980
S. cerevisiae (D4)	mitotic gene conversion	extracts of *Zea mays* (B37) exposed to metolachlor and atrazine (concentrations NR[e])	+ plant activation	negative	Plewa *et al.*, 1984
S. cerevisiae (D4)	mitotic gene conversion	water-soluble extracts of maize (WF9 x Bear 38) exposed to 30, 60, and 90 ppm[f]	+ plant activation	positive	Singh *et al.*, 1982
S. cerevisiae (D7)	mitotic gene conversion	NR	NR	negative	Franekic *et al.*, 1989
Schizo-saccharomyces pombe (NR)	forward mutations	6 mM[g]	+ plant activation (potato microsomes)	positive	Loprieno *et al.*, 1980; Adler, 1980
S. pombe (ade6)	forward mutations	3 mM/hour	+/- mouse liver S9 or maize extract activation	negative	Chollet *et al.*, 1982
Streptomyces coelicolor (NR)	forward mutations	NR	+ plant activation (potato microsomes)	positive	Adler, 1980
Aspergillus nidulans	reverse mutations	NR	NR	positve	EPA Genetox Program, 1988 as reported in RTECS, 1991

Table 13: Genotoxicity of Atrazine in Eukaryotic Systems (*In Vitro*) (Continued)

Test Organism (strain)	Genetic endpoint	Dose	Test Conditions[a]	Response[b]	Reference
Aspergillus nidulans	forward mutation and somatic segregation	NR	+/- plant activation (*Nicotitiana alana*)	positive for both endpoints with plant activation	Benigni *et al.*, 1979
A. nidulans	mitotic gene conversion	500-8000 ppm	tested with resting and germinating conidia	negative	Bertoldi *et al.*, 1980
A. nidulans	mitotic recombinations	460-4600 uM	+/- rat liver S9 activation	negative	Kappas, 1988
Neurospora crassa	meiotic aneuploidy	NR	NR	negative	Griffiths, 1981; EPA Genetox Program, 1988 as reported in RTECS, 1991
Neurospora crassa	meiotic aneuploidy	10-500 µg/ml	NR	positive	Griffiths *et al.*, 1986
Tetrahymena pyriformis	DNA damage	NR	- metabolic activation	negative	Mouton, 1981
Chinese hamster cells (V79)	forward mutations	3.0 mM [g]	+ plant activation (potato microsomes)	weak positive	Loprieno *et al.*, 1980; Adler, 1980
Chinese hamster cells (V79)	forward mutations	1.25-10.0 mM	+ liver microsomes (species NR)	negative	Adler, 1980
Chinese hamster ovary cells	chromosome breakage; sister chromatid exchange	1.25-10.0 mM	NR	negative	Adler, 1980
Chinese hamster lung fibroblasts	chromosomal aberrations	NR	+/- rat liver S9 activation	negative	Ishidate *et al.*, 1981
EUE human cells	unscheduled DNA synthesis	3.0 mM [g]	+ plant activation (potato microsomes)	positive	Loprieno *et al.*, 1980; Adler, 1980
Human epidermal carcinoma cells (KB)	DNA damage	NR	- metabolic activation	negative	Mouton, 1980
Human lymphocytes (NR)	sister chromatid exchange	0.001, 0.01, and 0.1 ppm	NR	negative	Ghiazza et al., 1984, as reported in USEPA, 1990
Monkey kidney cells (VERO)	DNA damage	NR	- metabolic activation	negative	Mouton, 1981

[a] Liver S9 fractions are from Aroclor-induced animals unless otherwise noted; + = with; - = without
[b] P values and levels of significance are included in the table when they were reported in the study
[c] Atrazine tested as the commercial preparation Gesaprim®
[d] Doses are reported for which the highest frequencies of mitotic gene conversion per survivor were observed
[e] NR = Not reported
[f] Atrazine tested as the commercial preparation Aatrex® 80W (80% atrazine)
[g] Lowest dose at which a positive response was observed

Table 14: *In Vivo* Genotoxicity of Atrazine in Prokaryotic and Eukaryotic Systems

Test Organism (strain and sex)	Genetic Endpoint	Dose and Route	Response[a]	Reference
Drosophila melanogaster (Oregon -R males)	sex-linked recessive lethals	0.01% by larval feeding[b] 0.012% injection to adults[b]	positive (P<0.05) negative	Murnik and Nash, 1977
	dominant lethals	0.01% by larval feeding[b]	positive (P<0.001)	
	Chromosomal breakage, disjunction, and loss	0.01% by larval feeding[b]	negative for breakage and disjuction; positive for loss (P<0.05)	
D. melanogaster (NR[c])	sex-linked recessive lethals	NR	negative	Gopalan and Njagi, 1981
D. melanogaster (NR)	sex-linked recessive lethals	5 + 10 mM[d] (route NR)	negative	Adler, 1980
D. melanogaster (NR)	sex-linked recessive lethals; nondisjunction; wgike sex chromosome loss	NR	Inconclusive	EPA Genetox Program, 1988 as reported in RTECS, 1991
Mouse/(101 x C3H, F1 hybrid males)	dominant lethal mutations in spermatozoa and spermatids	1500-2000 mg orally	positive	Ehling, 1980a
	chromosomal aberrations in bone marrow cells	2000 mg/kg orally	positive (4% chromosome breaks compared to 0.7% in controls)	
	somatic mutations	NR	negative	
Mouse (NR)	chromosomal aberrations in bone marrow cells	2000 mg/kg orally	positive	Loprieno *et al.*, 1980
Mouse (NR)	chromosome analysis of bone marrow cells; micronucleus test in bone marrow cells	NR NR	positive and dose-related negative	Kliesch and Adler, 1983
Mouse (NR)	chromosomal aberrations in germinal cells; dominant lethality	444 and 1332 mg/kg by gavage	negative	Hook and Muller, 1981, as reported in USEPA, 1990
Mouse (NR; males)	chromosomal aberrations in bone marrow cells, germinal cells, spermatagonia; diakinesis	6 mg/kg intraperitoneally or 1 ppm in drinking water for 7 weeks	negative	Chollet *et al.*, 1982
	dominant lethal mutations	6 mg/kg intraperitoneally or 1 ppm in drinking water for 7 weeks	negative for post-implantation lethality; positive for pre-implantation lethality after 4 weeks of treatment	

Table 14: *In Vivo* Genotoxicity of Atrazine in Prokaryotic and Eukaryotic Systems (Continued)

Organism (strain)	Genetic Endpoint	Dose and Route	Response[a]	Reference
Mouse (C57BL x C3H , F1, males)	abnormalities in sperm morphology	600-2400 mg/kg/day intraperitoneally for 5 days	negative	Osterloh *et al.*, 1983
Rat (Sprague-Dawley male)	DNA damage in stomach, kidney, liver and lung	875 mg/kg by gastric intubation or 350 mg/kg/day orally for 5 or 15 days	positive in cells from liver, kidney, stomach; negative in cells from lung	Pino *et al.*, 1988
Chinese hamsters (male and female)	chromosomal aberrations in somatic cells	282, 564, or 1128 mg/kg	negative	Hook *et al.*, 1981, as reported in USEPA, 1990
Host-mediated Assays:				
Rat (Wistar or BD) /Escherica coli indicator	forward mutations	100-600 mg/kg orally and intraperitoneally[e]	positive orally; negative intraperitoneally	Neal, 1980
mouse (strain NR)/ *S. pombe* indicator	forward mutations	1000 mg/kg orally[f]	positive	Loprieno *et al.*, 1980;Adler, 1980
mouse (Tuck TO)/ *Escherica coli* indicator	forward mutations	100-600 mg/kg orally and intraperitoneally	positive orally; negative intraperitoneally[e]	Neal, 1980; Solt and Neal, 1980
mouse (strain NR)/ *Salmonella typhimurium* TA1535, TA1538 indicator	gene mutationa	500-2,200 mg/kg orally; 275-1,100 mg/kg/day for 5 days	negative	Simmon *et al.*, 1977, as reported in USEPA, 1990
mouse (strain NR)/ *Salmonella typhimurium* indicator	gene mutations	1000 mg/kg[d] (route NR)	negative	Adler, 1980

[a] P values and levels of significance are included in the table when they were reported in the study
[b] Atrazine tested as the commercial preparation Aatrex® 80W (80% atrazine)
[c] NR = Not reported
[d] Lowest dose at which a positive response was observed
[e] The authors report that during several repetitions of these experiments, the results have proved inconsistent and require further investigation.
[f] Loprieno *et al.*, 1980 reported that atrazine was injected intrasanguineously to mice and tested on an unspecified strain of yeast in the host-mediated assay; Adler , 1980 reports that this laboratory conducted the host-mediated assay using *S. pombe*, and administered atrazine orally to the host animal.

Table 15: Genotoxicity of Atrazine in Plants

Test Organism (strain)	Genetic endpoint	Concentration	Results [a]	Reference
Zea mays (B14)	reverse gene mutations in male gametophytes	parent plant grown in soil treated with 35.3 mg	positive	Plewa and Gentile,1976a
Zea mays (W22, W23)	reverse gene mutations in male gametophytes	NR [b] (parent plant grown in plots sprayed with atrazine)	positive (10 x controls)	Plewa and Gentile,1976b
Zea mays (W22)	reverse gene mutations in male gametophytes	*in situ* treatment of corn with:		Plewa *et al.*, 1984; Plewa and Wagner, 1981
		atrazine (3.84 kg/hectare)	positive (P<0.005)	
		atrazine + metolachlor (2.4 + 3.0 kg/hectare)	positive (P<0.001)	
		atrazine + eradicane (1.92 + 3.6 kg/hectare)	positive (P<0.001)	
		atrazine + butylate (1.92 + 4.8 kg/hectare)	positive (P<0.001)	
Zea mays (B37)	sister-chromatid exchange	10 and 20 ppm	positive	Chou and Weber, 1981
Corn (VIR-27)	chromosomal aberrations (CA); chlorophyll mutations in M2 plants (CM)	soil sprayed with 4 kg/hectare (SD) or SD x 6; seeds soaked in 30 mg/150 ml H2O	negative for CA; positive for CM (P<0.001)	Morgun *et al.*, 1982
Hordeum vulgare L.	chromosomal aberrations in root tip cells from C1 generation	seeds soaked in 500, 1000, 1500 ppm for 6, 12, and 24 hours	positive (2.89 % compared to 0.68% in controls)	Wuu and Grant, 1966
	chromosomal aberrations in root tip cells from C2 generation	seeds soaked in 1000 ppm for 12 hours	positive (1.47 % compared to 0.0% in controls)	
Hordeum vulgare L.	chromosomal aberrations in pollen mother cells of C1 and C2 generation	seeds soaked in 1000 ppm for 12 hours [c]	positive (2.04 and 1.06 % compared to 0.22 and 0.49 % in controls, respectively)	Wuu and Grant, 1967
	chromosomal aberrations in meiotic cells	seedlings sprayed with 500 ppm[c]	positive(1.49 % compared to 0.66 % in controls)	
Hordeum vulgare L.	chromosomal aberrations in root tips	seeds soaked in 2.5 - 10 mM for 24 hours[d]	negative	Muller *et al.*, 1972
Vicia fabci	chromosomal aberrations in root tips	seeds soaked in 0.2 - 1.0 mM for 24 hours[e]	negative	Muller *et al.*, 1972

Table 15: Genotoxicity of Atrazine in Plants (Continued)

Test Organism (strain)	Genetic endpoint	Concentration	Results	Reference
Sorghum vulgare	chromosomal aberrations in pollen mother cells	seedlings sprayed with 5 kg /hectare[f]	negative	Muller *et al.*, 1972
Sorghum (KS3; Redlan; Martin)	chromosomal aberrations in pollen mother cells of microsporocytes	seedlings sprayed with 2.5 kg/hectare at different intervals for 7 weeks	positive	Liang and Liang, 1972
Tradescantia paladusa (clone 03)	micronuclei	5-200 ppm for 6 hours	negative	Ma *et al.*, 1981
Tradescantia (clone 4430)	stamen hair mutations	0.045 g/pot 6.9×10^{-5} M for 24 hours	negative positive (1% level of significance)	Van't Hof and Shairer, 1982
Tradescantia	micronuclei and stamen hair mutations	50, 100, 200 ppm for 6 hours	negative	Mohammed and Ma, 1983
Pelargonium zonale	chlorophyll defects; growth inhibition	10^{-3}M	negative	Pohlheim *et al.*, 1977

[a] P values and levels of significance are included in the table when they were reported in the study
[b] NR = not reported
[c] Atrazine tested as a commercial preparation composed of 50% atrazine.
[d] Atrazine tested as the commercial preparation Aatrex® 80W (80% atrazine).
[e] At concentrations of 0.25, 0.5 and 1.0 mM, atrazine tested as the commercial preparation Aatrex® 80W (80% atrazine; at concentrations of 0.2 and 0.4 mM, atrazine was tested as the pure substance.
[f] Atrazine tested as the commercial Preparation Gesaprim® 50 (50% atrazine).

G. Other Toxicological Effects

1. Endocrine Toxicology

In addition to the studies described below, endocrine studies are currently being conducted by Ciba-Geigy to investigate a possible endocrine basis for atrazine-related mammary tumor formation in female Sprague-Dawley rats (see section V.D.2). According to Ciba-Geigy, the preliminary data suggest that exposure of female Sprague-Dawley rats to high levels of atrazine may accelerate an existing endocrine imbalance that is naturally conducive to the development of mammary tumors. Results from these ongoing studies (on female Sprague-Dawley rats) include the following [Ciba-Geigy, 1991]:

— Circulating serum hormone levels, including estrogen and progesterone, are altered.
— Estrous cycle duration and pattern are changed.
— A reduction in the uterotropic response to estrogen has been noted, indicating a possible anti-estrogenic effect in that tissue.
— Estrogen receptor binding affinity is altered in the hypothalamus, pituitary, mammary gland, and uterus. Similar effects are noted *in vitro* with hypothalamic, pituitary, and uterine receptors.
— Interference with estrogen receptor binding appears to be competitive (*i.e.*, the affinity of estrogen for binding to its receptor is decreased while specific binding capacity is unaffected).

- ***oral, rats***

The effect of dietary atrazine on the *in-vivo* synthesis of prostaglandin E_1 (PGE_1) and thromboxane B_2 (TXB_2) by platelets from clotted blood was studied in 1-month-old male Wistar rats. Groups of rats were administered diets containing 500 mg atrazine/kg diet for 30-35 days, and serum PGE_1 and TXB_2 activity was determined by radioimmunoassay. The authors concluded that the effect of atrazine on the synthesis of PGE_1 and TXB_2 was not statistically significant ($P<0.05$) [Meydani *et al.*, 1984].

- ***oral, rats/rat anterior pituitary and hypothalamus cells***

The effects of atrazine and deethylatrazine (a primary metabolite of atrazine) on the enzymic systems responsible for testosterone metabolism in the anterior pituitary and hypothalamus were studied. Male Fisher rats were orally dosed with 6 or 12 mg/100g atrazine or deethylatrazine for 7 days. At the 12 mg/100g dose level, atrazine significantly ($P< 0.01$) increased the weight of the anterior pituitary with hyperemia and hypertrophy of chromophobic cells with vacuolar degeneration. At this dose, 5 alpha reductase (alpha-R) activity ($P<0.01/0.02$), and 3 alpha- and 17 beta-HSD activities ($P<0.01$) were inhibited in the anterior pituitary. The 12 mg/100 g dose of deethylatrazine significantly inhibited 5 alpha-R activity in the anterior pituitary ($P<0.01$). The authors suggested that, based on the similar reduction in 5 alpha-R activity caused by both atrazine and deethyl atrazine, the mechanism of inhibition may be similar. No changes in 3 alpha- and 17 beta-hydroxysteroid dehydrogenase (HSD) activities were observed in the high dose group compared to the controls. The lower dose of atrazine and deethylatrazine did not influence the

enzymic activities involved in testosterone conversion in the anterior pituitary. At the 12 mg/100 kg dose, atrazine significantly inhibited 5 alpha-R ($P< 0.02$) and 17 beta-HSD ($P<0.01$) activity in the hypothalamus tissue. At the 6 mg/100 kg dose, atrazine significantly ($P<0.01$) decreased 17 beta-HSD activity in the male rat hypothalamus. Deethylatrazine, at a dose of 12 mg/100g, significantly inhibited 5 alpha-R ($P<0.01$) and 17 beta-HSD ($P<0.01$) activities in the hypothalamus.

In addition to the oral study, an *in vitro* study on male Fisher rat anterior pituitary and hypothalamus cells was performed concurrently. Atrazine or deethylatrazine, was added into the incubation medium at a dose of .92 μM atrazine or deethylatrazine in 5 μl ethanol added into 2 ml of incubation medium. Addition of atrazine or deethylatrazine, significantly inhibited ($P < 0.01$) 5 alpha-R, 3 alpha- and 17 beta-HSD activities in the anterior pituitary. The inhibition of 5 alpha-R activity was more marked for atrazine. The *in vitro* addition of deethylatrazine was more effective in inhibiting 5 alpha-R, 3 alpha- and 17 beta-HSD ($P < 0.01$) in the male rat hypothalamus than atrazine. Atrazine was shown only to inhibit 3 alpha-HSD activity ($P < 0.01$) in the hypothalamus [Babic-Gojmerac *et al.*, 1989]. In an earlier study, Kniewald and Kniewald demonstrated that a mixture of atrazine and prometryne (0.56 mmol of each of the compounds) inhibited the formation of the active androgen metabolite in the male rat pituitary by 77% [Kniewald and Kniewald, 1983].

- ***subcutaneous, rats***

Kniewald *et al.*, studied the effect of atrazine and its metabolite, deethylatrazine, on the rat gonadotropic mechanism during the early postnatal period. An unspecified number of pregnant female fisher rats (~180 g) were treated subcutaneously with atrazine or deethylatrazine (in 0.1 ml paraffin oil/animal) at a concentration of 1.66 mg/100 g/ body weight/day from day 1 of pregnancy throughout gestation. A second group of pregnant rats was treated with this dosing regimen throughout pregnancy and during lactation. Nontreated and oil injected controls were run for each group. Body weights of the treated and control mothers as well as their offspring were recorded weekly. Offspring up to 28 days of age from both groups of does fed from their own mothers. Male and female offspring were sacrificed on either day 21 or 28 of life, their anterior pituitaries were removed, and the ventral prostrate or uterus tissues were collected from each group for cytosol preparation. The number of specific binding sites for the dihydrotestosterone (DHT)-receptor in the prostrate and estradiol-receptor in the uterus were determined. To assess pituitary enzymatic activities, the conversion of [4-^{14}C]-testosterone to the metabolites 5 alpha-androstane-3 alpha, 17 beta-diol (3 alpha-diol); 5 alpha-dihydro testosterone (5 alpa-DHT); androstat-4-ene-3,17-dione; and 5 alpha-androstane 3,17-dione was studied. No significant differences in maternal body weight or the body weight of offspring from either group were observed.

Daily atrazine and deethylatrazine injections to rat mothers during pregnancy did not induce any significant changes in pituitary 5 alpha-reductase, 3-oxy-steroid 4-ene-dehydrogenase C (5 alpha-R); 3 *a*-hydroxysteroid dehydrogenase (3 alpha-HSD), or 17 beta-hydroxysteroid dehydrogenase (17 beta-HSD) among 28-day old male offspring. Significantly ($P<0.05$ {atrazine}) increased levels of 5 alpha-R activity were observed among female offspring. Kniewald *et al.*, report that this data provides evidence that treatment of mothers during pregnancy induces, through the placenta, long-term effects in female offspring.

Treatment of does during both pregnancy and lactation with the two compounds induced a significant ($P<0.01${atrazine}, $P<0.05${deethylatrazine)) decrease in the formation of (3 alpha-diol) in 21-day-old male offspring. In addition, (5 alpha-DHT) formation was significantly ($P<0.05$) decreased by atrazine in 21-day-old males. Androstat-4-ene-3,17-dione and 5 alpha-androstane 3,17-dione formation were not affected by treatment with either chemical in male offspring. However, 21-day-old female offspring did exhibit significantly ($P<0.05$) decreased levels of androstat-4-ene-3,17-dione. The authors noted that while there were no differences in the weights of the pituitaries, these observed decreases in enzymatic activity were a direct result of treatment of mothers during pregnancy and lactation.

Concerning the results of hormone-receptor specific binding site determination in gonads of the 28-day-old offspring, treatment of dams with atrazine and deethylatrazine during pregnancy did not change the number of specific binding sites for the prostate DHT-receptor or for the uterus estradiol receptor. However, treatment with these compounds during both pregnancy and lactation induced a significant (P value not reported) reduction in the number of specific binding sites for prostate DHT-receptors in 21-day-old offspring. The authors conclude that, in rats, exposure to atrazine and its metabolite during pregnancy only, or during pregnancy and lactation, influences the pituitary-gonadal axis of male and female offspring [Kniewald *et al.*, 1987].

- ***unspecified route, rats***

Male Wistar rats were treated with 50 ppm/day atrazine (route not specified) for 15 days. Body, thyroid, adrenal and hypophysis weight were studied, and blood samples were collected for T_3, T_4 and lactic hydrogenase (LH) assays. Atrazine caused a slight decrease in thyroid weight, body weight, and weight of the hypophysis. T_3 and T_4 release in the serum was stimulated by atrazine while LH release in the serum was inhibited by atrazine. The amount of LH in the hypophysis showed an increase under the action of atrazine (P values not specified in study) [Ghinea *et al.*, 1979].

- ***thyroid cells , humans***

The effect of atrazine was studied in human thyroid cell cultures human thyroid to determine its influence on cell multiplication, protein synthesis, enzymatic activity of thyroid cells, and hormonal synthesis. Cultured cells were obtained by trypsinization from normal and pathological (Graves disease, thyroid nodule) human tissue which had been removed by surgery. Cell cultures were treated with doses of 0.0001, 0.001, 0.01, and 0.1 mg atrazine per culture tube containing 3×10^5 cells/1.5 ml medium. In normal cells, atrazine caused a strong inhibition of protein synthesis. In cell cultures of thyroid nodule, atrazine showed a slight stimulating effect on protein synthesis, and

in Graves disease cultures, no overall effect was noted. Effect on cell multiplication was similar to that for protein synthesis. At the 0.1 mg dose in the normal cells, atrazine caused cellular changes, cytoplasmic vacuoles, widely spaced nuclei, and thin-layered and extended cytoplasm. The Graves disease cells showed a decreased lactic dehydrogenase activity for all doses of atrazine. Atrazine caused inhibition of peroxidases at the high doses. Atrazine also caused an inhibition of RNA synthesis in all of the thyroid cell types. In the tube containing normal cells treated with 0.01 or 0.1 mg atrazine per tube, T_3 and T_4 cells were radioimmunologically assayed. Radioimmunologic assay of T_3 and T_4 cells in the culture medium of normal thyroid cells showed a slight stimulation of secretion of T_3 and T_4 cells induced by atrazine (P values not specified in study)[Ghinea *et al.*, 1979].

- ***embryo renal cells, humans***

The influence of atrazine on the conversion of T_4 to T_3 cells in human embryo renal cell cultures was studied. Human embryo renal cells were dosed with 0.1 or 1.0 µg/ml of atrazine, with varying levels of T_4, and with and without cysteine. Atrazine significantly decreased T_3 release ($P \equiv 1\%$) at both doses of T_4 [Ghinea *et al.*, 1986].

- ***thyroid cancer cells, humans***

The reactivity of thyroid cancer cells treated with estradiol and dehydroepiandrosterone (DHA) in the presence of other hormones (TSH, STH, insulin), myopeptides and pesticides, including atrazine, was studied. It was found that DHA exerted an inhibitory effect on protein synthesis, and that this effect was increased by the presence of atrazine. Also, release of Tg_1 in the culture medium was inhibited by atrazine (P values not specified) [Ghinea *et al.*, 1988].

- ***renal and liver cells, rats***

The influence of atrazine on the conversion of T_4 to T_3 cells in kidney and liver cell cultures, with and without cysteine present, was studied. Rat renal cell cultures and organotypic hepatic tissue cultures were dosed with 0.1 or 1.0 µg/ml of atrazine, with varying levels of T_4 , and with and without cysteine. Treated cultures showed an increase in cell density for the high dose, proportional with the dose of T_4 used. The enzymatic activity, the presence of lactic dehydrogenase, malic dehydrogenase, and glucose-6-phosphate dehydrogenase was increased in lots receiving 10 µg T_4 and atrazine (P value not specified, but increase was proportional to dose). In the rat renal cells, protein synthesis was inhibited by both doses of T_4, but atrazine produced a significant stimulation (P value not specified, but increase is proportional to dose). In the rat renal cell cultures, atrazine significantly increased T_3 release in the high T_4 groups (P=5% and 1%, respectively in the presence and absence of cysteine). This is in contrast to the results found in the human embryo renal cells (see above) [Ghinea *et al.*, 1986].

- ***pituitary, calves***

  Male and female calf pituitaries were collected and assayed for pituitary enzymatic activity. It was shown that atrazine induces inhibition of the enzymic activity in the male and female calf pituitary (P values not specified) [Kniewald and Kniewald, 1982].

2. Immunotoxicity

   - ***oral, rats***

     The immunotoxicity of atrazine was studied in 4 groups of 6 weanling male Riv: TOX (M) Wistar rats. A semisynthetic diet containing 0, 100, 300, or 900 mg/kg atrazine (97% technical) was provided *ad libitum* for three weeks. Body weight and food intake were recorded weekly. At the end of the 3-week period, exsanguination was done from the aorta, and the weights of the liver, kidney, pituitary, adrenals, thyroid, testes, thymus, spleen, and mesenteric and popliteal lymph nodes were determined. Samples of these organs were also fixed and stained. Finally, total leukocyte and differential leukocyte counts were carried out and serum IgM and IgG were quantified.

     Atrazine was found to cause a significant decrease in terminal body weight ($P<0.01$) and daily food intake ($P<0.001$) in the 900 mg/kg dose group only. The serum lymphocyte concentration of rats in the 100 mg/kg ($P<0.05$), 300 mg/kg ($P<0.05$), and 900 mg/kg ($P<0.01$) dose groups was also significantly reduced. Weights of the thyroid ($P<0.01$) and mesenteric lymph node ($P<0.05$) were significantly increased in the 900 mg/kg dose group only, while the weight of the thymus was significantly ($P<0.05$) decreased in this dose group. Histopathological effects, which were observed in only 1 animal from the high dose group, included homogeneous cytoplasm hepatocytes, slight cortex atrophy of the thymus, and slight depletion of PALS in the spleen. The authors concluded the alteration of immunological parameters was a sensitive indicator of atrazine-induced toxicity [Vos *et al.*, 1983].

3. Neurotoxicity

   - ***unspecified route, human***

     In 1980, a farmer suffering from a sensorimotor polyneuropathy disabling his lower extremities was admitted to a clinic in Milan, Italy. These symptoms of neurotoxicity were attributed to atrazine exposure. Although no other data was reported, it was stated that this incident prompted Castano *et al.*, to study the potential neurotoxic effects of atrazine in rats (see below) [Castano *et al.*, 1982].

- ***oral, rat***

  The neurotoxicological effects of technical grade atrazine (95% purity) were studied in 10 CFY male rats by dietary administration at concentrations 1/20 and 1/40 of the LD_{50} (75 mg/kg/day and 37.5 mg/kg/day, respectively) for 6 weeks. Behavioral experiments were carried out in a maze consisting of four T-shaped elements. After 15-hours of food deprivation, the rats were put into the starting point and their running time to the goal and the number of errors made were measured daily during the 6-week testing period. Atrazine was not found to affect either behavioral parameter [Dési, 1983].

- ***intraperitoneal, rat***

  Castano *et al.*, investigated the neurotoxic effects of atrazine in 40 Sprague-Dawley rats which were divided into 3 groups. Animals in groups 1 and 2 (n=14/group) received 40 mg and 20 mg of atrazine, respectively dissolved in 1 ml of dimethyl sulfoxide by intraperitoneal injection. A third group (n=12), which served as the control, was administered dimethyl sulfoxide only. For each group, atrazine, or the vehicle control, was injected twice weekly for a total period of 30 days. Half of the animals in each group were sacrificed at the end of treatment and the other half were sacrificed after a 30-day recovery period. Sciatic nerve, spinal ganglia, and spinal cord samples were removed and examined by electron microscopy. In addition, samples of the liver, kidney, lung and lymph nodes were examined microscopically.

  The only effect observed was a significant decrease in axonal areas, both in myelinated ($P>0.05$) and unmyelinated ($P>0.01$) fibers among rats treated with 40 mg atrazine and sacrificed immediately following treatment. After a recovery period of 30 days, axonal areas had returned to normal. No pathological changes were noted in the liver, kidney, lung, or lymph node. The authors concluded that high doses of atrazine have a reversible neurotoxic effect, primarily involving unmyelinated fibers [Castano *et al.*, 1982].

4. Biochemical Toxicology

   - ***oral, rats***

     The acute enzymotoxic effects of atrazine were examined in groups of 8 female Wistar rats following two oral doses of 0.75 g/kg atrazine in a glycerol-water mixture administered two weeks apart (on days 1 and 14). Control animals received equal doses of the vehicle. Animals were sacrificed 2 and 6 hours, and 1, 2, 3, 5, 7, and 14 days after the first dose, and 1, 3, and 7 days after the second dose. Liver, spleen, and kidney homogenates were prepared for the determination of ceruloplasmin and acid phosphatase activity. In the liver, a sharp decrease in ceruloplasmin activity and a simultaneous mild increase of acid phosphatase activity occurred after the first dose, whereas the second dose caused a sudden rise of both enzymatic levels. In the kidney, ceruloplasmin decreased and acid phosphatase slightly increased on the 2nd and 3rd days after the first dose; however, the second dose caused an increase in ceruloplasmin and a very slight decrease in acid phosphatase levels. In the spleen, the levels of ceruloplasmin generally remained within the limits of the control values following both doses. Acid phosphatase activity, however, significantly increased (P

value not reported) after the first dose, and then was rapidly restored to normal levels by the second dose. The authors concluded that atrazine shows different effects on the activities of the enzymes studied, and that the liver was the most sensitive organ to the influence of this compound. The authors also state that the results seen following the second dose indicate that atrazine undergoes rapid degradation and elimination [Worth, *et al.*, 1982].

- ***oral, rats***

  Male and female Wistar rats were used to examine the effects of atrazine on organ glucose-6-phosphate dehydrogenase (G-6-PDH) and aldolase (ALD) activities following two oral doses of 750 mg atrazine/kg two weeks apart (on days 1 and 14). Atrazine was administered in 2 ml of a glycerol-water mixture. Control animals received equal doses of the vehicle. Animals were sacrificed 2 and 6 hours, and 1, 2, 3, 5, 7, and 14 days after the first dose, and 1, 3, and 7 days after the second dose. Liver, spleen, and kidney homogenates were prepared for the determination of enzyme activity. The results demonstrated that G-6-PDH activity decreased in the liver only 2 hours after the first dose of atrazine, but returned to the normal by day 14. In both the kidney and the spleen, the first dose had no effect on the G-6-PDH activity, and in all three organs, the G-6-PDH activity was lowered on the first day after the second dose and then returned to normal after 3 days. There was no apparent change in the ALD activity during either dose in the liver and the spleen; however, in the kidney, the second dose produced a marked decrease in ALD activity within one day of administration. The authors concluded that the quick recovery of enzyme activities to normal levels was presumably due to rapid elimination of atrazine. Also, a second dose administered after recovery from the first, produced transient enzymotoxic effects [Radovcic *et al.*, 1978].

- ***oral, rats***

  In a study examining the effects of atrazine on 5 alpha-dihydrotestosterone (DHT) receptor complex formation, groups of 8-12 male Fischer rats (28- and 90-days-old) were given daily oral doses of atrazine (12 mg/100 g body weight) in paraffin oil for 7 consecutive days. Control animals were given the same volume of vehicle. Animals were sacrificed 1-22 days after treatment, the prostrate gland was removed and homogenized, and the samples were analyzed for cytosol DHT receptors. Atrazine was found to inhibit DHT receptor complex formation in both 28- and 90-day-old animals; however, the inhibition was stronger in the young rats than in the sexually mature adults. The effect of atrazine on this process was reversible, and returned to normal 22 and 14 days after dosing was stopped in young and adult rats, respectively [Simic *et al.*, 1991]

- ***oral, rats***

  The effect of atrazine on monoamine oxidase (MAO) activity was studied in male Wistar rats. Groups of 7-12 animals were given daily oral doses of 220 mg atrazine/kg for 6 days, and MAO activity was determined in brain and liver tissues 2 hours after the sixth dose. Treatment with atrazine significantly inhibited ($P<0.01$) MAO activity in the brain, and significantly increased ($P<0.01$) the activity in the liver [Bainova *et al.*, 1979].

- ***oral/intraperitoneal, rats***

  The effects of atrazine on rat liver glycogen metabolism were investigated in female Sprague-Dawley rats following oral or intraperitoneal administration. Animals were either fed a diet containing 0.05% atrazine (0.3g/20 ml acetone mixed with 600g of standard diet) for 4 to 7 days, or were given an intraperitoneal injection of 25 mg atrazine (suspended in corn oil)/100 g rat. Control animals were fed a diet that did not contain atrazine or were administered injections of corn oil. Cyclic AMP activity was determined in the liver at unspecified times after oral dosing and at 5, 10, and 15 hours after the intraperitoneal dose. Liver glycogen phosphorylase, adenylate cyclase and phosphodiesterase activities; and liver glycogen and blood glucose levels were determined 5, 10, and 15 hours after intraperitoneal administration.

  Results demonstrated an increase in cyclic AMP levels following both routes of atrazine administration; however, the increase was more pronounced after the intraperitoneal injection than after oral dosing. The increase in liver cyclic AMP was highest 4 hours after injection, and was followed one hour later by a three-fold increase in glycogen phosphorylase activity. Intraperitoneal administration of atrazine also caused a decrease in liver glycogen and a 50% increase in blood glucose content within 4 hours of dosing. However, there were no changes in adenylate cyclase and phosphodiesterase activities. The authors stated that from these results it is evident that atrazine alters the intermediary metabolism of the rat [Messner *et al.*, 1979].

- ***in vitro, mice***

  The effect of atrazine on the active transport of glucose was studied in an isolated intestine from an unspecified strain of mice. A suspension of 0.1 mM atrazine was introduced to a 4-cm section of small intestine removed from a six-week-old male mouse, and was incubated for 80 minutes at 30 degrees. The results showed that atrazine significantly inhibited glucose transport in the serosal fluid ($P<0.05$) and the gut ($P<0.01$) [Guthrie *et al.*, 1974].

H. Aquatic Toxicity

LC_{50} data (96-hour) for atrazine in fresh and salt water invertebrates and fish are presented below in Table 16. Additional effects of acute, prechronic, and chronic atrazine exposure on these organisms are detailed in Table 17.

In aquatic microcosm studies, the introduction of atrazine at concentrations as low as 20 µg/L had adverse effects on freshwater fauna. For example, the dietary habits and reproductive success of three species of fish were negatively affected after exposure for 136 days in ponds containing 20 µg atrazine/L. The reproduction of channel catfish and gizzard shad failed, and that of bluegills, as measured by the number of young per pond, was reduced more than 95%. Also, the number of prey items in the stomachs of bluegills was significantly lower in fish from treated ponds than in fish from control ponds. Macrophyte communities in treated ponds were reduced more than 60% in 2 months; this reduction probably led to impoverished diets and more cannibalism by adult bluegills [USDI, 1989].

Bioconcentration of atrazine in aquatic organisms is most likely limited [Howard, 1991; USDI, 1989], and reported bioconcentration factors (BCFs) from field and laboratory studies were generally low [EC, 1990]. BCFs of 7.5, 11.0, and 76.0 have been reported for snails, fish, and algae, respectively. Also, BCFs of 4.4 and 2.2 were reported for daphnids in water containing 0.01 and 0.08 mg atrazine/L, respectively. Bioaccumulation was not reported for brook trout, fathead minnows, or bluegill sunfish exposed to 0.094-0.74 mg/l for 43-44 weeks. Investigations of the mechanism of atrazine accumulation in mollusks and fish indicate that the majority of atrazine uptake was via the gills, and body tissues were contaminated by the blood. BCFs of 3 and 4 were found for the mollusk and a BCF of 2.8 was found for fish. It was also found that fish accumulated atrazine very rapidly and reached the concentration of atrazine in water within one hour [EC, 1990].

Table 16: Acute LC_{50} Data for Atrazine in Aquatic Organisms

Organism	LC_{50} (mg/L)[a]	Reference
Fresh Water Invertebrate		
Cladoceran (*Daphnia magna*)	3.6-6.9[b]	USDI, 1989; EC, 1990
Scud (*Gammarus fasciatus*)	5,700 (3.6-8.0)[b]	USDI, 1989; EC, 1990
Midge (*Chironomus tentans*)	0.720 (.36-1.44)[b]	USDI, 1989; EC, 1990
Marine Invertebrates		
Mysid shrimp (*Mysidopsis bahia*)	1.0 (.65-3.10)	USDI, 1989; EC, 1990
Copepod (*Acartia tonsa*)	0.094 (0.052-0.167)	USDI, 1989; EC, 1990
Pink shrimp (*Penaeus duorarum*)	6.9 (4.1-12.0)	USDI, 1989; EC, 1990
Grass shrimp (*Palaemonetes pugio*)	9.0 (5.3-16.0)	USDI, 1989; EC, 1990
Fiddler crab (*Uca pugilator*)	>29	USDI, 1989; EC, 1990
Mud crab (*Neopanope texana*)	1,000	USDI, 1989
Shore crab (*Carcinus maenus*)	>100[c]	EC, 1990
Cockle (*Cardium edule*)	>100[c]	EC, 1990
Fresh Water Fish		
Bluegill (*Lepomis macrochirus*)	6-42	USDI, 1989; EC, 1990
Fathead minnow (*Pimephales promelas*)	0.52	USDI, 1989; EC, 1990
Rainbow trout (*Salmo gairdneri*)	4.5-24.0	USDI, 1989; EC, 1990
Brook trout (*Salvelinus fontinalis*)	6.3 (4.1-9.7)	EC, 1990
Crucian carp (*Carassius carassius*)	76	EC, 1990
Goldfish (*Carassius auratus*)	60	EC, 1990
Catfish (*Ictalurus melas*)	7.6	EC, 1990
Perch (*Perca* sp.)	16	EC, 1990
Guppy (*Lebistes reticulata*)	4.3	EC, 1990
Marine Fish		
Sheepshead minnow (*Cyprinodon variegatus*)	>16	USDI, 1989
Spot (*Leiostomus xanthurus*)	8.5 (6.5-12.0)	USDI, 1989; EC, 1990

[a] The values reported are for 96-hour LC_{50}s, unless otherwise specified

[b] 48-hour LC_{50}

[c] Duration unknown

Table 17: The Effects of Acute, Prechronic, and Chronic Exposure to Atrazine in Aquatic Organisms

Organism	Dose(mg/L)/ Duration	Effects	Reference
Invertebrates			
Cladoceran (*Daphnia magna*)	0.2/six generations	reduction in number of young in generations 4, 5, and 6	USDI, 1989
Cladoceran (*Daphnia magna*)	NS/28 days and entire lifespan	reduction in longevity at 20 mg/L, and in reproduction at 1 mg/L	EC, 1990
Cladoceran (*Daphnia magna*)	NS/21 days	reduction in mean # of young/female	EC, 1990
Amphipod (*Gammatus fasciatus*)	NS/30 days-17 weeks	reduction in survival at 0.94 mg/L; reproductive effects at 0.14 mg/L	EC, 1990
Midge (*Chironomus tentans*)	≥ 0.23/2 generations	reduction in hatching; increased larval mortality, retarded larval development; reduced pupation and emergence	EC, 1990
Scud (Gammarus fasciatus)	0.14/119 days	reproductive effects; impaired larval development	EC, 1990
Leech (*Glossiphonia complanata* and *Helobdella stagnalis*)	<1/NS	reduction in growth, food intake, and egg production	USDI, 1989
Brown shrimp (*Penaeus aztecus*)	1/48 hrs.	50% immobilized	USDI, 1989
Oyster (*Crassostrea virginica*)	>1/96 hrs.	reduction in growth	USDI, 1989
Fiddler crab (*Uca pugnax*)	1000/8 weeks	death; interference with escape response	EC, 1990
Fiddler crab (*Uca pugnax*)	10,000/10 weeks	reduced survival	EC, 1990
Fish			
Brook trout (*Salvelinus fontinalis*)	0.45/NS	reduction in embryo incubation time	USDI, 1989
Brook trout (*Salvelinus fontinalis*)	0.24-0.72/90 days	reduction in fry growth and survival	EC, 1990
Bluegill (*Lepomis macrochirus*)	0.5/28 hrs.	lethargy, poor eating, erratic swimming	EC, 1990
Smallmouth bass (*Micropterus dolomieui*)	10/72 hrs.	death within 3 days	EC, 1990
Channel catfish (*Ictalurus punctatus*)	0.05-46.7/spawning-96 hrs. post-hatch	4-100% of hatched fish had unspecified teratogenic effects	EC, 1990

Table 17: The Effects of Acute, Prechronic, and Chronic Exposure to Atrazine in Aquatic Organisms (continued)

Organism	Dose (mg/L)/ Duration	Effects	Reference
Rainbow trout (*Salmo punctatus*)	0.05-50.9/spawning-96 hrs. post-hatch	3-62% of hatched fish had unspecified teratogenic effects; no hatching at high dose	EC, 1990
Carp (*Cyprinus carpio*)	0.1/6-72 hrs.	increased serum glucose and cortisol; decreased serum protein, cholesterol, and liver glycogen	EC, 1990
Other Vertebrates			
Bullfrog (*Rana catesbeiana*)	0.4-45.8/spawning-96 hrs. post-hatch	3-100% of hatched toads had unspecified teratogenic effects	EC, 1990
American toad (*Bufo americanus*)	10.8-48.2/spawning-96 hrs. post-hatch	3-17% of hatched toads had unspecified teratogenic effects	EC, 1990
Leopard frog/tadpoles (*Rana pipiens*)	0.31-12.0/54 days	increased mortality after 27 days; growth retardation	EC, 1990

NS = Not specified

I. Avian Toxicity

The acute avian toxicity (LD_{50}) of atrazine is presented below in Table 18. Additional effects of acute/subacute and reproductive/teratogenic exposure to atrazine in birds are summarized in Tables 19 and 20, respectively.

Table 18: The Acute Toxicity (LC_{50}) of Atrazine in Avian Species

Organism	LC_{50} (duration of dosing)	Reference
Mallard duck (eggs)	>375 lbs./A (30 sec. immersion)	Hoffman and Albers, 1984
Mallard duck	19,650 mg/kg in feed (8 days)	EC, 1990; USDI, 1989
Mallard duck	>5000 mg/kg in feed (5 days)	EC, 1990
Japanese quail	>5000 mg/kg in feed (5 days)	EC, 1990
Ring-necked pheasant	>5000 mg/kg in feed (5 days)	EC, 1990
Bobwhite pheasant	>5000 mg/kg in feed (5 days)	EC, 1990
Bobwhite pheasant	5760 mg/kg in feed (8 days)	EC, 1990; USDI, 1989
Bobwhite pheasant	700-800 mg/kg (5 days)	EC, 1990

Table 19: Effects of Acute and Subacute Exposure to Chlorpyrifos in Avian Species

Route	Species (Strain/sex)	Dose	Effect	Reference
Oral	Chicken (Gallus/ laying hens)	100 mg/kg for 7 days	No effect on eggs; atrazine detected in excreta until day 11	USDI, 1989
Oral	Chicken (Gallus/ NS)	100 mg/kg for 7 days	Tissue residues ranged from 0.04 (muscle) to 38.8 (abdominal fat)	USDI, 1989
Oral	Pheasant (Ring-necked/males)	2,000 mg/kg;	weakness, hyperexcitability, ataxia, tremors; remission by day 5	USDI, 1989
Oral	Duck (Mallard/ females)	2,000 mg/kg	weakness, tremors, ataxia, weight loss; symptoms persisted up to 11 days	USDI, 1989
Oral	Bird (Coturnix/ NS; chicks)	5,000 mg/kg	1/14 birds died on day 3; no other effects were observed	USDI, 1986

Table 20: Reproductive and Teratogic Effects of Atrazine in Avian Species

Species (Strain)	Test System	Dose	Parameter	Result	Reference
Hen (NS)	Egg injection technique	10-500 ppm atrazine HCl in methanol	Embryotoxicity (hatching rate)	Negative	Dunachie and Fletcher, 1967; Dunachie and Fletcher, 1970
			Teratogenicity (external effects)	Negative	
Duck (mallard)	30-sec. egg immersion	1.5-3.75 lbs/A (conc. based on lbs. active ingredient/100 gal/A)	Embryotoxicity (mortality, weight, crown-rump length)	Negative	Hoffman and Albers, 1984
			Teratogenicity (external effects)	Negative	

VI. STRUCTURE ACTIVITY RELATIONSHIPS

Atrazine is a member of the *s*-triazine herbicide class. Structurally related *s*-triazine herbicides include simazine, propazine, and cyanazine (See Figure 3). These triazine compounds have not been tested by the National Toxicology Program (NTP). According to studies by Innes *et al.*, (1969), propazine and simazine are not tumorigenic [Hayes, 1982]. However, according to results of another study (1970), simazine caused equivocal evidence of carcinogenicity following subcutaneous administration in rats (16 gm/kg for 61 weeks) and mice (35 gm/kg for 87 weeks) [RTECS, 1992]. In a two year rat feeding study conducted by Ciba-Geigy, propazine (1000 ppm) caused a significant increase in mammary tumors in females, but not males [USEPA, 1992]. Simazine and propazine are currently being reviewed for evidence of human carcinogenic potential by the EPA.

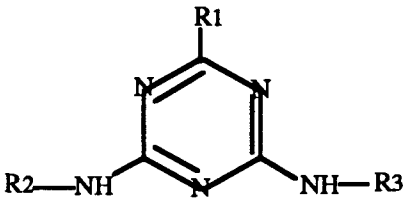
As reported by Murnik and Nash (1977), simazine increased the frequency of X-linked lethals following injection into male *Drosophila melanogaster*, but failed to do so when fed to larvae [Hayes, 1982]. Simazine also induced sister chromatid exchange in cultured human lymphocytes and was mutagenic in cultured mouse lymphocytes. In addition, cyanazine has been found to be positive in *D. melanogaster* by both parenteral and oral routes of administration. Other tests for mutagenicity in this species were negative [Hayes, 1982].

Cyanazine is under review by the EPA for developmental effects. This herbicide was found to cause decreased pup viability and decreased mean pup body weight during lactation in a 2-generation reproductive study in rats conducted by DuPont. In a study conducted by Shell Chemical, cyanazine also caused developmental effects in rats (significant alterations in skeletal variations, abnormal developments of the diaphragm, and anophthalmia/ microphthalmia) and fetotoxic effects in rabbits (increased number of post-implantation losses, decreased number of live fetuses/dam). In addition, propazine caused a reduction in mean pup body weight in a three generation reproduction study conducted by Ciba-Geigy [USEPA, 1992b]. This compound also induced developmental effects (delayed ossification of cranial structures) in the offspring of rats administered by gavage on days 6-15 of gestation. In a 1981 study, simazine caused effects on fertility, fetotoxicity, and developmental parameters following oral administration to rats on days 6-15 of gestation [RTECS, 1992].

Atrazine has been found to form the N-nitroso derivative, N-nitrosoatrazine (2-chloro-4-(N-nitroso-N-ethylamino)-6-isopropylamino-s-triazine under experimental conditions both in vitro [Wolfe et al., 1976; Eisenbrand, et al., 1975a,b; Janzowski et al., 1980] and in vivo using mice.[13] The N-nitroso derivative of this herbicide has been reported by some authors to be carcinogenic in unspecified animals [Kruall et al., 1980]. However, in a recent study, Weisenburger et al., reported that N-nitrosoatrazine was noncarcinogenic in female Swiss mice and female Wistar rats treated with either N-nitrosoatrazine by gavage twice weekly, or with atrazine and NaNO2 in their drinking water for 96 weeks (mice) or 104 weeks (rats). These authors also report that N-nitrosoatrazine was found to be mutagenic in the Ames assay using hamster liver S9 activation, but was not mutagenic in the absence of metabolic activation [Weisenburger et al., 1990b].

[13]According to Ciba-Geigy, based on available data, N-nitrosoatrazine residues do not occur in plants, animals, or the environment. Considering the inherent instability of the compound and the belief that the necessary environmental conditions do not exist in nature, it is Ciba-Geigy's position that the use of atrazine will not result in the formation of N-nitrosoatrazine [Ciba-Geigy, 1991].

Figure 3. Structurally Related *S*-Triazine Herbicides



Herbicide	R_1	R_2	R_3
simazine	Cl	C_2H_5	C_2H_5
cyanazine	Cl	C2H5	$C(CH_3)_2CN$
propazine	Cl	$(CH3)_2CH$	$CH(CH_3)_2$

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APPENDIX I. ON-LINE DATABASES SEARCHED

Date of Search: **December, 1991**

CIS:
TSCAPP (TSCA Plant and Production Search System)
TSCATS

DIALOG:
Chem Bus Newsbase
Chem Safe Newsbase
Medline
Toxline
Toxline 65

MEAD:
Lexis Gen Fed; CFR

NLM:
Chemid
Chemline
CCRIS
DART
Eticback
Emicback
HSDB
IRIS*
RTECS

STN:
Beilstein
CA (Toxlit, Toxlit 65)
CApreviews
Chemlist
CSchem
Registry

*Database Search Updated February, 1992.

APPENDIX II. SAFETY INFORMATION

- **HANDLING AND STORAGE**

  Atrazine is stable in slightly acidic or basic media. This compound is hydrolyzed to inactive hydroxy derivatives by alkali or mineral acids [Budavari, 1989]. Atrazine has a shelf-life of 3 years when stored in unopened containers in a well-ventilated area away from sources of heat and light [Meister, 1990]. Atrazine is subject to decomposition, but under normal conditions this effect is small [WSSA, 1983]. Atrazine is a non-corrosive [RSOC, 1987].

  See sections II.C., Chemical and Physical Properties and IV. D., Regulatory Status, for additional information.

- **EMERGENCY FIRST AID PROCEDURES**

  Eye: First check the victim for contact lenses and remove if present. Flush victim's eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control center. Do not put any ointments, oils, or medication in the victim's eyes without specific instructions from a physician. If symptoms (such as redness or irritation) develop, immediately transport the victim to a hospital.

  Skin: IMMEDIATELY flood affected skin with water while removing and isolating all contaminated clothing. Gently was affected skin areas thoroughly with soap and water. If symptoms such as inflammation or irritation develop, IMMEDIATELY call a physician or go to a hospital for treatment.

Inhalation: IMMEDIATELY leave the contaminated area and take deep breaths of fresh air. If symptoms (such as wheezing, coughing, shortness of breath, or burning in the mouth, throat, or chest) develop, call a physician and be prepared to transport the victim to a hospital.

Provide proper respiratory protection to rescuers entering an unknown atmosphere. Whenever possible, Self-Contained Breathing Apparatus (SCBA) should be used.

Ingestion: If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and IMMEDIATELY call a hospital or poison control center. Be prepared to transport the victim to a hospital if advised by a physician.

If the victim is convulsing or unconscious, do not give anything by mouth, ensure that the victim's airway is open and lay the victim on his/her side with the head lower than the body. DO NOT INDUCE VOMITING. IMMEDIATELY TRANSPORT THE VICTIM TO A HOSPITAL.

- **PROTECTIVE EQUIPMENT**

  Eye: Safety goggles.

  Gloves: Two pairs of dissimilar protective gloves shall be worn when handling the neat chemical, otherwise one pair. When contact with this chemical has been known to occur, change gloves immediately.

  Clothing: Minimally, a disposable laboratory suit (e.g. Tyvek ®) shall be worn, as specified in the most current NTP Statement of Work or the NTP Health and Safety Minimum Requirements.

  Respiratory Protection: A NIOSH-approved chemical cartridge respirator with an organic vapor and high-efficiency particulate filter cartridge.

- **EXTINGUISHANT**

  Dry chemical, carbon dioxide or halon extinguisher.

- **MONITORING PROCEDURES**

  There is no NIOSH analytical method reported in the NIOSH Manual of Analytical Methods for atrazine.

- **SPILLS AND LEAKAGE**

  Persons not wearing the appropriate protective equipment and clothing shall be restricted from areas of spills until cleanup has been completed. When exposure to unknown concentrations may occur, air-purifying respirators may not be used. Chemical cartridge respirators with organic vapor cartridges may not be used when airborne concentrations exceed 1000 ppm.

  If atrazine is spilled the following steps shall be taken:

  1. In order to prevent dust formation, use moistened paper towels to clean up a solid spill. Avoid dry sweeping.

  2. If a liquid solution is spilled, use vermiculite, sodium bicarbonate, sand, or paper towels to contain and absorb the spill.

  3. Clean the spill area with dilute alcohol (approximately 60-70%) followed by a strong soap and warm water washing.

  4. Dispose of all absorbed material as hazardous waste.

- **DECONTAMINATION OF LABORATORY EQUIPMENT**

  TDMS Terminal: Whenever feasible, a protective covering (e.g.,plastic wrap) shall be placed over the keyboard when in use.

  General Equipment: Before removing general laboratory equipment (i.e., lab carts, portable hoods and balances) from animal dosing rooms and/or chemical preparation areas, a decontamination process shall be conducted in addition to routine housekeeping procedures.

- **WASTE MANAGEMENT AND DISPOSAL PROCEDURES**

  Waste Management: If an inhalation study is to be conducted, all exhaust air from the inhalation chamber must be cleaned with appropriate air cleaning devices unless the laboratory has informed local and state air pollution regulatory agencies of both the laboratory's operating practices and the potential hazards of the chemicals in use. Compliance with all federal, state and local air pollution laws and regulations is required. A specific air cleaning system design must consider the specific conditions of the laboratory (eg., air flow rates and volumes, mixing of exhaust streams, size of inhalation chamber, etc.) and the dosing regimen selected. Air cleaning systems designs must be described by the laboratory and approved by the NTP Office of Laboratory Health and Safety.

  Waste Disposal: Securely package and label, in double bags, all waste material. All potentially contaminated material (i.e., carcasses, bedding, disposable cages, labware) shall be disposed of by incineration in a manner consistent with federal (EPA), state, and local regulations or disposed of in a licensed hazardous waste landfill.